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THE

TERCENTENARY

OF THE

COMPOUND MICROSCOPE:

AN

INAUGURAL ADDRESS

DELIVERED NOVEMBER 7, 1890

TO THE

SCOTTISH MICROSCOPICAL SOCIETY.

 $\mathbf{B}\mathbf{Y}$

PROFESSOR W. RUTHERFORD,

M.D., F.R.SS. L. AND E., ANNUAL PRESIDENT OF THE SOCIETY.

EDINBURGH:

PRINTED BY NEILL AND COMPANY.

1891.



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IN

JOURNAL OF ANATOMY AND PHYSIOLOGY, Vol. XXV. GENTLEMEN,—I have to thank you for the honour of being elected President of this Society for the session which we open this evening.

This year happens to be the tercentenary of the invention of the compound microscope, and it seems appropriate that I should on this occasion briefly review the history of the microscope, its present position, and the difficulties that impede its further progress.

The microscope far surpasses all other instruments in the help it has rendered to biological science. Without its aid the cellular structure of plants and animals would still have been unknown; the minute structure of their organs would still have been matter for conjecture; the wonderful changes that characterise the development of complex organisms would still have been a mystery; and the theory of evolution which has to a large extent sprung from the law of cellular development might not yet have been advanced. Without the microscope, the nature of many diseased processes would still have been unknown, and the physician still baffled in his search for rational methods of treatment; without the microscope, some of the most dangerous causes of disease would still have remained undetected, and the rational principles of antiseptic surgery would not yet have been enunciated; and, without its help in medico-legal inquiry, the ends of justice would on several occasions have been defeated. Through no avenue of sensation, so much as through the eye, has our knowledge of nature been gained, and marvellous though the efforts of nature have been in evolving our wonderful organ of sight, it is to the glory of science that she has brought the telescope and the microscope to its aid-the one to penetrate the veil of distance and reveal the nature of the stellar universe and the gigantic scope of creation; the other to break the seal covering objects of small dimension, and to reveal to us many of their wonderful properties, and some of the great laws that govern the operations of living nature. With the range of human vision thus extended the mind has been enabled in some measure to realise the wonders of nature revealed in her greatest and in her smallest objects, and to perceive some of the great laws that have dominion over them.

No one knows exactly when the simple microscope was invented. Burning glasses were so common in Greece that they were sold as curiosities in the toy shops. Such glasses must have been convex, and could therefore serve as magnifying as well as burning glasses. It has been argued with good reason that the engraving of ancient gems could not have been executed without the help of a magnifying glass; while on the other hand it has been contended, but with questionable logic, that if magnifying glasses had been known to the ancients they could scarcely have failed to use them as spectacles, and to have alluded in their medical writings to the help which magnifying glasses can give to sight. But it appears that no such allusion is ever made by any Greek or Latin author, or indeed by modern authors, until the close of the thirteenth century, when spectacles were invented by Armati of Florence about the year 1285. It seems to me quite possible that a single lens might have been used by an engraver of gems long before the advantage of placing such a lens in front of each eye became known.

The compound microscope dates from the year 1590, as nearly as can be ascertained. Hans Janssen, or his son Zacharie, spectacle makers at Middelburg, in Holland, discovered that the magnified image of an object produced by a convex lens can be still further magnified by looking at it with a similar lens. They discovered the principle of the indirect amplification of an object, and thus invented the double microscope. Eighteen years afterwards (1608) the telecope was also invented in Holland. Neither of these instruments was invented in Italy by Galileo, although he constructed both of them. Recently an Italian physicist, Professor Govi,¹ has claimed the invention of the double microscope for Galileo, but there is sufficient evidence that he arranged lenses to form a double microscope after he had obtained a telescope from Holland ; while as a matter of fact the double microscope was invented in Holland eighteen years before the telescope. There need therefore be no doubt that the credit of the invention of both instruments belongs to the Dutch.

Some seventy years after Janssen's invention, Robert Hooke, secretary of the Royal Society of London, devoted much attention to the microscope, and made several suggestions worthy of note. His work, entitled Micrographia, published in 1665, contains many curious observations on plants and animals and on other subjects, and is illustrated by elaborate drawings. He principally used a double microscope with one lens for the object-glass and another for the eyeglass; but it is to be observed that his eye-glass was much wider in proportion to the object-glass than in the Janssen microscope, for a reason that must be obvious. He tells us that when he desired to see a greater extent of the surface of an object, he introduced a third lens in the position of what is now termed the field glass,² but whenever he wished to see the object very clearly, to examine its minute details, he took out the third glass, because, as he said, "the fewer the refractions, the brighter and clearer the object." The Dutch optician, Huygens, the celebrated author of the undulatory theory of light, had previously used such a glass in the eyepiece of the telescope, and therefore his name is commonly associated with an eyepiece so constructed.

Hooke was so persuaded that the only way to see clearly with the microscope is to diminish the number of refractions, that he invented one in which the space between the object-glass and eye-glass was *filled with water*, so that the refraction at the upper surface of the object-glass and lower surface of the eye-glass might be greatly lessened. With water in place of air, the image of an object viewed through such a microscope was more brightly illuminated because of diminished loss of light by reflection at the inner glass surfaces, but

¹ See abstract and criticism in Jour. Roy. Mic. Soc., 1889, p. 574.

² R. Hooke, Micrographia, London, 1665.

as its employment was inconvenient, it was abandoned. By that invention Hooke showed his perception of a principle that afterwards led to the construction of immersion lenses. He complained that the apertures of object-glasses were so small that "very few rays were admitted, and that many of them were so false that the object appeared indistinct." He believed "that these inconveniences are inseparable from all spherical glasses, and to diminish them he recommended a bright illumination of the object by a convex glass, such as a globe of water, or a *deep plano-convex lens whose convex surface is turned to the window, and its plane surface to the object.* But he was obliged to admit that a solid bead of clear Venice glass fixed in a small hole in a metal plate would magnify more highly, and show some objects more distinctly than any of the great microscopes.

The celebrated Dutch observer Leeuwenhoeck appears to have arrived at a similar conclusion, for most of his microscopical observations were made with a simple globule of glass, mounted between two metal plates, pierced with a minute aperture to allow rays to pass only through the central portion of the lens.

Previous to the introduction of achromatism the compound microscope underwent some improvements in the hands of Campani, Divini, and others; but I must pass them over to briefly notice the additions made by a London optician, Benjamin Martin. His work on optics,¹ published in 1740, is a lucid exposition of the subject, and admirably illustrated. His construction of the mechanical parts of the microscope had the great excellence ever since maintained by English opticians. He added a rack and pinion for moving the body of the instrument; a nosepiece for carrying different lenses; a double mirror to illuminate transparent objects; and a stage with rectangular movements accomplished by fine screws with graduated heads, so that the extent of motion in any direction could be accurately determined as in the movable stages now made.

For more than two centuries the microscope awaited the correction of the great faults of spherical and chromatic aberration inherent in its lenses. The great step that led the way to its modern development was the invention of achromatic lenses by the English optician, John Dollond. His celebrated "Account of some Experiments concerning the different Refrangibility of Light" was published in the Philosophical Transactions for 1759. The account is remarkably brief and concise. One requires to read it in order to realise that in discovering the different optical properties of flint and crown glass, which enabled him to construct an achromatic lens, he had to prove Sir Isaac Newton to be in error, and all the opticians who adopted his teaching and believed that the refractive and dispersive powers of an optical medium are always equal. Dollond proved that to be an error by comparing the refractive and dispersive powers of glass and water. His experiment was essentially this: he took a triangular glass prism and a wedge-shaped trough made of plate glass and filled with water. He arranged them with bases reversed, so that the one

¹ A New and Compendious System of Optics, by B. Martin, London, 1740.

might counteract the other. His glass prism had an angle of 60°, and he experimentally ascertained the angle required for the water prism to compensate the refraction of a beam of light by the glass. On looking at a white object through both prisms thus arranged, he observed that although the object did not appear shifted in position it seemed to be coloured. Therefore, although the water had compensated the refraction which the mean rays of the luminous beam had suffered in the glass prism, *it had not recomposed white light*, and he perceived this to be due to the dispersive power of glass being nearly twice as great as that of water. He then increased the angle of his water prism until it compensated the dispersion produced by the glass, and recomposed white light; but he found that although the object now appeared colourless, it seemed shifted in position, because the ray emerging from the prisms had been refracted from the plane of the incident ray.

By these simple experiments Dollond proved that the refractive and dispersive powers of an optical medium may be unequal, and that it is possible to compensate the chromatic dispersion of light and still obtain a final bending of the beam. Dollond made these experiments in 1757, two years before he published his paper, and he immediately proceeded to construct new object - glasses for the telescope. He at first made them of "two spherical glasses with water between them," and he found them "free from chromatic errors." But the errors due to spherical aberration were still so great that he gave up all hope of success with lenses of such construction. He was led to "suspect that different sorts of glass might possibly be found to show differences in the ratio of their refractive and dispersive powers." So that he says (p. 739) "the next business to be undertaken was to grind wedges of different kinds of glass and apply them together so that the refractions might be made in contrary directions, in order to discover whether the refraction and the divergence or dispersion of the colours would vanish together." He then says (p. 740), "I discovered a difference far beyond my hopes, in the refractive qualities of different kinds of glass, with respect to their divergency of colours. The yellow or straw-coloured foreign sort, commonly called Venice glass, and the English crown glass, are very near alike in that respect, though in general the crown glass seems to diverge the light rather the least of the two. The common plate glass made in England diverges more, and the white crystal or flint English glass, as it is called, most of all. It was not now my business to examine into the particular qualities of every kind of glass that I could come at, much less to amuse myself with conjectures about the cause, but to fix upon such two sorts as their difference was the greatest, which I soon found to be the crown glass."

Dollond's communication is remarkable for lucidity and brevity; probably few other papers limited to nine octavo pages have chronicled an advance so important in practical science. But Dollond's originality has been questioned. Thomas Young, in his *Lectures on Natural Philosophy*, published in 1807, states¹ "that

¹ P. 380, Kelland's edition, published 1845.

Dollond was led to make experiments on the refraction of different kinds of glass in consequence of a discussion with Euler, Klingenstierna, and some other mathematicians," but although Dollond probably received suggestions from others, to himself alone appears to belong the credit of having made the first achromatic lens. Euler, in his *Letters to a German Princess*, wrote on the 19th of August 1760—a year after Dollond's paper was published—that, although chromatic aberration could be remedied by combining lenses of different substances, "neither theory nor practice have hitherto been carried to the degree of perfection necessary to the execution of a structure which should remedy the defect."

From the extract I have read from Dollond's paper, it appears that he aimed at the correction of both spherical and chromatic aberration in lenses by correcting the positive aberration of a biconvex of crown glass by the negative aberration of a plano-concave of flint glass—and he succeeded in doing both—to a large extent. He relied on the degree of concavity in the flint lens to remove the positive spherical error of the crown lens, and on the nature of the material, as well as on the curvature, to remove the chromatic error. As a matter of fact, however, the chromatic fault, although greatly diminished, was never completely removed, nor was the spherical fault ever completely abolished ; nor could they be by the simple combination of a single convex and a concave lens of crown and flint glass.

Dollond's achromatic lenses were made for the telescope in 1758, but, strange to say, their application to the microscope seems to have been very tardy, apparently because Dollond devoted his attention specially to the telescope. Achromatic objectives of crown and flint glass on Dollond's principle were at length made for the microscope by Frauenhofer of Munich in 1811, and Amici of Modena in 1815, but apparently with no great success.

The achromatic microscope as now commonly employed, really dates from 1823, when Chevalier of Paris, instructed by Selligue, made high power objectives on the principle of superimposing several achromatic doublets, instead of attempting to obtain a high power from a single lens. This method was well known in the simple microscope, and it is remarkable that its application to the compound instrument was not sooner thought of. Another novel feature was the cementing together of the flint and crown lenses in each doublet, whereby the loss of light by reflection within the doublet was greatly diminished. At first the convex surface of each lens was turned to the object, with the consequent production of great spherical aberration that rendered the lens of little value, unless the pencil of light was made very narrow by a diaphragm. Chevalier afterwards shortened the focus of each doublet, turned their plane surfaces towards the object, and thus greatly reduced the spherical aberration.

Within a year after the appearance of Selligue's lenses, the London optician, Tully (1824), made triple achromatic object-glasses, which defined with "great sharpness;" but the impulse which enabled English opticians to rise to their prominent place as makers of achromatic lenses appears to have been given by Joseph Jackson Lister in a paper published in 1830,¹ in which he showed that Selligue's method of superposition of achromatic doublets in the objective was capable of yielding results not hitherto attained, because the key to them had not previously been found. Lister laid due stress on the principle that an objective must have a large angle of aperture to give a brilliant and distinct image. He showed that the marginal rays of a luminous pencil are those which specially serve to resolve fine closely adjacent lines such as those on the scales of Lepidoptera, as may be proved by the fact that some of the most difficult of these lines are best seen when only the marginal rays are employed and the central rays stopped out. He stated that the "great requisite for the object-glass of a compound microscope is a large focal pencil free from aberration ; that the field should be flat and well defined throughout, and that the light admitted should as far as possible be only that necessary for the formation of the picture, and that it should not be intercepted or diffused over the field by too many reflections." He said that the prominent obstacle to obtaining a sufficient pencil of light for high powers by one objectglass of large aperture and deep curves is, that the correction for spherical aberration by the concave lens is proportionally greater for the marginal than for the central rays, so that there is over-correction of the marginal rays and the image consequently rendered indistinct, and at the same time coloured. It, therefore, becomes necessary to cut off the marginal rays, and so diminish the aperture of the lens. Lister was the first to show that by superposition of achromatic doublets the effective aperture of the series of lenses can be widened by a precise adjustment of the distance between them, and by accurately centering them around the optic axis. In combining several lenses together he says "it is often convenient to transmit an under-corrected pencil from the front glass, and to counteract its error by over-correction in the middle one" (p. 199). These apparently simple indications were what he termed the "key" to the improvement of achromatic objectives.

Lister's suggestions were promptly acted on by London opticians, more especially by Smith and Beck, Andrew Ross, and Hugh Powell, whose ingenuity and skill rapidly raised English microscopes to the first rank for optical as well as mechanical excellence. No better testimony could be given to Lister's acuteness in 1830, than that furnished by Professor Abbe² in 1879 in his paper on the correction of spherical and chromatic aberration, where he shows the great importance of the relative distance between the lenses of an objective, and the excellent results attainable when an under-corrected pencil of light is transmitted to over-corrected lenses placed at a suitable distance above the under-corrected front lens, the compensating power

¹ "On some Properties in Achromatic Object Glasses applicable to the Improvement of the Microscope," by J. J. Lister, *Phil. Trans.*, London, 1830, part i., p. 187.

² Abbe "On New Methods for Improving Spherical Correction applied to the construction of Wide-Angled Object-Glasses," Jour. Roy. Mic. Soc., 1879, p. 812.

of the upper lens depending to a considerable extent on the relative distance between them and the lower lens. The ordinary high power objectives devised by Abbe consist of an uncorrected lens placed in front, and three over-corrected doublets placed behind it, in which the spherical and chromatic errors of the front lens are removed.

Few points in microscopical optics have been more discussed than the aperture of the object-glass. Let me remind you that the angle of aperture of an objective is the angle formed between the most external rays that can penetrate the entire system of lenses from a luminous point in the focus. But in many object-glasses the lenses cannot be used to the full extent of their aperture, owing to the serious increase of spherical error in the marginal portion of the lens. Therefore it is frequently necessary to intercept the passage of rays through the marginal portion, and thus to curtail the aperture. Indeed, the angle of aperture of a lens is in practice reduced to the angle between the most peripheral rays that are capable of forming a correct image. Therefore a large angle of aperture is only possible when the spherical and chromatic faults of a system of lenses have been so well corrected that rays transmitted by the outer zones of the lens can form a distinct image.

The power of an object-glass in resolving fine adjacent lines increases with its angle of aperture as Lister pointed out. Therefore opticians have been constantly striving to produce lenses of large aperture. The lens with the highest angle yet produced is Abbe's apochromatic oil immersion of 134°. Abbe has shown that it is vain to attempt to increase the angle appreciably beyond this, because the focus of the lens is so short, and the incidence of the peripheral rays so oblique, that with a higher angle the lens would be practically useless. Objectives of large aperture are valuable for the study of bacteria, muscle fibrils, and all objects with fine markings, but they are not so serviceable as those of small angle for the ordinary study of the structure of tissues and organs, because the penetrating power, that is, the power of seeing objects a little above, and also below, the exact focus, diminishes as the angle of aperture is increased. The method of indicating the aperture of an object-glass by stating its angle has been found inconvenient, because the angles have a relative value only when lenses are used in the same medium. be it air, water, or oil. An angle of 100° for a water-immersion lens indicates a greater resolving power than a similar angle in a dry lens, Abbe has therefore proposed to supersede the angular designation of aperture by the more convenient method of indicating the aperture numerically. The numerical aperture is obtained by multiplying the sine of half the angle of aperture by the refractive index of the medium through which the light reaches the lens, be it air, water, or oil. The numerical aperture is an index of the resolving power of an objective, irrespective of the medium in which it is immersed. Thus a lens whose power of resolving fine lines is indicated by the numerical aperture 1.0, would have, in air, an angle of 180°; in water, an angle of 97° 31"; and in cedar oil, an angle of 82° 17". That

illustration is sufficient to show what confusion may be avoided by adopting the numerical aperture as an index of resolving power. The numerical aperture of objectives may vary from as low as 0.05 to the comparatively high figure of 1.4, the aperture of the highest apochromatic oil-immersion lens made by Zeiss.

The remarkable increase of the resolving power of lenses, which has taken place in recent years, would have been impossible but for the adoption of the immersion principle. We are indebted to Amici of Modena for the invention of immersion lenses in 1840, or shortly after. To him belongs the credit of having been the first to aim at the construction of homogeneous immersion lenses. Knowing that certain oils have a refractive index similar to that of glass, he constructed lenses to be immersed in them, so that the refraction and reflection at the upper surface of cover glass and lower surface of objective might practically be abolished. It was a similar idea that led Robert Hooke nearly two centuries before to fill the tube of the microscope with water. The Italian microscopists, however, found the oil attack the surface of the lens, so that Amici was obliged to abandon it and make lenses for immersion in water. In making lenses for oil immersion fifty years ago, Amici was before his time, because powerful objectives with a sufficiently large angle of aperture to take advantage of the homogeneous immersion principle had not then been constructed. Amici exhibited his water-immersion lenses in Paris, and similar objectives were made there, but they were soon surpassed by the immersion systems made in this country by Powell and Lealand. The adoption of the immersion principle for high power objectives has permitted of their construction with a larger aperture, so that their resolving power is increased; but for very high power lenses water has now given place to thickened cedar oil, having refractive index 1.512, which is so nearly that of crown glass (1.53), that when interposed between the cover glass and lens it virtually forms with them a homogeneous optical system, in which there is practically no reflection or refraction at upper surface of cover and lower surface of lens: consequently illumination and definition are improved, and resolving power increased, because the oil permits of the lens being constructed with a larger aperture than is possible with a water lens. The highest numerical aperture of the oil lenses made under Abbe's direction is 1.4, which is regarded by him as the highest useful aperture. He believes it impracticable to attempt any further increase of aperture, because of the difficulty of overcoming spherical aberration, which increases with the aperture.

The first oil-immersion lens in the recent period of the microscope was constructed by Zeiss from a formula calculated by Abbe, —at the suggestion of Mr J. W. Stephenson, treasurer of the Royal Microscopical Society. The celebrity so rapidly attained by the firm of Zeiss, as practical opticians, is mainly due to their association with a skilled mathematician and master of optics in the person of Professor Abbe. The association of the mathematical theorist with the practical optician is always desirable, and in these times no optician need hope to accomplish anything remarkable unless he is himself

deeply versed in mathematics, or instructed by a mathematician willing to turn his attention to optics. The University and the factory seldom join hands, but the results of such union achieved in the small town of Jena show how much the honour of a country, as a producer of philosophical instruments, may be promoted by such combination. All who use the microscope must ever remain indebted to Abbe for having solved some of the most embarrassing difficulties in practical optics that completely baffled all who preceded him. Until he devoted his mind to the subject, there was no such thing as a compound microscope in which spherical and chromatic aberration was completely corrected, consequently the image produced by the objective was so faulty that deep eyepiecing was ineffective, and owing to yellow and violet rays being brought to different foci, it was difficult to use the microscope in photography. The main difficulty arose from the peculiar difference in the dispersive powers of flint and crown glass. If a prism of flint and another of crown glass are made of such angles that the same beam of light transmitted through each prism gives rise to a spectrum of the same length in each case, and the two spectra are shown side by side, it is found that they are not identical. The junction of the green and blue is nearer the violet end in the crown glass spectrum, and nearer the red end in the flint Therefore, since the several parts of the two spectra are spectrum. not in complete correspondence, the chromatic dispersion of crown glass cannot be completely corrected by that of flint glass; there must always be a residue of non-achromatised light, which produces what is termed a secondary spectrum, and therefore gives rise to a slightly coloured image. Consequently, in the best ordinary achromatic objectives, the chromatic error has never been corrected for more than two colours of the spectrum-the red and yellow; while spherical aberration was not corrected for more than one part of the spectrum-viz., the yellow line D given by a sodium flame. It was undercorrected for the red and overcorrected for the blue rays. residue of imperfectly corrected spherical aberration is more detrimental than uncorrected chromatic aberration, because it impairs the definition of the object, and renders the image incapable of being highly magnified with advantage.

It was felt impossible to remove these difficulties without the aid of glass differing in composition from that of the old crown and flint glass commonly employed. Abbe therefore sought the aid of Dr Schott, a chemist experienced in glass making, and numerous experiments were undertaken. As many as a thousand specimens of glass of different composition were prepared ; a prism made from each, and its refractive and dispersive powers determined, with the result that as many as forty-four different sorts of optical glass were obtained, nineteen of them being entirely new. The old flint glass consists chiefly of silicates of potash and lead, while crown glass consists of silicates of potash and lime. Abbe and Schott have made new sorts of flint and crown glass, which have a dispersive power very nearly in the same ratio for all parts of the spectrum, so that the secondary spectrum of achromatic combinations can be almost completely eliminated. The

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Rev. W. Harcourt had previously ascertained that glass containing salts of boracic acid has peculiar properties, an observation that has been turned to good account at the Jena glassworks. Different sorts of glass containing borates, and others containing phosphates have been obtained, and are now used with siliceous glass in the finest lenses. The new optical glass has from the first been generously supplied to all opticians, and a marked improvement in the microscopes produced in London and elsewhere has been the result of Abbe and Schott's experiments in glass-making.

With the help of the new glass Abbe has been able to devise the finest lenses that have yet been made. They consist of five lenses, in which the new crown and flint glass, and glass containing borates and phosphates are used. He has termed the new lenses apochromatic, because they are practically free from chromatic and spherical faults. They bring the red and violet and other rays of the spectrum to precisely the same focus, so that they are of the greatest value for photography. When the lens is so arranged that the object is most clearly seen on the plate of the camera, further adjustment is unnecessary, because the chemical rays are in the same focus as the most visible rays. Spherical aberration is corrected for two colours of the spectrum instead of one as in previous lenses, so that it is practically abolished even when the full aperture of the lens is used. By bringing practically all the rays of the spectrum into one focus, the image is better defined, and can bear deep eyepiecing. However, I think, I may safely say that the chief value of these lenses is in photography, where they certainly give us great help; but for ordinary microscopic observation the advantage they give over the old lenses is not so great as one might have anticipated. The apochromatic objectives are intended to be used with eyepieces of new construction, termed compensating, because they have been designed by Abbe to compensate the slight residual faults of the objective. They are a great improvement on the old oculars, and give a sharper image even when used with the old objectives. With reference to the eyepiece, we must not forget our indebtedness to Huygens for the double eyepiece he invented for the telescope, and which has now for so long a time been used in the compound microscope.

It is evident that within the last few years the compound microscope has made remarkable progress notwithstanding difficulties that for a long time appeared insurmountable. Within the brief period of some twelve years the principle of homogeneous immersion has been carried into practice; new kinds of optical glass have been compounded, and apochromatic objectives and compensating oculars invented. It is only just to say that we owe these valuable results mainly to the mathematical skill and deep insight of Abbe, but notwithstanding the rapid advances recently made, we are not permitted by him to anticipate any great future advance in the microscope's power of resolving fine details. Its power of so doing is not capable of indefinite extension; the nature of light itself prevents it. The very light that so readily reveals the objects around us will not allow us to see the interval between a pair of lines if it is less than half the wave length of the light; indeed, with *central* illumination the interval must not be less than a wave length to be rendered visible. Therefore, the light of the *visible* part of the spectrum renders the microscope unable to resolve parallel lines of more than 118,000 to an inch; indeed, for a serviceable lens the lines must be not more than 95,000 to an inch. This limitation chiefly results from the phenomena of *diffraction*.

The interference and diffraction of light have so great an influence on the appearances presented by certain objects under the microscope that I would ask your attention to that subject for a little. You will find on the table an oxyhydrogen lantern, with its ordinary condenser inside, and an adjustable metal slit in its aperture. The lantern slit has been opened to the extent of two or three millimetres to get a thin beam of light, and if you place your eye in its path, and look through an adjustable slit held close to the eye with its long axis parallel with the lantern slit, and opened only to the extent of a millimetre or less, you will readily observe a central bright band, and on either side of it a fringe of fainter bands gradually disappearing at the sides. The bright central band is produced by the principal rays passing through the slits, while the fringes of alternate light and dark bands arises from diffraction of a portion of the light in passing through the narrow slit before the eye, and from the mutual interference of the diffracted rays (fig. 1). If you vary the



Fig. 1.—Principal (p) and diffraction bands (d) produced by the same slit with red (r), green (g), and violet light (v).—Ganot's *Physique*.

width of the slit before the eye you will find on making it *narrower* that the principal and the diffraction bands all *widen* out and become separated to a greater distance owing to a similar widening of the dark interference bands. If a circular aperture is substituted for the slit at the lantern, you find on looking through the ocular slit a central bright spot, and on each side of it a row of crescentic diffraction bands extending *laterally* from the slit in whatever position it is placed, proving convincingly that the diffraction phenomena in both cases are produced by the narrow slit close to the eye, and vary with its width. You will further observe that with ordinary light every diffraction band is a spectrum with the red always on the side farthest from the principal band.

If you successively place behind the lantern slit plates of red,

green, and blue glass, you find that all the light and dark bands are broadest with red, and narrowest with blue, and intermediate with green light. The difference arises from the waves of red being longer than those of green, and these in turn longer than those of blue light.¹

The undulations of light, like those on the surface of a liquid, are transverse to the path they pursue. They radiate as spherical waves from the luminous source. Wave systems from countless luminous points can pursue their several paths, and intersect each other without hindrance. But when two waves intermingle, the molecular movements on which they severally depend must be compounded, for the same molecule cannot move forwards and also backwards at the same moment. Therefore, when the opposite phases of the undulatory movement are exactly counter-balanced, there is rest by mutual *interference*, while the coincidence of similar phases *amplifies* the resulting wave. One may observe these effects in water waves, but they are still more evident on the surface of mercury. If in an oval trough half filled with mercury one suddenly dimple the surface at one of the foci, the waves spread to the sides, and are reflected to and fro, producing a beautiful system of interference lines.

Luminous undulations give rise to similar though much less evident results, because of the extreme shortness of the waves, and the invisibility of the medium in which they are propelled. The interference of light may be shown by several methods. The most intelligible is one of those devised by Fresnel, in which a system of waves of red or green light slightly diverging from a common source is received on two closely adjacent straight-edged plain mirrors of steel, or of plate-glass blackened behind, and inclined towards each other at an angle of 180° or less (fig. 2, M, N). The waves falling on the inclined surfaces are reflected in two systems that intersect each other at an angle suitable for the production of interference phenomena. When the light falls on a screen of ground glass there is a bright central band, with a fringe of alternate dark and light bands on each side, the dark bands arising from the coincidence of opposite phases, the light bands from the coincidence of similar phases of the two sets of waves.

The phenomena produced by Fresnel's mirrors arise simply from the interference of luminous waves; those produced by a slit arise from the *diffraction* as well as the *interference* of the wave motion. Diffraction is not peculiar to light. All undulatory movements may be diffracted. The *principal wave* that spreads from a point of disturbance on the surface of a liquid is the resultant of an infinite number of *elementary motions* of the molecules. Every molecule implicated in the spreading wave successively becomes a centre from which an elementary wave system radiates. But in the mutual

¹ These phenomena may be more simply, though less clearly, shown by the ordinary method of placing a slit in a piece of black cardboard or paper before a lamp, and looking at it through a line drawn with a needle across a slip of smoked glass. The blue diffraction bands cannot be seen, however, unless the lamp is completely shaded to cut off all diffuse light.

intermingling of the elementary waves there is an infinite labyrinth of interference and reinforcement that results in the formation of the principal wave.



Fig. 2.—Fresnet's experiment with "interference mirrors" M, N. Luminouspencil of monochromatic light diverging from the focus of a lens (S); s,s', virtual foci of the cones of rays reflected from the mirrors; a,a', d,d', e,e', coincidence of similar phases of undulation strengthening the light; m,m', n.n', coincidence of opposite phases producing darkness. (This fig. from Ganot's *Physique*, 20th ed., errs in having the luminous point S so near and so high that no light could reach mirror M.)

When a screen with a wide slit in it is placed across a trough of water or of mercury, and waves propelled against it, a fraction of each wave passes on through the slit, but in so doing gives rise to a new system of secondary waves spreading in arcs of circles from each margin of the slit into the space protected from the principal waves behind the screen, and also across the path of the principal waves that have passed on through the slit. The secondary waves arise at the slit from the elementary motions of the on-passing portion of the principal waves being no longer restricted laterally by the elementary motions of the arrested portion of the wave. The undulations of light behave in a similar manner. The socalled "rays" are merely the paths pursued by the undulations as they travel from a luminous point. The principal waves (fig. 3, p)



Fig. 3.—p, principal wave arriving at a slit in a screen, s; elementary undulations in principal wave, e.

result from an infinitude of *elementary* wave systems (e) radiating from every molecule of the ether. If the so-called rays are divergent, the principal wave front is convex; if they are parallel, the wave front is flat. When a beam, it matters not whether of divergent or



Fig. 4.—Schematic representation of effect of slit ab in a screen s on principal waves p of divergent light; d, diffracted waves. Only those diffracted from margins of slit are shown; similar diffracted waves proceed from every point in whole width of slit. (Original drawing.)

of parallel light, impinges on an opaque screen (s) with a fine slit, the principal waves pass on through the opening, but owing to their

fracture the elementary motions of the on-passing waves immediately give rise to new systems of secondary or diffracted waves, not merely at the margins of the slit where the fracture occurs, but throughout its entire width. Every molecule of the vibrating ether in the slit is affected, and helps to form diffracted waves, while at the same moment helping to transmit the principal waves. The paths or rays of the diffracted waves diverge in all directions from the slit. From every point in its whole breadth they spread bilaterally into the geometrical shadow of one side, and across the principal rays into the shadow on the other side, so that on each side of the slit there are diffracted rays that have travelled through different small distances from various points in the slit (fig. 4, d). When the decussating waves are half a wave length behind each other, there is darkness from interference, and when similar phases coincide there is reinforcement, so that alternate dark and light bands are produced by the diffracted rays. Since the undulations of green are longer than those of blue light, it follows that the coincidence of similar and of opposite phases of undulation must render the diffraction bands broader with green than with blue or violet light, and still broader with red light. The diffracted rays also produce interference effects with the principal rays (p), but feeble monochromatic lights (preferably green) is required to show them.

I have thus briefly sketched the principles involved in the diffraction of light, in the hope that those of you who have not had the



Fig. 5.-Abbe's grating. The lines at α are 15

opportunity of previously studying these subjects may be enabled to follow the gist of what I have still to say.

Diffraction phenomena may be beautifully shown with a series of parallel slits termed a grating. You will find Abbe's grating under a microscope on the table. It is a cover-glass silvered on one side, and the silver film then cut into equi-distant lines with a diamond. The silvered surface is inverted and cemented with balsam to a slide. There are two sets of lines, the intervals between them in one set being 15 micro-millimetres (fig. 5, a), and only half as wide in the other set (b). The objective used is Zeiss aa, as recommended by Abbe; with a No. 3 eyepiece it magnifies about 50 diameters. With transmitted light you simply micros. apart, those at see a number of clear lines in a dark field, each b are 7.5 micros. apart. line being a fine slit in the silver film. The

grating may be used to show diffraction fringes, and also to illustrate Abbe's theory of the microscopic image of such lines.

If you place a very small aperture of a stop diaphragm on a level with the upper surface of the stage, and focus the broader spaces of the grating (a), and then lower the lens to focus the margin of the aperture in the diaphragm, you of course lose sight of the grating slits no longer in focus, and the margin of the diaphragm is remarkably sharp considering that it is seen through the grating. You will see a bright round disc due to the principal rays of the aperture, with a series of coloured diffraction fringes on each side overlapping each other and the central disc, and extending at a right angle to the lines of the grating (fig. 6, A). Gradually increase the distance between the diaphragm and the grating, and at the same time lower the lens to keep the margin of the aperture sharply in focus, and you will find the diffraction fringes more laterally (B), and eventually become separate discs, if the aperture is sufficiently small, and sufficiently far from the grating. The diffraction discs no longer overlapping show each a pure spectrum, the violet being always on the side nearest the principal disc. If you now bring the finer grating (fig. 5, b) over the aperture, the diffraction discs move still further apart (fig. 6, D). Using the lens I have mentioned, you will probably find that the diffraction discs have been thrown so far aside that only the inner one of each series is now visible in the field (D, d'). Evidently a still finer grating could throw all the diffraction discs out of the field.



Fig. 6.—Diffraction phenomena produced by Abbe's grating with ordinary light. Aperture of diaphragm seen with Zeiss obj. aa, and oc. No. 3; p, principal; d, diffracted discs; corresponding discs indicated by ', ", ""; A.B.C., aperture seen through grating (fig. 5, a); D, the same seen through finer grating (fig. 5, b). (Drawn from the object.)

I have now to explain Abbe's theory of the formation of the microscopic image of the grating. You will find it easiest to perform the experiment in proof of his theory with monochromatic light. Therefore, place a plate of "signal" green glass on the stage under the grating. Focus the broader spaces (fig. 5, a), and place the same small aperture of the diaphragm sufficiently near to illuminate the field diffusely, but not too brightly. On removing the eyepiece and

looking down the tube with the eye shaded from collateral light, you will see a row of small green discs crossing the field at a right angle to the slits of the grating. The discs will be so close together that confusion is apt to arise. It is well, therefore, to bring the finer grating (b) under the lens, and a central disc due to the principal or dioptric rays will be seen in the centre, with two diffraction discs on each side of it (fig. 7, a). Abbe has shown that the eyepiece reunites the principal and diffracted rays, and thus produces the microscopic image. You will find a slot in a collar intercalated above the objective, and if you place in it a diaphragm with a slit in the middle just large enough to permit one of the circular beams to pass, you will find on replacing the eyepiece that the position of the grating is marked by a diffuse light band in which no lines are visible, even though the slit is arranged to allow the principal or dioptric beam to pass (fig 7, b). But if a wider slit, capable of admitting two of the discs, be substituted, the lines are visible, because the reunion of two sets of rays is necessary for the formation of their image. The slit may be placed to permit the principal beam and portions of the inner diffracted beams to pass (fig. 7, c), or the principal beam and





a. The finer grating (fig. 4, b), illuminated with small aperture of diaphragm same as that used in fig. 5, is sharply focussed and eyepiece then removed. Dioptric beam p, and four diffraction beams d, seen on looking down tube.

b. A diaphragm with slit introduced above lens, cutting off all the diffraction beams, and allowing only dioptric beam to pass. On replacing eyepiece, no lines of grating visible.

c. A wider slit allowing dioptric and one diffractive beam or portions of two diffraction beams to pass. On replacing eyepiece lines are visible. (Drawn from the object.)

one of the inner diffracted beams may be taken, or the principal beam and the diffraction beams on one side of it may all be excluded, and the lines still be visible, provided the remaining two diffraction beams are permitted to pass to the eyepiece. The resolution of the lines, however, is most complete when all the beams are utilised in producing the image on the eyepiece.¹

¹ One may perform this experiment in a slightly different way by removing the diaphragm and placing a lamp with a flat wick at some distance from the microscope, with the edge of the flame turned towards it, the principal and diffracted rays produce oval images when the eyepiece is removed, and by placing the lamp sufficiently far away the beams can be separated to a greater extent than when the diaphragm is used. Of course, if the coloured glass is removed the diffracted beams form spectra, while the principal beam is mainly white.

From these experiments it follows that for the resolution of the lines of the grating the principal or dioptric rays are insufficient without the addition of diffracted rays. I intentionally omit further detail¹ regarding these experiments, for I have stated the main points which will carry me to the explanation of the limitation of microscopic vision. It follows from what I have stated that if the lines of a grating are so close that they throw the principal and diffracted beams so far apart that both cannot appear in the field at the same time, the resolution of the lines would be impossible, because one beam of rays is insufficient. It follows that a wide-angled lens must admit a pair of more divergent beams from such an object than would be possible with a lens of small angle, and must therefore have a greater resolving power. It also follows that violet light must, because of its shorter waves, be able to resolve what green and red light cannot, for we have seen that with blue or violet the interval between the principal and diffracted beams is narrower than with green or red light; therefore a grating fine enough to throw the diffracted rays of red or green light out of the field could still be resolved by violet, and still more by ultra-violet light, provided at least a portion of the inner diffracted beam is not thrown from the field. Hence it is that photography can, by utilising violet and ultra-violet light, render visible what cannot be seen without its aid. On this account photomicrography is destined to play an increasingly important part in microscopy, and the apochromatic lenses of Abbe have already greatly facilitated the practice of a method hitherto much neglected, because of the difficulty in finding the focus of the chemical rays with the old lenses.

Gentlemen, it would be difficult to prophecy what new achievments may mark the fourth century of the compound microscope, but it is scarcely to be expected that they can be so remarkable as those of the century now closed, in which science has advanced so rapidly, and has pursued so many paths leading to the attainment of possible results, and leaving superlative difficulties for the future.

¹ For further detail of Abbe's experiments see "Observations on Professor Abbe's Experiments, &c.," by J. W. Stevenson, F.R.A.S., *Monthly Microscop. Journ.*, vol. xvii. p. 82.





