V.—*On the Limits of the Optical Capacity of the Microscope.*

By Professor HELMHOLTZ; with a Preface by Dr. H. FRIPP.

THE last number of the 'Proceedings of the Bristol Naturalists' Society' contained a translation of Professor Abbe's article on the "Theory of the Microscope," originally published in Schultze's 'Archives.' In that article Professor Abbe stated the general conclusions at which he had arrived after a prolonged investigation of the optical laws affecting the transmission of light through the lenses of the microscope. These laws relate to—1. The divergence of the rays of light forming a geometrical image; 2. The brightness of that image; 3. The dispersion of coloured rays, and its consequences; and 4. The diffraction of light occasioned by minute particles in the *objects* placed under (or before) the microscope. In explanation of these several phenomena, a theory of the microscope was stated in general terms, the mathematical demonstration of this theory, and its various applications, being reserved for a future communication.

Simultaneously with Professor Abbe's researches, a most interesting investigation of the same subject was completed by Professor Helmholtz, and appeared in Poggendorff's 'Annals' (1874). The theoretical grounds taken by these two authors are identical, and their results, so far as the researches were directed to the same points, also agree. But in each essay the mode of treatment is thoroughly independent, and the experimental proof of the conclusions respectively obtained is conducted by each writer in a separate and original method. The mathematical demonstrations omitted in Professor Abbe's article are fortunately supplied by Professor Helmholtz, and the two essays are confirmatory and supplementary to each other in several other respects, whilst in both we recognize that clearness of thought and precise knowledge of the subject treated, which justifies entire confidence in the conclusions. It seems therefore to me that Professor Helmholtz's essay should naturally follow in this number of our 'Proceedings.' For, taken together, these two essays form the most complete and authoritative exposition of the optical principles involved in the action of microscope objectives, and the most trustworthy interpretation of that action, and consequently of the capacity of performance of such objectives, that have as yet been made public.

In introducing the first of these essays to the notice of our readers, I expressed my strong conviction of its high value as a contribution of really scientific character to the theory of the microscope. The essay of Professor Helmholtz deals somewhat more fully with that aspect of optical science which is known as physiological optics, and of which no physicist of our times has a more

profound knowledge. This point of view had not been neglected by Dr. Abbe, but in my translation two short sections of his essay, which referred to brightness of image, and to certain inquiries connected with illumination of the image, were, for reasons mentioned in the preface, omitted. It is therefore so much the more satisfactory that Professor Helmholtz's essay enters fully into the subject. The peculiar conditions under which objects are seen when magnified by the microscope, can only be understood by studying both aspects, physical and physiological, in connection with each other. The laws of formation of optical images (when amplified by interposition of lenses), and the laws of dispersion of the rays by which these images are formed, help us to an interpretation of the physical agencies at work, and show us also why the extreme amplifications employed render vision through the microscope more imperfect than through any other optical instrument, such as telescope or camera. But the analysis of these physical agencies and effects involves the consideration of the eye itself, as an optical instrument through which the microscope image must pass to reach the perceiving organ. And apart from the imperfections arising from aberrations and dispersions of rays in the instrument, other imperfections of the retinal image will be found in considering the more or less favourable conditions under which the microscope image enters the eye. The area into which the microscope image is collected at the eye spot (over the ocular), varies in size with the amplification, and is smaller in proportion as the amplification is greater. And this variation of size is accompanied by variation in brightness of image and distinctness of detail. If the area of illuminated image entering the pupil is smaller than that of the pupillary aperture, loss of brightness is felt. For the condition of most effective illumination (brightness of image) is that which obtains when the area of image at the eye spot, and the area of the pupil, are equal. On the other hand, a small and intensely bright spot of light in front of the pupil presents the exact condition under which entoptic shadows obscuring the image are thrown with it on the retina. But as brightness of image is as necessary to distinct vision as any mere amplification of detail can be, it follows that a suitable relation of "aperture" to "magnifying power" must be maintained in every good objective; for "aperture" in this particular case means the measure of light admitted with the image-forming rays; and as a larger measure of light is required in proportion to the increase of magnifying power, so it is only when these two factors are suitably proportioned that details in the objective will be rendered clearly visible in its microscope image. And again, as respects the bundle of rays collected into a smaller or larger area at their entrance to the pupil, the regulation of illumination from without is better maintained with a large

"aperture" of objective by means of diaphragm openings and stops than by using stronger light with diminished aperture. Thus the management of illumination, and manipulation of the microscope to obtain good definition, though for the most part left to empirical practice, would be more easily and thoroughly acquired if the physiological laws were carefully studied. But another and far more serious deterioration of definition arises from excessive diminution of area of the image entering the pupil. This contracted area —the necessary consequence of the optical combinations used to obtain high amplification—has the same effect as any minute aperture through which a luminous object is viewed, and occasions, as is well known in physics, those diffractive effects which obscure the outlines of an image by making them overlap each other. On this fact is founded the whole argument of Professors Helmholtz and Abbe respecting the limits of microscopic vision, as well as the corollary which directly follows from it respecting the ultimate limits of minuteness to be assigned for vision of any and every kind of material atoms with the optical apparatus and materials yet employed. The theory of the microscope as interpreted by Helmholtz and Abbe on identical physical and physiological bases, is therefore of great importance in its general bearing on physical science, and the precise and comprehensive treatment of it in the following pages worthy of careful study.

As respects the translation now offered, it is only necessary to add that it was undertaken at the same time as that of Professor Abbe's essay, and with exactly the same motives. Our readers will, it is hoped, bear in mind that the translator's object was simply to make known to those who could not otherwise so readily inform themselves, the views of scientific men abroad, whose authority on these subjects is at all events high in their own country, and whose teaching he had himself accepted with pleasure. No mention of English cotemporary work was needed therefore in the brief introductory notice of Dr. Abbe's article. Since its publication, however, the translator has been questioned respecting English contributions to the theory of the microscope, and he therefore ventures to add a few words on this subject.

One may be well excused from referring to the meagre optical chapters in our handbooks on the microscope, which might perhaps suit the 'Boys' Own Book,' but which contain neither demonstration nor diagram of the course of rays through any sort of modern lens system, nor even a rough application of its very elementary statements respecting refraction and reflexion to any special formulæ of constructions, according to which the lens combination of an objective would be worked, or by which its performance would be tested. Nor can the favourite descriptive chapter of the instruments of various makers help anyone to a theory of the micro-

scope. The opinions expressed by experts and authorities on definition, penetration, resolution, aperture, &c., as being so many separate *powers* or qualities, besides savouring strongly of a mythological period in the history of the microscope, have only retarded the search in the right direction, viz. by physical analysis and physiological study of optical phenomena for true causes of the effects observed. And in fine it must be confessed that our handbooks fail greatly in respect to theories of the microscope, however valuable their information on practical and mechanical subjects, and more especially on all branches of science involving skilful *use* of the instrument.

In the absence of such handbooks as the German students possess, and of which the work of Nägeli and Schwendener might be cited with admiration as an example, the scattered articles and shorter notices in our serials rise into comparative importance. But it will scarcely be contended that such desultory and disconnected communications and such remarkable disputes respecting easily determined facts, should be accepted as an equivalent of the systematic theory and practical demonstration which distinguish foreign study of optics applied to the microscope, from our yet unlearnt, or at least unwritten, micrographic science.

Various communications bearing more or less on the optical capacity of lens systems constructed on given formulæ or for employment as "dry" or "immersion" objectives, have appeared in the 'Monthly Microscopical Journal,' the 'Quarterly Journal of Microscopical Science,' and the 'Transactions of the Royal Society' during present and preceding years. Of these, one series of papers published by Dr. R. Pigott claims to be a mathematical exposition of optical laws governing the divergence and dispersion of rays of light transmitted through different kinds of glass. Another series of papers by Mr. Wenham takes the practical direction to which English microscopists mostly incline. The communications of Mr. Sorby have enriched microscopic science with the most ingenious and successful applications of spectrum analysis that any country can boast. To all these gentlemen the English student may feel equally indebted for their respective labours. And the mention of these in juxtaposition with the work of so great an authority as Professor Helmholtz and so conscientious a workman as Professor Abbe, is not only due as a recognition of the individual services, but also as a proof of the higher direction of study now being pursued in England by amateur microscopists. As a humble member of this numerous class, the present writer ventures to refer to the early date of Mr. Wenham's communications when he stood almost alone as the pioneer of a future micrographic science, and to bear thankful testimony to the practical experience and sterling value of all

that he has written. And he also cordially recognizes the high aim and zealous study of Dr. R. Pigott, the direction of whose labours must ultimately prove most serviceable to all who desire to understand the real power and possible perfection of their favourite instrument. Any unfair spirit of criticism of matters so little appreciated by some of his critics is to be earnestly deprecated. One can only regret, whilst profiting by the opportunity of hearing all sides of a question, to be reminded of the woeful sentiment "*tantæne celestibus iræ.*" The vexatious partisanship of "aperture" and the disputed estimates of the performance of lenses constructed by this or that maker, must appear as overstrained and even ridiculous to the optician who can best gauge his own or any other maker's work, as to those who care only to understand the principles of construction and to form a rational judgment of their action.

It is to be hoped that a more general agreement on the essential parts of the theory of the microscope will soon prevail, and that the exaggerated significance of certain matters too long discussed in our journals, will fade to its proper vanishing point.

The Theoretical Limits of Optical Capacity of the Microscope.

In Poggendorff's 'Annalen' for 1874, Professor Helmholtz published an article, of which the following is a translation.

Whether, and to what extent, the optical performance of the microscope is capable of further improvement, is a question of the greatest moment for many branches of natural history. Doubtless, some progress, and notably through the revival of Amici's suggestion of immersion lenses adopted and carried out with such success by Hartnack, has been made, but each onward step is slow and faltering. We have, it is clear, arrived now at a point at which any trifling gain is effected with a disproportionate effort of mental as well as mechanical labour. And yet, so far as I can see, no one has been able to give any reason why this should be, excepting the common belief that the difficulty lies in overcoming the spherical aberration of lenses so small and of such quick curvation as is needed for objectives of very high magnifying power. It is not long since Herr Listing, one of the most eminent authorities on this subject, discussed * the means by which it might be possible to obtain amplifications ranging from 25 to 50,000 diameters, whilst in actual practice the ordinary range of *serviceable* amplification is at the present moment limited to from 400 to 800 diameters. Moreover, the collective experience obtained by repeated efforts of practical opticians has taught us that all high amplifications combined with good definition (i. e. sharp delinea-

* Poggendorff's 'Ann.' vol. cxxxvi.

tion) are obtainable only by instruments in which the objective admits a cone of light of very large angular aperture from each point of the object.

We have gradually arrived at that stage of improvement in the construction of instruments in which rays of light whose direction is nearly perpendicular to the axis of the instrument are passed into and through the objective, and transmitted towards the ocular. This, it is true, happens only when a lens is used dry (i. e. the front surface in contact with air), in which case rays inclined to the axis at angles up to $87\frac{1}{2}°$ actually do enter a well-constructed immersion lens. This angle, however, diminishes to about 48° * when the lens is used wet, that is, when water is dropped between lens and covering glass as in the ordinary practice. This last-named angle is nevertheless of far higher amount than any angle of aperture in the lens system of a telescope, or photograph camera, because with such oblique incidence the spherical aberration, even in the carefully calculated and accurately executed lenses of these instruments would be simply intolerable. Why then, notwithstanding this, is the large incident cone of light in the microscope more advantageous than a narrow one of more intense light which would deliver an equal absolute quantity? The answer hitherto given to this question appears to me unsatisfactory. For the so-called "penetration" (i. e. the power of delineating by light and shadow and so rendering visible to the eye particles whose refractive quality differs but slightly from that of the matter surrounding them) depends solely upon the proportion of the aperture of *illuminating* cone to that of the cone passing from points of the object into the lens. Sufficient delineating shadow can only be got by narrowing the aperture of the illuminating cone; and a comparatively large cone can only be applied beneath the object when the cones of light passing from it into the objective are also large.

Now there does, in point of fact, exist in the microscope a special cause which under the conditions here given produces a far greater aberration of rays from the focal plane than is occasioned by spherical and chromatic aberration, and which makes itself most felt just when the cones of incident light are smallest. This cause is diffraction.

If, perhaps, occasional allusion has been made to diffraction as a cause of deterioration of the microscopic image, I have yet nowhere found any methodical investigation into the nature and amount of its influence; but such an investigation shows, as will here appear, that diffraction necessarily and inevitably increases with the increase of magnifying power, and at length presents an

* These figures, it must be borne in mind, denote in each case the angle included between outermost incident ray and axis of instrument, that is, *half* the so-called "*angle of aperture.*"

impassable limit to the further extension of microscopic vision, which limit, moreover, has been already closely approached in our newest and best instruments.

That diffraction and consequent obscurity of microscopic image must necessarily increase with increasing amplifications of the image, and this quite independently of any particular construction of the instrument, rests as a fact upon a general law which applies to all optical apparatus, and which was first formularized by La Grange * for combinations of any kind of "infinitely thin" lenses. This law has apparently remained almost unknown, perhaps because La Grange enunciated it in equations whose coefficients have not characters which readily present clear ideas to the mind. In my treatise on physiological optics, I have given expression to this law in a somewhat more general form, namely, for centred systems of refracting curved surfaces with any singly refracting medium between them, and have endeavoured to formularize it in readily intelligible physical characters. I shall therefore recapitulate as briefly as possible this theorem and its demonstration. It holds good for every centred system of spherical refracting or reflecting surfaces through which rays pass with angles of incidence so fine as to form punctiform images of punctiform objects; that is to say, refracts homocentric rays, homocentrically.

By the term centred system, I designate one in which the centres of the curves of each refracting or reflecting spherical surface lie in the same straight line, the "axis" of the system. In front of such a system, and situate in its axis, let us suppose a luminous point belonging to some object lying in a plane at right angles to the axis, and from which rays pass through the system. The angle formed between any one of such rays and the axis, we shall call the divergence angle of that particular ray. Any plane supposed to extend through the axis and along the ray, constitutes the incidence plane of that ray at the first refraction, and will include, therefore, the same ray after its next refraction, and consequently after every subsequent refraction. Of this plane, which will be divided in crossing the axis into two halves, one half will be treated as positive, the other as negative, and in correspondence therewith, the divergence angle of the ray as positive or negative, according as the ray proceeds towards the positive or negative half of the plane. These postulates being settled, the rule may be thus stated:—

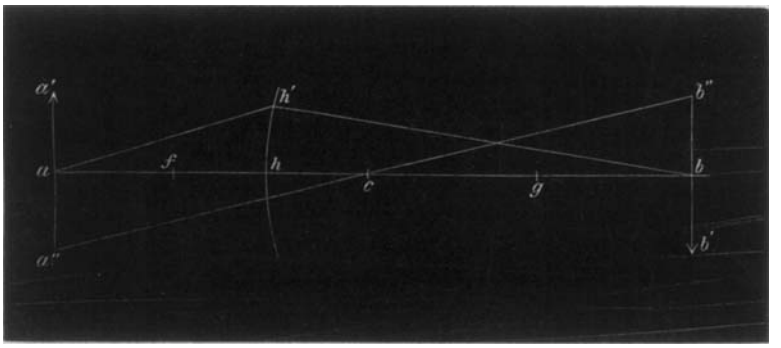
THEOREM.

In a centred system of spherical refracting or reflecting surfaces the product of the divergence angle of any ray, the refraction indeæ of the medium through which that ray passes, and the

* "Sur une Loi générale d'Optique," 'Mémoires de l'Académie de Berlin,' 1803.

magnitude of the image to which the rays passing through that medium belong, remain unchanged by every refraction, provided always that the conditions of production of an accurate image are duly preserved. This product will therefore have the same value after emergence of the rays as it had before they entered the system of lenses.

DEMONSTRATION.



Let $a\ b$ be the axis of a lens system,
$h\ h'$ one of the refracting surfaces,
c the centre of its curve,
a the point of convergence of rays, incident on $h\ h'$,
b the point of reunion of rays refracted by $h\ h'$,
f the front principal focus,
g the back principal focus.

Further, let n' represent the ratio of refractions of the medium in front of $h\ h'$,
n'' represent the ratio of refractions of the medium behind $h\ h'$,
a' the positive divergence angle $h'\,a\,h$ of the ray passing in first medium through h',
a'' the negative divergence angle, in second medium $-h'\,b\,h$,
β' the magnitude of image $a\ a''$ belonging to the rays of the first medium,
β'' the magnitude of image—$b'\ b''$ belonging to the rays of the second medium.

Firstly, we have, from similarity of triangles $a\,a''\,c$ and $b\,b''\,c$,

$$\frac{\beta'}{\beta''} = -\frac{a\,c}{c\,b}. \qquad [1]$$

Again, if we consider the short arc $h\,h'$ of the refracting surface as a straight line at right angles to the axis $a\,b$,

$$h\,h' = a\,h \,.\, \text{tan.}\ \alpha' = -\,b\,h \,.\, \text{tan.}\ \alpha''.$$

Or substituting the angles for the tangents, which is allowable here on account of the smallness of the angle,

$$\frac{\alpha'}{\alpha''} = \frac{b\,h}{a\,h}. \qquad [2]$$

Multiplying equations [1] and [2], we get

$$\frac{\alpha' \,.\, \beta'}{\alpha'' \,.\, \beta''} = \frac{a\,c \,.\, b\,h}{b\,c \,.\, a\,h}. \qquad [3]$$

Now, according to the known laws of refraction at a spherical surface, whose radius $h\,c = r$, the value of their principal focus is

$$\mathrm{F}' = hf = \frac{n'\,r}{n'' - n'}; \quad \mathrm{F}'' = hg = \frac{n''\,r}{n'' - n'}; \qquad [4]$$

from which follow

$$\frac{\mathrm{F}'}{\mathrm{F}''} = \frac{n'}{n''}; \qquad [4^a]$$

$$\mathrm{F}'' - \mathrm{F}' = r. \qquad [4^b]$$

Further,

$$\frac{\mathrm{F}'}{a\,h} + \frac{\mathrm{F}''}{b\,h} = 1, \quad \text{and} \quad \frac{\mathrm{F}''}{a\,c} + \frac{\mathrm{F}'}{b\,c} = 1;$$

or

$$\frac{b\,h}{a\,h} = \frac{b\,h - \mathrm{F}''}{\mathrm{F}'}, \quad \text{and} \quad \frac{b\,c}{a\,c} = \frac{b\,c - \mathrm{F}'}{\mathrm{F}''}.$$

Division of the last two equations gives

$$\frac{b\,h \,.\, a\,c}{a\,h \,.\, b\,c} = \frac{\mathrm{F}''\,(b\,h - \mathrm{F}'')}{\mathrm{F}'\,(b\,c - \mathrm{F}')};$$

but by equation [4^b],

$$b\,h = b\,c + r = b\,c + \mathrm{F}'' - \mathrm{F}',$$

and

$$b\,h - \mathrm{F}'' = b\,c - \mathrm{F}'.$$

Hence

$$\frac{b\,h \,.\, a\,c}{a\,h \,.\, b\,c} = \frac{\mathrm{F}''}{\mathrm{F}'} = \frac{n''}{n'}, \text{ according to equation } [4^a].$$

Therefore equation [3],

$$\frac{\alpha' \,.\, \beta'}{\alpha'' \,.\, \beta''} = \frac{n''}{n'},$$

or

$$n' \,.\, \alpha' \,.\, \beta' = n'' \,.\, \alpha'' \,.\, \beta''. \qquad [5]$$

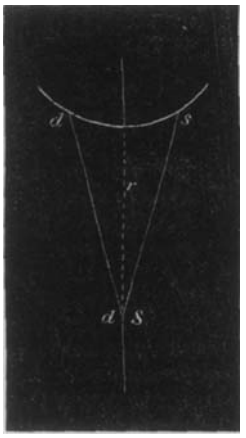
q. e. d.

From this theorem it follows—

Firstly, that when a ray, B, proceeding from a luminous point has an absolute smaller divergence angle than the ray A, the divergence angle of B will, after subsequent refraction, remain always less than that of A, because the product obtained by our theorem for B is from the beginning less than that obtained for A, and for the same reason must continue to be smaller after each refraction.

Secondly, when two rays, starting from the same point on the axis, with equal angles of divergence, but following planes which extend in opposite directions through the axis, their divergence angles continue to be equal after each refraction, a result which appears indeed at once evident from the symmetrical disposition of a lens system round its axis.

If now we imagine the illuminating rays, on their way to the object, to be circumscribed by interposing a diaphragm pierced with a circular opening whose centre coincides with the axial line, the plane of the diaphragm being at right angles with the optical axis, then those rays which pass through the opening close to its margin have all alike the largest divergence angle, and retain the same relation after each fresh refraction. These rays obviously occupy the exterior outline of cones having a circular base, and whose axis is the optical axis of the lens system, and they constitute the boundary of the cone of light proceeding from the luminous point. The divergence angle of these border rays is, in this case, throughout their entire course, the angle which the semi-aperture of the conical surface bounding the illuminating cone measures.



From this there follow, firstly, certain important results in regard to the *photometric* conditions of the microscope image.

According to known laws of photometry, we may equate L the quantity of light sent forth from the luminous point $d\,\mathrm{S}$ upon another point $d\,s$, whose distance is r, as follows, where (r, N) and (r, n) represent the angles formed between the line r and the normals N and n.

$$\mathrm{L} = \mathrm{J}\,\frac{d\,\mathrm{S}\,.\,d\,s}{r^2}\,.\,\cos.\,(r, \mathrm{N})\,.\,\cos.\,(r, n). \quad [6]$$

If now we understand by $d\,s$ the circular aperture of the cone of rays at one of the refracting surfaces, and by $d\,\mathrm{S}$ a luminous point intersected by the axis so that r falls in the axial line,

Then cos. $(r, n) = 1$, and $d\,\mathrm{S} \,.\, \cos.\,(r, \mathrm{N})$ is the projection of $d\,\mathrm{S}$ on a plane normal to the axis.

Let α be the angle of divergence of the rays directed to the periphery of $d\,s$, then $d\,s = \pi \,.\, r^2 \,.\, \alpha^2$.

$$\mathrm{L} = \mathrm{J} \,.\, \pi \,.\, \alpha^2 \,.\, d\,\mathrm{S} \,.\, \cos.\,(r, \mathrm{N}). \qquad [6^a]$$

The same amount of light must also be contained in the same cone of rays continued through the following medium. And if we indicate the corresponding quantities by the signs J′, α', $d\,\mathrm{S}'$, N′, then

$$\mathrm{L} = \mathrm{J}' \,.\, \pi\, \alpha'^2 \,.\, d\,\mathrm{S}' \,.\, \cos.\,(r, \mathrm{N}'). \qquad [6^b]$$

Now, $d\,\mathrm{S}'$ is the image of $d\,s$, and its projection—normal to the axis—$d\,\mathrm{S}' \,.\, \cos.\,(r, \mathrm{N}')$ is the image of the corresponding projection of $d\,\mathrm{S}$. We have therefore the proportion

$$d\,\mathrm{S} \,.\, \cos.\,(r, \mathrm{N}) : d\,\mathrm{S}' \,.\, \cos.\,(r, \mathrm{N}') = \beta^2 : \beta'^2.$$

From which follows

$$\mathrm{J} \,.\, \alpha^2 \,.\, \beta^2 = \mathrm{J}' \,.\, \alpha'^2 \,.\, \beta'^2;$$

and by equation [5],

$$\mathrm{J} : \mathrm{J}' = n^2 : n'^2. \qquad [6^c]$$

This gives the brightness with which the surface of image included within the outline of the illuminating cone shines, independent of the direction which $d\,\mathrm{S}$ and $d\,\mathrm{S}'$ have in relation to the axis, and of their distances from the surface of the curve (of lens).

From this image ($d\,\mathrm{S}'$) we might pass on to consider a second, $d\,\mathrm{S}''$, and so forth. It is obvious that between each following image and $d\,\mathrm{S}$ a similar equation would arise.

If we suppose the object and the image to lie in the same medium, then *the brightness of the optical image produced by rays which incline at very slight angles to the axis and perpendicular will always be equal to* (i. e. *neither more nor less than*) *the brightness of the object, except in so far as loss of light by reflexion and absorption may occur.*

But this law should hold good without limitation of divergence angle. For if it were possible to throw an image of any bright point sending forth its light according to the conditions above expressed (namely, of rays circumscribed by a diaphragm aperture), which image should shine with greater intensity than the rule above given admits; then we could cause this bundle of rays to pass on as parallel rays through a plane end-surface into the air, and to fall into the eye of an observer; and in such case it would happen that an object would be seen more brightly illuminated through an

optical instrument than it was before,—a thing contrary to all experience, whatever kind of transparent refracting material be used. Now, if this were possible with light it would also be true of heat, as might be shown by application of similar reasoning; and then the law of equal radiation of bodies possessing equal temperature would be impugned.

But the equation which premised very slight divergence angles of incident rays may be more precisely formulated, and so express the same result in the case of wide divergence angles.

A more precise expression of the Law of Divergence Angles.—In equation [5] it is a matter of indifference whether we substitute for α its sine or tangent or similar functions which for indefinitely small α would be its equivalent. If we assume larger divergence angles of a pencil of rays whose section is a circle, then

$$L = J\,dS \int_0^{\alpha} 2\pi \,.\, \cos.\,\alpha \,.\, \sin.\,\alpha \,.\, d\alpha = \pi J\,dS \,.\, \sin.^2\alpha.$$

If after a series of refractions the surface dS_1 is completely and accurately imaged in dS_1 with the brightness $\frac{n_1^2}{n^2} J_1$ and α_1 of the respectively appertaining divergence angles, then the amount of light must be

$$L = \pi J \frac{n_1^2}{n^2} \,.\, dS_1 \,.\, \sin.^2\alpha_1 \,.$$

As now, $dS : dS_1 = \beta^2 : \beta_1^2$, there follows from these equations,

$$n \,.\, \beta \,.\, \sin.\,\alpha = n_1 \,.\, \beta_1 \,.\, \sin.\,\alpha_1, \qquad [7]$$

which renders this formula of equation [5] valid for larger angles of divergence, assuming that β and β_1 are two images exactly reproducing each other, and whose surfaces are perpendicular to the axis.

Brightness of Image.—When the pupil of the observer's eye is fully immersed in the pencil of rays proceeding from any point of an image, the observer will see the image illuminated as brightly as the object. This result was already announced by La Grange. Unfortunately he had not investigated a second case, which happens to be more common just when high powers are used, namely, when the pencil of rays does not entirely occupy the pupil of the eye.

If a pencil of light having only small divergence angle α_1 does not entirely fill the pupil when the image β_1 is situate at the proper distance of distinct vision, then the brightness H of the retinal image in that eye will be less than that entering the free eye H_0, whose pupil is entirely filled with light.

Let s indicate the distance of vision, p the radius of the pupil, then the area of its surface will be πp^2, the cross section of the pencil of light $\pi s^2 . \sin.^2 \alpha_1$ and the general relation will be

$$H : H_0 = s^2 . \sin.^2 \alpha_1 : p^2.$$

Or using equation [7],

$$H = H_0 . \frac{s^2}{p^2} . \frac{n^2}{n_1^2} . \frac{\beta^2}{\beta_1^2} \sin.^2 \alpha.$$

The last medium in front of the eye must necessarily be air, therefore $n_1 = 1$, and if we indicate by α_0 the angle of divergence of the instrument measured in air according to Lister's method, then $\sin. \alpha_0 = n . \sin. \alpha$. Putting the amplification $\frac{\beta_1}{\beta} = N$, then

$$H = H_0 \frac{s^2 . \sin.^2 \alpha_0}{p^2 . N_2}.$$

With an amplification N_0 by which the cone of light just fills the pupillary opening, and which we shall call the normal amplification of the instrument, $H = H_0$. Hence

$$N_0 = \frac{s}{p} \sin. \alpha_0. \qquad [8]$$

And if α_0 remains constant,

$$H : H_0 = N_0^2 : N^2. \qquad [8^a]$$

If as was assumed

$$N > N_0.$$

Whilst $H = H_0$ when

$$N \leqq N_0.$$

That is to say,

*The brightness of an image seen through the microscope is equal to that of light filling the unoccupied eye * when the amplification is less (or not greater) than the "normal" amplification* (i. e. when the area of the ocular image just fills the pupil); *otherwise, with the same constant divergence of incident rays, the brightness is inversely proportional to the amplification of image.*

The normal amplification increases with the increase of the sine of the divergence angle whose greatest value is 1 when this angle approaches a right angle (as is the case with the widest-angled objectives).

Assuming 10 inches as the distance of clear vision for calculation

* Daylight is of course supposed, and a monocular microscope in use.

of the amplified image, and 1½ mm. as radius of pupil for bright illumination, the normal amplification is represented by the figures 166·7, and the brightness of image follows the following rates:

For an amplification of	333·3	¼ brightness.
„ „	500·0	$\frac{1}{9}$ „
„ „	666·7	$\frac{1}{16}$ „

which shows how rapidly the brightness must necessarily decrease with increasing amplifications.

Were it possible to conduct a hemispherical cone of light from an object lying in water into an immersion lens, and form therewith a correct image, all these amplifications might be raised in the proportion 1·335 to 1 whilst the brightness of image remained the same. But, as already remarked, every instrument hitherto constructed admits in air only, and not in water, a cone of incident light at all approaching to the hemispherical (180°).

The sectional area of the pencil of light entering the pupil may be determined empirically with ease. Focus the instrument on a bright field, and withdraw the eye from the ocular (keeping the direction of the axis of the microscope) and look at the ocular itself. Just in front of it will be seen a small bright circle against a dark ground. This is the optical image of the objective lens which the ocular (i. e. chiefly its field glass) forms. All light which comes through the objective and has passed the ocular must be collected in this image of the objective. It corresponds, therefore, to the area in which the several cones of light, transmitted from the bright points of the object, are collected at this spot. To gather all this light and thus get the largest and clearest field of vision, the pupil of the eye must be brought to this spot. The relation between the area of the image and that of the pupil gives at once the ratio by which the brightness of the image is less than that of the object when looked at with the unarmed eye. The same brightness of image as of object exists only when the size of the image is equal to or larger than that of the pupil.

In the instance of the telescope, La Grange had already stated that the relation of size between the diameter of the objective and that of the picture of the objective formed by the ocular, is directly as the amplification, and he proposed to employ this ratio as a means of determining the amplification. With the telescope, however, such a decrease of brightness is not a necessary accompaniment of increased amplification, because the amount of incident light may be augmented indefinitely by enlarging the object-glass or reflector. The aperture of the cone of light entering the microscope is, on the contrary, definitely restricted by the limits of the angle measuring that aperture.

So far, our demonstration shows that the relation between brightness of image and amplification is entirely independent of any particular construction of the instrument, provided only that it gives well-defined images. An increase of amplification would only be possible, therefore, when a more intense illumination, e. g. direct sunlight were employed, as indeed Listing had in view in the methods proposed by him for obtaining enormous amplifications. But here other difficulties present themselves, which arise from the very slight divergence angle of the emerging rays, as appears in all cases of high amplification from the conditions of the equation representing the course of rays that enter an objective with wide divergence angle.

The first difficulty is, that shadows of entoptic objects throng the field more densely as the area of this field at the eye spot (ocular image of the objective) becomes smaller. The retina is illuminated from this area as if it were the source of light from which proceeded all the rays that enter the eye. This area is at the same time the basis of the collective pencils which belong to the several points of the object, and of its image on the retina, and its diameter, as before shown, varies in inverse proportion of the amplification. But the very conditions which must be fulfilled in order to obtain sharply defined shadows of objects within the eye are exactly what occur here, namely, that a strong light should enter the eye from a relatively small surface.

Whoever has, at any time, attempted to illumine the field of the microscope with direct sunlight, when employing a high amplification, will remember the peculiar spotty appearance of the field so obtained. Some of these spots remain fixed in the field, but others move with the motion of the eye. The first class of spots is due to dirt particles or imperfect polish of the ocular lenses; the second arises from shades caused by intervening opacities in the tissues of the eye—conjunctiva, cornea, crystalline lens, or vitreous humour.* This method has even been used to discover their existence, and is, in truth, a very suitable one. In proportion, however, as entoptic objects become more noticeable, will a greater number of finer details of microscope objects become obscured.

A second and inevitable disadvantage arising from the narrow divergence angle of the emerging rays shows itself in the occurrence of *diffraction phenomena*, whereby the outlines of visible objects are effaced, and at the same time doubled or further multiplied. We have to deal here chiefly with diffraction phenomena as they appear when we look through a minute circular opening. A bright point of light (reflexion of sun on the bulb of a thermometer) viewed through a pin-point hole pierced in a card appears as a

* But mainly from the retinal vessels, as shown by Heinrich Muller, vide Wurzburg Verhandlungen,' vol. v. p. 411.—H. E. F.

bright disk surrounded by alternate bright and dark circles. The apparent breadth of these rings, reckoned from minimum to minimum, corresponds very nearly to a visual angle whose sine is equal to $\frac{\lambda}{a}$, where λ expresses the respective wave-length of the light, and a the diameter of the opening. The outermost rings have exactly these dimensions, the inner are a little wider, and the radius of the innermost bright ring is $1{\cdot}220\,\frac{\lambda}{a}$. Now, as the smallest visual angle under which we can possibly distinguish two fine bright lines from each other may be fixed at 1 minute, the figures of the brightest yellow-green light, whose wave-length $= 0{\cdot}00055$ mm., will be visible when $d = 1{\cdot}89$ mm. Even with a somewhat larger opening the dispersion of a bright point into a circle or of a bright line into a streak must be noticeable.

When we look through such an aperture at any object which shows luminous points, the diffraction figures of the separate points partially cover each other, so that the fringe of dispersion circle of each single point, taken by itself, may not be recognizable. The effect, however, of this diffraction, since it changes every point into a small dispersion circle, obviously causes effacement of the true outline, just as happens when the accommodation of the eye is imperfect, in consequence of which very minute objects, which can be perceived only when the image on the retina is sharply defined, are unrecognizable. We may convince ourselves that this is the fact by a simple experiment. The retina is most sensitively impressed by such objects as gratings, consisting of alternate dark and light parallel lines, whether printed on paper, or made of wire-work, or drawn on glass. Let the observer place himself at such a distance from the grating that, with the aid of spectacles giving perfect accommodation of the eye, he may just be able to distinguish the bars or lines separately from each other. Then let him place before his eye a card in which fine apertures of different diameters have been pierced, and observe whether he still sees the lines or sees them as well with as without the card. The grating must be brilliantly illuminated (e. g. by exposing lines printed on paper to direct sunlight), in order that the picture seen through the aperture may remain sufficiently bright. On trying the experiment myself, I find that a notable deterioration of the image is caused by an aperture of $1{\cdot}72$ mm. diameter, and the deterioration is much more striking with still narrower apertures.

Instead of a series of lines printed letters may be used, the same conditions being fulfilled, namely, by observing the point at such a distance that the single letters may be just distinguished. On looking at them through an aperture of 1 mm. diameter, they will be scarcely or not at all legible. This experiment is, however, not

so sensitive as the first. But in all cases the best accommodation of the eye must be carefully maintained, otherwise the act of passing a card, pierced with an aperture, before the eye may, when there is imperfect accommodation, actually improve vision by diminishing the dispersion.

The theory of diffraction of rays in the microscope leads, as will be shown in the following pages, to the conclusion that any single point of light in *the object* must, when viewed through the microscope, appear exactly as if an actual luminous point, situate in the *image of the object*, were observed through an aperture corresponding in size and position to the ocular images (at the so-called eye spot) of the respective narrowest diaphragm aperture.

Hence it follows, firstly, that diffraction phenomena must be visible when the ocular image has a diameter less than 1·89 mm., and that the size of the dispersion circle, caused by diffraction, must increase in inverse proportion to the diameter of this ocular aperture, consequently in direct proportion to the amplification, supposing that the incident light from each point in the object remains unchanged. Under such circumstances then, the image will not, even with higher amplifications, suffer *further* loss of sharpness of outline from diffraction, inasmuch as the dispersion circles preserve, throughout, the same relation to the apparent magnitude of the object. On the other hand, the deterioration arising from diminished brightness and multiplication of darker entoptic shadows, must increase with the amplification. From this it follows, therefore, that, as a general rule, that amount of amplification will show most detail by which the minutest points that are visible at all in the image, shall be presented under the most suitable visual angle, namely, somewhat larger than that at which an observer can distinguish the minutest objects visible to him under any circumstances.

Calculated by the equation before mentioned, the diameter (1·89 mm.) of the area of light rays entering the pupil, when the light incident on the objective (in air) spreads out to nearly 180°, corresponds to an amplification of 264½. For objectives with less aperture the amplification must be set down at a lower figure. In H. v. Mohl's handbook of the microscope it is stated that amplifications varying between 300 and 400 allow most detail to be seen, whilst Harting, speaking of more recent instruments with large angular aperture, found amplifications of 430 to 450 most serviceable.

If now it be required to determine the magnitude of the minutest recognizable object as a standard by which to measure the accuracy of the microscopic image, we must not take for our unit the measured diameter of such objects as bright single spots or lines on

a dark field, or *vice versâ*, for the reasons which I have already given in my 'Handbook of Physiological Optics' (p. 217), in discussing the capacity of the eye for distinct vision. For in the cases above mentioned the result depends not only on the proportional magnitudes of the images, but also on the susceptibility of the retina to slight differences of light. The most suitable objects are, here also, fine gratings which show alternate clear and dark stripes. Such indeed are in common use, as in the examples of Nobert's lines, and the line systems of diatoms and insect scales. But as the light of the bright stripes is doubtless strongly dispersed before it becomes quite undiscernible, dependence can be placed only on the measurement of the space between the centres of two contiguous stripes, and not upon the measurement of space occupied by the stripes (wide or narrow) as originally distributed. I select, therefore, as the measure of the minutest distinguishable objects, that smallest appreciable interspace between the centres of two contiguous stripes by which these stripes can still be recognized as separate.

When diffraction is caused by a fine network of square meshes, it can be proved that the network must appear as a uniformly illuminated surface when the breadth of fringe of diffracted light is equal to that of the open space of the network. For circular meshes, the integration for calculating the distribution of light is tediously diffuse. When the diameter of a circular mesh is equal to the length of one side of a square mesh, the outermost fringes in the spectrum of a bright spot are of equal width, but the innermost fringes are wider in the circular meshwork. If therefore the fringes of the square meshes are so broad as to efface all impression of separate bright lines of the network when the measured widths of fringe and mesh are equal, the same thing must happen with the circular meshwork, a portion of whose diffraction fringes is still wider. For this reason I have, in the following demonstrations, taken the width of the outermost fringes of a circular meshwork as the lower limit of distinguishable distances in an object. It is not, however, impossible that by some fortuitous overlapping of images, objects of still smaller dimensions might occasionally be half seen, half guessed at. But safe and certain recognition will scarcely be possible.

Let now

ϵ be the magnitude of the smallest recognizable interspace,

λ wave-length of the medium,

α divergence angle of the rays incident in that medium,

λ_0 α_0 the values of the last-named magnitudes (λ and α) for air.

Then by the formulæ deduced in a subsequent page,

$$\epsilon = \frac{\lambda}{2 \sin. \alpha} = \frac{\lambda_0}{2 \sin. \alpha_0}.$$

For white light we may, as before, take the wave-length of the medium bright rays.

$$\lambda_0 = 0{\cdot}00055 \text{ mm.}$$

If $a_0 = 90°$, then

$$\epsilon = \frac{\lambda_0}{2} = 0{\cdot}000275 \text{ mm.} = \tfrac{1}{3636} \text{ mm., or } \tfrac{1}{92000} \text{ inch.}$$

Were it possible to obtain with an immersion lens the transmission of rays = 180° of divergence aperture (in water), a would then = 90° and λ nearly $\frac{3}{4}\lambda_0$; and hence

$$\epsilon = \tfrac{1}{4848} \text{ mm.} = (\tfrac{1}{122000} \text{ inch}).$$

According to measurements of Harting,* the magnitude of the smallest distances taken with No. 10 objective of Hartnack, reckoned by our formula, is

$$\epsilon = \tfrac{1}{3313} \text{ mm.}$$

The figures $\frac{1}{5210}$ mm. given by Harting refer to the width of the dark space *between* the lines. In close accordance with the above are the measurements by Herr L. Dippel,† of fine diatoms, who found that the closest series of lines that he could distinguish $= \frac{1}{3500}$ mm., and the finer Nobert lines $= \frac{1}{3600}$ ($\frac{1}{90000}$ inch). Earlier measurements, 1853, of Messrs. Sollitt and Harrison,‡ count much higher. Recognizable lines of *Navicula Arcus* are said to have been counted at 5120 to the mm. ($\frac{1}{129000}$ inch). This far exceeds the theoretical limits for objects in air. But since all later measurements remain much lower than these, I do not know that they are trustworthy. Harting also, who cites them, doubts their accuracy.

Besides any possible further increase of angular aperture in the case of objects lying in water, the capacity of performance might perhaps be increased by employing blue rays only.§

In photography, blue light is chiefly active, and photographs appear actually to perform more than the eye can with white light. In a photograph of *Surirella gemma*, executed by Dr. Stindi, with an objective of Gundlach's, giving $\frac{1000}{1}$ amplification, lines are visible which may be counted at 3800 to 4000 in the millimeter ($\frac{1}{100000}$ of English inch).

Thus it appears to me beyond doubt that diffraction of the rays is the principal cause of the limitation of sharpness of the microscope image. In comparison with diffraction, chromatic and spherical

* Published in vol. cxiv. of Poggendorff's 'Annals.'

† In his work on the Microscope: Brunswick, 1867.

‡ 'Quarterly Journal Microscopical Society,' vol. v. p. 62.

§ Hartnack made an illuminating apparatus for use of blue rays only, and exhibited it in the Vienna Exhibition, 1874.

aberrations appear to exert but an inconsiderable influence, in spite of the very large angles of incidence and divergence of rays. Considering the extreme care expended on calculation and execution of lenses for telescopes and the photograph camera, it is justly a matter of surprise that with the lenses of the microscope, which are so much more difficult to construct according to prescribed dimensions, and which have so large an aperture, spherical aberration makes itself so little felt. I have, however, already pointed out that when there is water between the object and covering glass, and also between this and the objective, the divergence angle is not $87\frac{1}{2}°$, as usually stated, but only $48\frac{1}{2}°$. With dry mounted objects an angle of $87\frac{1}{2}°$ can indeed be in action, but *only through the minute distance between the object and covering glass*, so that the spherical aberration arising therefrom is of no importance.

As wide pencils of light are needed to keep diffraction within moderate limits, the illuminating apparatus should also be capable of emitting pencils of the same angle, in order to show clearly the contour lines of dark objects.

If there happen to be particles in the object which act like lenses, these may of course convert a small illuminating pencil of rays into strongly divergent rays, and so become clearly visible. Otherwise nothing is seen but a confusion of diffractions at and in the object on one part, and in the (optical) aperture of the microscope on the other part.

Here lies obviously the explanation why microscopes, otherwise good, but whose illuminating apparatus is not specially arranged for the purpose, yield, with artificial illumination, e. g. a flame, such unserviceable images of the outlines of dark objects. For an immersions lens, the best illuminating apparatus is one constructed according to the same principle—that is to say, a lens of the same kind reversed. The readiest mode of finding whether the illuminating apparatus gives sufficiently wide pencils of light is to examine the ocular image with a magnifying lens after the instrument has been focussed.

I must now relate here the *failure of an attempted improvement*, the negative result of which is significant. I thought myself justified in inferring theoretically that the diffraction of the microscope might be neutralized if the points of the narrow aperture which causes this diffraction were made singly and separately luminous, and that this could be effected by causing a sharply defined optical image of the source of light (e. g. sun illumined cloud) to be thrown by a lens on the plane of this aperture. Years ago I tried experiments of this kind on a Nobert microscope, provided with immersion lenses, giving excellent definition. The result of this trial showed that it was perfectly indifferent whether the image of the source of light fell on the plane of the object or

of the objective. The diffraction fringes caused by the use of a very deep ocular remained uncorrected. More recently I have convinced myself by fresh trials made with larger lenses, that such a procedure is useless. When a good achromatic lens of about 18 inches focus is so placed as to show a sharp image of the source of light (as in this case a bright sky cloud) upon the surface of a system of lines scratched on glass, the images of many separate luminous points will be thrown upon the variously transparent clefts of this grating, and it might be supposed that the interference of rays which had passed through neighbouring clefts would cease. If, however, we look through the grating towards the lens, and place before the lens pieces of card pierced with fine slits, we see with the naked eye just the same diffraction fringes, as well at these slits as at the outer edges of the cards, as would be seen if the lens were removed, or the grating set out of focus.

Instead of the lines, I then made trial of two fine linear slits cut in cardboard, with an interspace of about 1 mm., and through which I could see with the naked eye a system of very fine interference lines belonging to the diffraction image of another slit which was cut with the lines at a very small acute angle, sufficiently narrow to produce the interference lines at the point of this angle. But these did not disappear when I threw an optical image of the incident light on the plane of the double (parallel) slit. In this experiment not the slightest suspicion could be entertained that chromatic or spherical aberration had dispersed the rays over an interspace of 1 mm. width. The only explanation I can offer is, that the light from the lens which passed through the acute angle of the slit serving here as object, suffers so strong a diffraction that it subsequently reaches the two openings of the doubly-slit card with a corresponding wave-phase and therefore sends interfering bundles through both openings. In order to be able to see the interference lines, it is necessary that their minima shall appear at a wider distance from each other than the width of the lines of which they are images, and when this condition is fulfilled theory does in fact show that the central clear portion of the diffraction figure of the simple slit forms a line of light which is broader than the distance between the two slits of the doubly-slit card.

Similar relations take place (although more difficult to subject to calculation) when the fine edge of a dark screen is used as the object. It is known that from such an edge, bundles of interrupted rays (in linear formation) likewise bend themselves into the dark field, which have corresponding phases of movement, and so when bent by a second screen can exhibit regular interference. That the resultant effect cannot become *nil* appears clearly from the fact that the effect of a bright line may be represented as the product of the action of two endless half-planes bounded by straight

lines the edges of which half-planes slightly overlap each other, minus the action of an equally bright whole plane. As the latter causes no interference phenomena, the bright line of itself could not cause interference in any part of the field, unless each of the half-planes also produced such interference. It follows therefore that the light bent away from a straight edge must also spread itself out with notable strength to the same width as would the light from a slit in the card bounded by two other slits.

Theory of Diffraction in the Microscope.

In conclusion, I shall here show a method by which the diffraction of rays passing through the microscope may be theoretically calculated. Instead of the simple lengths of rectilinear rays, as taken into consideration by the theory of diffraction of light which passes through one medium only, the *optical lengths* of the rays must be taken, that is to say, the lengths obtained by adding together the product of each portion of a ray multiplied by the index of refraction of the medium through which it passes.

The wave-phases of two rays that have started from the same luminous point, and have equal optical lengths, are also equal at the other terminal point, because the wave-lengths in different media are inversely proportional to the refractive indices. Further, it is known * that the optical length of all rays between two conjugate foci of the same pencil in which a perfect reunion of these rays is accomplished is equally great.

In order to calculate the diffraction through the (relatively) narrowest aperture of the microscope, each point (c) in the plane of this aperture must be treated as a ray centre whose phase is determined by the optical length of the normally refracted ray, which, starting from the luminous point (a), has arrived at c. This length I designate with $a\,c$. On the other hand, the difference of phase between c and the point b in the surface of the image whose brightness is to be determined depends on the optical length $c\,b$ found for the normally refracted ray travelling from c to b. The phase of movement continued from a, through c as a new centre of the ray, to b, will therefore depend on the sum of the optical lengths $a\,c + c\,b$. The share which this ray has in the movement in the point b will be given by an expression in the form

$$A \sin. \left\{\frac{2\pi}{\lambda}[a\,c + a\,b - a\,t] + \text{constant}\right\},$$

where λ is the wave-length in empty space, A the speed of progressing movement, t the time. The sum of these quantities

* The proof of the law here adduced is to be found in my 'Handbook of Physiological Optics,' and elsewhere.

taken for every point c of the aperture (in which the factor a can be considered as approximatively independent of c) will finally determine the movement at b.

If now we suppose the rays passing from (a) and (b) to the point (c) of the relatively narrowest aperture to be prolonged in the direction which they have at the point (c) until they intersect each other in the points (α) and (β), these last points will be the images of the points (a) and (b), formed in the medium of (c). Since, then, from what has been said above, the optical lengths $(a\,\alpha)$ and $(b\,\beta)$, being lengths measured between conjugate foci, are constant, we may put

$$(a\,c) = (a\,\alpha) - (c\,\alpha)$$
$$(c\,b) = (\beta\,b) - (\beta\,c).$$

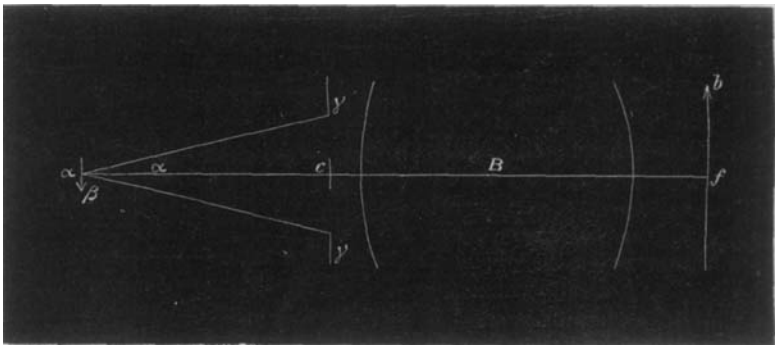
The direction of movement of the ray must be conceived as always advancing from the first to the second letters; and therefore

$(c\,\alpha)$ be put $= -(a\,c)$, as also $(\beta\,c) = -(c\,\beta)$.

Then the expression for the effect of each separate ray on the point (b) becomes

$$\text{A sin.}\left\{\frac{2\pi}{\lambda}[(a\,c) - (\beta\,c) - \frac{t}{a} + (a\,\alpha) + (\beta\,b)] + \text{constant}\right\}.$$

The only terms amongst the signs bracketed under the sine that vary with the point c are $(a\,c) - (\beta\,c)$. These optical lengths, however, lie wholly in the medium of (c), and are therefore straight lines; consequently, the diffraction effect of the light from



(a) at the point (b), apart from the factor A, which expresses its total intensity, will be the same as that of the light from a for the point β. But the latter can be calculated according to the known method valid for rectilinear rays.

Let $\gamma\gamma'$ be the relatively narrowest aperture, and (c) its middle point, B the portion of the optical system immediately behind this aperture, and let a be the image of the axis point a of the object; further, let $a\beta$ be its image lying in the medium $\gamma\gamma'$ and fb the image formed by B in the last medium.

When light proceeds from a, and is viewed through the aperture $\gamma\gamma'$ whose radius is ρ, interference fringes will appear around a, in which the distance δ between each two neighbouring maxima (excepting the two first) will be according to known laws, if, as before, α represents the divergence angle $ca\gamma$, which by assumption is very small.

$$\delta = \frac{(ac)\lambda}{2\rho} = \tfrac{1}{2}\frac{\lambda}{\alpha}.$$

If N be the amplification of the image bf in comparison to $a\beta$, the breadth of fringe δ' of bf will be

$$\delta' = \mathrm{N}\delta = \tfrac{1}{2}\mathrm{N}\frac{\lambda}{\alpha} \qquad [8]$$

or as $\mathrm{N} = \dfrac{n\,\alpha}{n'\,\alpha'}$ when α' expresses the divergence angle of the emergent ray, n' the refractive index of the last medium, n that of the medium at (c).

$$\delta' = \frac{n}{2n'} \times \frac{\lambda}{\alpha'}. \qquad [8^a]$$

If $n = n'$, then the form in which this value of the breadth of fringe of image bf is expressed is exactly analogous with that for $a\beta$, and shows that the fringes in the last image are of just the same dimensions as if seen through the aperture which determines the divergence angle α' of the cone of rays $\gamma a \gamma$, or, in other words, through the ocular image of narrowest aperture.

The above demonstration presupposes that the relatively narrowest aperture of diaphragm is situate where the divergence angles of the pencil of rays are very small. It may, however, be situate at any part of the instrument. With an immersion microscope this condition is indeed not fulfilled when the surface of front lens is the relatively narrowest aperture. But it would be fulfilled if the aperture were situate on the upper side of the second or third lens. Thus if there were no lateral outspread of the advancing rays on their passage through the front lens of the objective where the pencil is still diverging strongly, then from the point where the divergence is weak, or convergence commences, its lateral limitation, whether occasioned by a diaphragm actually situate at the place, or only conditioned by the previous course of the rays, must nevertheless produce a diffraction.

As regards the final result, it makes no difference whether the aperture at the circumference of the pencil of rays be supposed to be situate a little more to the front or to the back. The image of this aperture formed by the ocular lenses will be very slightly larger when it is situate at the back lens than when it lies in the front lens, but the difference is without any practical significance.

In equation [8] δ' is the breadth of fringe in the last image, a the divergence angle in the medium where the aperture lies, λ the wave-length at the same place, N the amplification of the last image, as distinguished from that formed by the rays passing the aperture.

If, on the other hand, we put N_1 for the amplification of the last image referring to the object λ_1, and n_1 for the wave-length, and refraction index for the medium in which the object lies, we may according to equation [7] make, as a is, by assumption, small,

$$\frac{n_1}{N_1} \sin. a_1 = \frac{n}{N} \cdot a.$$

a_1 is the divergence angle in the first medium.

Putting the value of $\frac{a}{N}$ in equation [8], it becomes

$$\frac{\delta'}{N_1} = \tfrac{1}{2} \lambda \frac{n}{n_1} \cdot \frac{1}{\sin. a_1} = \epsilon;$$

or, as $\lambda n = \lambda_1 n_1 = \lambda_0 n_0$, which last refers to air medium, we have

$$\frac{\delta'}{N_1} = \frac{\lambda_1}{2 \sin. a_1} = \frac{\lambda_0}{2 \sin. a_0} = \epsilon.$$

This ϵ is the true magnitude of those lengths in the object, which in the magnified image of the fringes appear equal, and will therefore be effaced. Therefore, ϵ may be considered the measure of the smallest distinguishable distances in the object. ϵ will be smallest when a_0 is largest,—that is to say, when amounting to a right angle. In that case

$$\epsilon = \tfrac{1}{2} \lambda_0. \qquad [9]$$

This determination of limit is likewise, as may be seen, independent of the construction of the optical instrument. It holds just as valid for a photographic apparatus as for the relation of the microscope to the eye of the observer. These are the formulæ which were applied in the calculations previously given.—*From the Proceedings of the Bristol Naturalists' Society*, New Series, vol. i. part 3.