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VI.—*Theories of Microscopical Vision.*

(SECOND PAPER.)

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(Read June 21st, 1905.)

IN a former paper\* I endeavoured to show that the explanation of microscopical images must always be sought on the basis of Professor Abbe's theory; in other words, that the detail is brought out by the light diffracted by the object. I further showed how the formation of the image of a simple plane grating could be fully accounted for on the basis of Abbe's theory, and that the objections which have at different times been raised against that theory are unsound.

In establishing these results I introduced two essentially new propositions, the first referring to certain interesting differences of phase between different spectra from any one grating, the second serving to explain how the want of definite focus in an elementary diffraction-image is replaced by the much desired well defined focus under the usual working conditions.

I now proceed to the consideration of more complicated structures—dot and cross-lined patterns—on the same basis.

It will be remembered that it is with such patterns that the most startling false images are secured in the experiments with the "Diffraction-plate"; it will therefore be extremely interesting to study the images obtainable with such gratings under "normal working conditions," i.e. when the simple circular form of the aperture of the object-glass is not interfered with.

\* J.R.M.S., 1904, p. 610-633.

Our first step must be to determine the diffraction-spectra produced by such structures. We know that a grating of simple, straight, and narrow slits like fig. 123 gives a row of diffraction-spectra lying at right angles to the direction of the slits. Supposing we place another simple grating across this one in the manner shown in fig. 124, the result will be that the light from those parts of each original slit which are covered by the bars of the second grating is cut off; but the light from the portions of the original slits which remain uncovered necessarily continues in the same phase-relation as before, and therefore produces precisely the same row of spectra, only proportionately weakened in brightness. Hence a row of bright dots produces essentially the same diffraction-spectra as the unbroken slit, of which the dots may be considered to be intermittent portions. But this deduction immediately leads us to another; for if the dots are arranged in any perfectly regular order, they will range themselves into straight rows in a number of different ways and directions, and thus we are justified in laying it down that a dot-pattern produces rows of diffraction-spectra corresponding to all simple line-gratings, the slits of which have a direction in which the dots form themselves into straight rows. We will study two concrete cases to make this abstract proposition clearer. Let us first take bright spots (or perforations) arranged in perfect squares (fig. 125). We can range these—

1. Into horizontal rows  $a, a \dots$ , corresponding to a vertical row of diffraction-spectra  $A_1, A_2$ , etc., in fig. 125A.

2. Into vertical rows  $b, b \dots$ , corresponding to a horizontal row of diffraction-spectra  $B_1, B_2$ , etc., in fig. 125A.

3. Into two oblique rows,  $c, c$  and  $d, d \dots$ , with corresponding rows  $C_1, C_2 \dots, D_1, D_2 \dots$ , of diffraction-spectra; and we note that the lines  $c, c$  and  $d, d$  are closer together, hence the corresponding diffraction-spectra are further apart.

4. We can arrange those dots into rows which are in the relation of a knight's move on a chessboard—with four possible directions  $e, f, g$  and  $h$ ; the distance between these rows will be still smaller than that found in case (3), and the diffraction-spectra  $E, F, G, H$ , will be correspondingly further apart.

Evidently this may be carried further and further; the principle will, however, now be perfectly clear.

The result is that the dot pattern here considered gives a set of diffraction-spectra precisely similar in arrangement to the pattern itself; for a simple mathematical investigation shows that the increasing closeness of the oblique rows of successive orders is such as to cause the corresponding diffraction-spectra to be spread out to the proper distances to cover the right places in our pattern.

It should be pointed out that both fig. 125 and fig. 126 show the dots black instead of white, after the manner of a photographic negative.

The second case to which we will pay attention will be a dot-pattern arranged in equilateral triangles, as shown in fig. 126. Similar reasoning to that just applied to the square pattern shows us three directions, *a, b, c*, in which the dots arrange themselves into rows with a maximum distance apart; next three directions, *d, e, f*, of closer rows of dots; and so on, the result being again that a set of diffraction-spectra, as illustrated in fig. 126A, is formed similar in arrangement to the pattern itself.

The reasoning here used is *directly* applicable only to very small perforations which can be considered as parts of *separate* and distinct lines, *a, b, c, d*, etc. But as the diffraction-spectra are

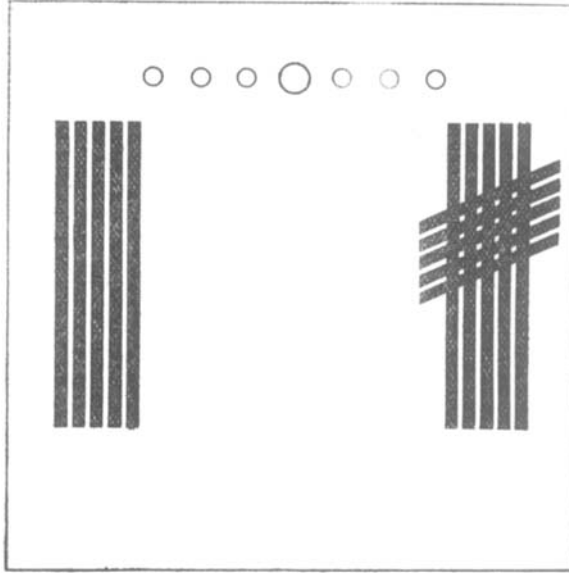


FIG. 123.

FIG. 124.

formed by light from all the perforations meeting with differences of phase expressed by a *whole* number of wave-lengths, and as *this* phase-relation will not be disturbed if all the perforations are *uniformly* increased in size, it will be seen that the *arrangement* of the diffraction-spectra must remain the same no matter how large the perforation may become; for that arrangement is determined by the configuration of similarly situated points in the individual perforations.

The relative brightness of, and the phase-relation between the direct light and the different spectra will, however, depend upon the relative size and upon the shape of the dots or perforations;

*this*, therefore, remains to be investigated in each individual case, and will have to be attacked by applying the Huyghenian principle in the same manner in which I applied it to simple gratings in my first paper.

Before proceeding to this we must, however, study another class of gratings, viz., those consisting of bright line-patterns, or of opaque dots. At first sight this looks a more formidable problem than that of the perforation-patterns, but it can be dealt with at once by the application of Babinet's theorem concerning "reciprocal gratings." Two gratings are said to be reciprocal when the

FIG. 125.

FIG. 125A.

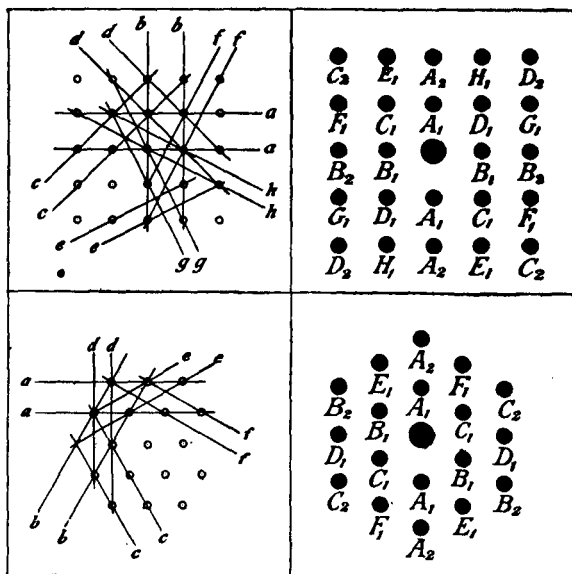


FIG. 126.

FIG. 126A.

opaque portions of one are precisely similar to the transparent portions of the other, and when it is therefore *just possible* to so superpose one upon the other as to produce uniform opacity. In other words, a grating and its reciprocal stand in the exact relation to each other of a photographic negative and the corresponding positive transparency.

The simple process of reasoning first applied by Babinet then leads to the discovery of a very simple and valuable relation between the diffraction-spectra from reciprocal gratings.

Supposing we have an aperture fitted with two screens in such a manner that either one or both together may be applied or removed, screen No. 1 having perforations of any shape and design

whatever, whilst screen No. 2 is so cut and adjusted that when superposed it exactly covers the apertures in No. 1. Therefore, if we apply screen No. 1 by itself we shall have the set of apertures cut in it; if we apply screen No. 2 by itself we shall have a new set of apertures corresponding precisely to the dark portions of screen No. 1; screen No. 2 therefore represents a grating reciprocal to that formed on screen No. 1.

The apertures in screen No. 1 will produce a set of diffraction-spectra peculiar to their shape and configuration; the apertures in screen No. 2 will also produce a set of diffraction-spectra. If now we let both sets of apertures act at the same time, we are justified by the Huyghenian principle in stating that the diffraction-effects of both sets are now superposed. But the uncovering of both sets of apertures means the removal of both our screens with the consequent exposure of a simple large aperture producing no sensible diffraction-effect; in other words, we are driven to the conclusion that the diffraction-spectra produced by the apertures in screen No. 2 exactly blot out or neutralise those produced by the reciprocal screen No. 1. According to the undulatory theory, this can only be explained on the assumption that the light diffracted by screen No. 2 is precisely equal in intensity, but also exactly opposed in phase to the light diffracted by the reciprocal screen or grating, which we designated as No. 1.

This, then, is Babinet's theorem; it states that reciprocal gratings produce diffraction-spectra in the same directions and of the same intensities, but opposed to each other—*cæteris paribus*—in phase.

It will be seen that this convenient theorem enables us to determine the complete diffraction-pattern produced by any bright line device by first ascertaining that of a perforation pattern having perforations exactly corresponding to the opaque dots of the bright line device, and then attributing to the latter diffraction-spectra of the same distribution and intensity, but of the opposite phase when referred to some definite point of reference such as the centre of the dots. Babinet's theorem does not, however, give us any direct information about the intensity of the *direct* light; this, therefore, remains to be determined in each individual case.

Having learned how the diffraction produced by the complicated structures now under consideration may be completely determined, we are in a position to discuss the image resulting from the co-operation of a greater or lesser number of the diffraction-spectra in the field of a Microscope directed and focused upon such structures.

We will first take a pattern consisting of relatively small perforations arranged in perfect squares such as we have represented (as a *negative*) in fig. 125. Owing to the smallness of the dots, they may be considered as intermittent portions of relatively narrow slits, and, in accordance with the reasoning given in my

former paper, we may safely assume that all the spectra immediately surrounding the direct light will leave the centres of the dots in exact phase with the direct light, and will, in accordance with the fundamental principle of the equality of optical paths, arrive in the same phase-relation at the centres of the ideal geometrical images of the dots. Hence we see that in this case also, in precise analogy to what I proved to be the case with simple line-gratings, the centres of the bright dots must be represented in the image by maxima of brightness exactly coinciding with the geometrical images of the dots, and that the position of the latter will, therefore, be correctly indicated.

FIG. 127A.

FIG. 127B.

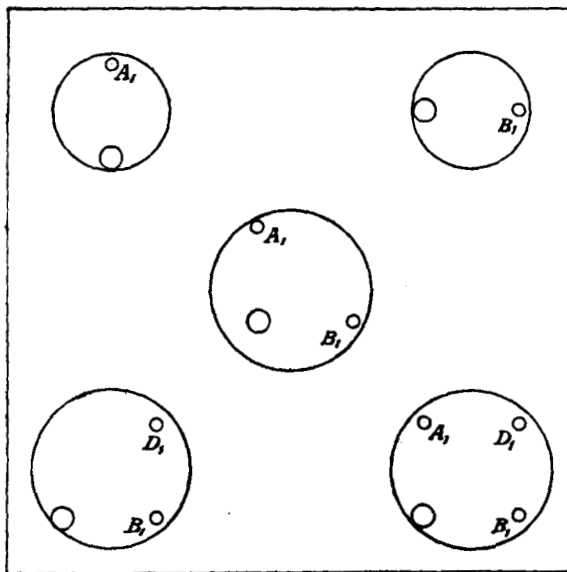


FIG. 127C.

FIG. 127D.

FIG. 127E.

It will be highly instructive to study the image in its gradual evolution as the aperture of the Microscope object-glass is increased.

In order that any structure may be shown at all, we must have at least two maxima entering the objective—say the direct light and one of the innermost spectra. If we admit the direct light and the spectrum  $A_1$  in the manner illustrated in fig. 127A, the Microscope will show the lines  $a$  in fig. 125, from which the spectra  $A_1$ ,  $A_2$ , etc., are derived. If we admit the direct light and the spectrum  $B_1$  as shown in fig. 127B, we obtain an image displaying the corresponding lines  $b$  of fig. 125. Evidently, either of these images is

unsatisfactory, inasmuch as it discloses only a part of what is capable of being shown by the object-glass. And this leads us to the discovery of an important advantage to be derived from the use of an extended source of light—or, in other words, of a large cone of illumination. For in that manner we can obtain the effects shown in fig. 127A and fig. 127B simultaneously and superposed, leading to the formation of an image showing bright lines corresponding to  $a$  and  $b$  of fig. 125, and with the points of intersection, which will be noted to correspond to the actual dots, specially bright as the light of both systems of lines is there added together. The simple expedient of using a wide cone of illumination, being equivalent to oblique light in all directions, has, therefore, at once produced a tolerably good indication of the actual nature of the object. We can derive yet another lesson from this observation. On inspecting figs. 127A and 127B it will be seen that direct light in the central part of the aperture is useless for the purpose of showing any structure, because no corresponding diffraction-spectrum can enter through the available aperture of the object-glass. Such light can, therefore, only form a general bright illumination of the field; cutting it off by a central stop, and thus producing *annular illumination*, must improve the clearness of the image, and this would appear to be a perfectly legitimate means of attaining the utmost distinctness in the image of structures close to the limit of resolution of an object-glass.

We proceed to study the effect of an increase of aperture.

No new spectra can enter unless the aperture is at least equal to the diagonal of the squares into which the spectra of fig. 125A arrange themselves. When that aperture is slightly exceeded, we have the possibility of three distinct combinations of maxima which can enter the increased aperture, viz. :—

1. In accordance with fig. 127C we can have a beam of direct light, and the two diffracted beams  $A_1$  and  $B_1$  derived from it. We have thus three separate beams capable of interfering with each other. The direct light and  $A_1$  alone would meet in equal phase and produce bright *lines* corresponding to  $a$  in fig. 125; the direct light and  $B_1$  would similarly produce *lines* like  $b$  in fig. 125. When all three are admitted at once, then they will all meet in the same phase and produce a very pronounced maximum of brightness at the *points* of intersection of lines  $a$  and lines  $b$  in fig. 125; in other words, these three maxima lead to the formation of the correct *dot* pattern. When added to the crossed-line effect—with enhanced points of intersection—resulting from the combinations illustrated in figs. 127A and 127B, they will further accentuate the dots, and thus improve the verisimilitude of the image.

2. We may have groups like that in fig. 127D—i.e. the direct light, the spectrum,  $B_1$  (or  $A_1$ ), and one of the remoter spectra,  $D_1$  (or  $C_1$ ). By similar reasoning we find that the points of inter-

section of lines *b* and lines *d* will be brought out as bright dots; reference to fig. 125 shows that these again exactly correspond to the true position of the actual perforations; this new combination of maxima, therefore, further improves the image.

3. Finally, we may have four maxima such as direct light and spectra,  $A_1$ ,  $B_1$  and  $D$ , admitted simultaneously—fig. 127E.

We again obtain very bright dots at the points of intersection of lines *a*, *b* and *d* in fig. 3—i.e. in the position of the actual dots.

This small increase of aperture is, therefore, sufficient to emphasise the bright dots to such an extent as to render them unmistakable.

The qualitative method of discussing the results to which I am

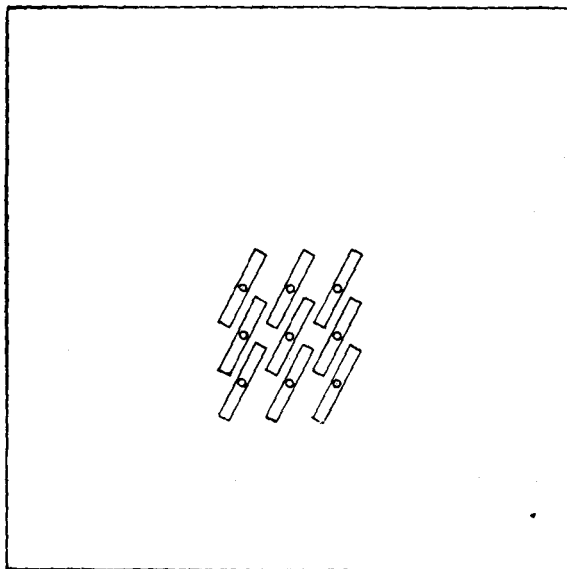


FIG. 128.

at present limiting myself is not adapted to bring out all peculiarities of the different partial images, such as the secondary maxima (intercostals) resulting with combinations of maxima like figs. 127C, 127D, and 127E, nor to show that these secondary effects are different with the different combinations, and are more or less neutralised when they are all superposed. These finer points must be left for a rigorous quantitative treatment of a few judiciously selected cases which I hope to bring forward on a future occasion. What has been stated above will suffice for the present to show that with a perforation pattern also there must be rapid improvement of the image with increasing aperture.



I purposely chose a pattern of *small* perforations; with large perforations we have the possibility of reversed phase in some of the spectra—in fact, we may have perforations such that even some of the innermost spectra will be reversed in phase. I must leave these to be dealt with separately, merely pointing out now that it is found that the phase-reversals again prove to be the agency through which the finer peculiarities of the structure are brought out in the image. Fig. 128 illustrates a possible case of this kind: the long oblique perforations will be noted to be formed round the centres of the small dots of fig. 125; they are, therefore, arranged in the same configuration as fig. 125, and give diffraction-spectra arranged in the manner of fig. 125A, but it is not difficult to see on reference to my former paper that the spectra  $A_1$  will be reversed in phase.

We will next briefly study the image to be obtained of the *reciprocal* grating corresponding to the one discussed above, i.e. a *black dot* design of which fig. 125 would be an actual (positive) representation. From what has been said concerning Babinet's theorem, it will be clear that, referred to the lines connecting the black dots, all the inner spectra will now be *opposed* in phase to the direct light, i.e. the light of all these inner spectra will arrive at the corresponding lines of the image in the phase opposite to that in which the direct light reaches them, and there will, on the other hand, be a tendency to form bright lines *midway between* the lines *a, b, c*, etc., of fig. 125. In the cases represented in figs. 127A and 127B we shall thus obtain intersecting bright lines midway between the lines *a* and *b* respectively which leave dark spots between them precisely corresponding to the real dots of the object. Similarly, the combinations of spectra 3 and 4 shown in figs. 127C, 127D, and 127E, now lead to the formation of bright dots at various points *between* the dark dots of the pattern, thus leading to a more and more uniform filling with light of the spaces outside the true images of the black dots, and to a corresponding improvement in the verisimilitude of the image of the black dot pattern.

The triangular pattern represented in fig. 126 may be similarly discussed; we again find first intersecting lines indicating fairly accurately the position of the actual bright or black dot, and next, as soon as more than two maxima are admitted by the object-glass, the formation of bright dot images which are so distributed as to improve the resemblance between object and image. The only difference is that a very much smaller increase of aperture leads to this latter result in the case of the triangular pattern than in the more fully discussed case of a pattern arranged in perfect squares, for the simple reason that a circle only slightly larger than that required to enclose say the direct light and spectrum  $A_1$  of fig. 126A will suffice to embrace three adjacent maxima, such as direct light and spectra  $A_1$  and  $B_1$ .

On the other hand, it will be seen by reference to fig. 126A that

the triangular pattern is at a disadvantage when a still further increase in the number of spectra admitted is aimed at; it evidently requires a very considerable increase of aperture to bring into action any of the outer circle of spectra. Both these peculiarities of a triangular design are well exemplified in the case of *Pleurosigma angulatum*. Any objective which resolves the structure at all—and a numerical aperture equal to 0.55 will do this—will show the familiar dots, provided the objective be well corrected. On the other hand, it is very difficult to attain a pronounced advance on that image, even with oil-immersion objectives.

It would be useless to attempt a very precise discussion of the image of any dot-pattern by simple reasoning; this must be left for another occasion, when I propose to treat concrete cases mathematically.

We will instead try to draw some further conclusions from the above general discussion.

In the previous paper I showed that one important advantage resulting from the use of an extended source of light, or of a wide illuminating cone, was that the want of focus of an elementary diffraction-image was overcome and replaced by a well-defined focus, such as one expects with an optical instrument. The study of dot-patterns enables us to see another and even greater advantage. In order to obtain extreme resolution with a narrow beam of light, we must let it enter obliquely, through the marginal zone of the object-glass. But that gives us the high resolving power in one direction only—along the diameter of the object-glass having the direct light at one of its ends; it leads to the formation of a misleading image, inasmuch as fine detail is shown in that one particular direction, whilst detail no finer, perhaps even considerably coarser, in other directions is not even hinted at. *A well-centred illuminating cone overcomes this; it gives us equal resolving power in all directions, and thus brings into view everything that a given objective can resolve, no matter in what directions the structural details may be arranged.* Here, then, is a full explanation of the necessity of a uniformly bright and well-centred cone of illumination. Any want of centring, any dark or coloured portions in the circle of light at the back of the objective, imply a want of symmetry in the image, and a corresponding danger of misleading images. It will, indeed, be found, when the nature of "critical illumination" is impartially examined, that the type of image looked for with such illumination is invariably obtained when, on looking down the tube, a uniformly bright and perfectly centred circle of illumination is seen—*no matter how obtained*—and that the critical image is as invariably absent when examination of the back-lens shows any want of uniformity or symmetry in the said circle, no matter how brought about.

I indeed venture to suggest that "aplanatic cones" or "critical light" would be more scientifically described and specified as "concentric illumination."

There is yet another advantage accompanying the use of extended cones of illumination, viz., the certainty that the objective is free from serious rests of spherical aberration, for only a well-corrected objective will bear a wide illuminating cone. The danger of utterly false images is a very grave one, when only a very narrow beam of light is employed; we may then obtain a sharp image although there is considerable spherical aberration, and as the latter is equivalent to inequality of the optical paths between conjugate points, it will be seen that the phase-relation between the direct light and the diffraction-spectra, which I have shown to play a most important part in the formation of images, will be entirely falsified by spherical aberration, and that misleading images must result.

It only remains to bring forward some strong evidence in favour of the position which I took up in the early part of my former paper, i.e. the claim that *all* microscopical images were due to the diffraction produced by the object.

The chief *theoretical* arguments in favour of this somewhat revolutionary postulate were given in the former paper, and have not as yet been called into question; there is, however, *experimental evidence* tending in the same direction.

The first of these experimental facts is one of which I myself often make practical use in the testing of Microscope objectives. It is this: if we examine a *broken* specimen of *Pleurosigma angulatum* (showing the familiar postage-stamp fracture) with a wide "aplanatic cone" of light, using a dry objective, we obtain a remarkable result if spherical aberration is present, i.e. if the wrong tube length is employed.

At one focal adjustment the broken edge is clearly discernible, whilst by varying the adjustment the dots may be brought into view. As the fracture and the structure are really in the same plane, this is utterly inexplicable on the basis of the spurious disk theory; it is irreconcilable with the assumption that the object behaved as if it were self-luminous, for in that case all parts of the object would have their images formed by the same process and in the same plane.

The diffraction-theory on the other hand explains this quite easily and naturally.

The broken edge produces a narrow fan of diffracted light closely surrounding any ray of direct light; the image of the broken edge is due to such confined pencils of diffracted light passing through the axial portion of the object-glass; for owing to spherical aberration affecting (when of fairly moderate magnitude)

only the outermost zone of an object-glass, a large central portion of the aperture is capable of yielding a good image of such a coarse structure, which is only "fogged" by the scattered light which has passed through the marginal zone. The dots, on the other hand, are brought out by the regular diffraction-spectra corresponding to them, and in accordance with figs. 127A to 127D, combinations of these can only enter through the marginal zone; the image of the dots therefore is formed by, and indicates the focus of, the marginal zone, whilst the image of the outline is due to light passing through the axial portion of the object-glass.

This peculiarity of the image formed by an under- or over-corrected objective may therefore be claimed as constituting a proof that objects do *not* behave as if they were self-luminous.

An even more remarkable fact bearing on the subject is mentioned in our standard handbook of Microscopy.\* It is that with difficult diatoms resolution is sometimes emphasised when an analyser is interposed between the object and the eye. As it is universally accepted as a criterion of a self-luminous object that the light from it is quite free from any trace of polarisation, this observation again proves that the object does not behave like a self-luminous body. At the same time it is a remarkable piece of evidence in favour of the Abbe theory; for when the effect of gratings is studied more rigorously than by the usual more or less elementary approximation, *the result is arrived at that the diffracted pencils are polarised*, the amount of the polarisation depending largely on the angle between the direct and the diffracted light, but also on the nature of the edges of the slits, etc. This observation, which in the above quoted passage is put forward as a puzzling one, is therefore a direct refutation of the spurious disk theory and an equally direct proof of the correctness of the Abbe theory.

The chief results of this inquiry into the theory of microscopical vision may now be summarised as follows:

1. The spurious disk theory, being based on the inadmissible assumption that microscopical objects could be made to behave as if they were self-luminous, must be abandoned.

2. The images obtainable from plane gratings of various types can be fully accounted for by the Abbe theory, provided that the phase-relation as well as the intensity of the diffraction-spectra is taken into consideration.

3. The advantages derivable from so-called aplanatic cones of light are:

- (a) That the image acquires that fixity of focus which is desirable and indeed necessary in order to distinguish spurious "ghosts" from the image formed in the plane of the geometrical image.

\* Carpenter, Dallinger, 8th edition, bottom of page 381.

- (b) That we obtain equal resolving power in all directions, and can therefore see simultaneously everything that a given combination of condenser and objective can show.
- (c) That false images, due to a badly corrected object-glass, are not likely to deceive the observer, because such objectives will not bear this mode of illumination.

4. One other important result has been arrived at since this inquiry was opened, and is, I believe, largely due to it.

It is a *warning against dark-ground illumination*. In supplying an experimental proof of the phase-reversal in diffraction-spectra I also showed that with dark-ground illumination a grating may be seen *reversed*, i.e. bright where it ought to be dark, and *vice versâ*.

Mr. Rheinberg has shown an even more remarkable experiment at the Royal Institution and again at the Quekett Club, viz. that with dark-ground illumination we may see a *grating doubled* under otherwise perfectly normal conditions.