XVII.—THE PHOTOMETRY OF A BULL'S-EYE LENS FOR ILLUMINATING MICROSCOPIC OBJECTS.

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FIVE TEXT-FIGURES.

THE bull's-eye first used to increase the illumination of opaque objects has since been employed in connexion with substage condensers and dark-ground illuminators.

The term bull's-eye is here understood to mean a powerful convex lens used for the purpose of condensing light to a focus, or for collecting it from a point source and rendering it parallel. Those used with the microscope are generally between $2\frac{1}{2}$ in. and $3\frac{1}{2}$ in. in focal length, and of a diameter only slightly less than their focal length.

The method by which they concentrate light upon an object cannot be properly explained by the ordinary optical text-book diagrams which illustrate the passage of light through a lens. Such diagrams show the light as a point, and illustrate what happens to the light which emerges from that point as it passes through the lens; but a light, except in the case of a distant star, is never a point, but an area of a definite and often a considerable size.

Fig. 1 shows the ordinary text-book illustrations in which O is a point source of light, and S is a screen illuminated by the light at (a) without a bull's-eye; at (b), where the bull's-eye is so placed as to give out so-called parallel light; at (c) and (d) in the two positions where it can be placed in order to focus the light to a point on the screen. Such diagrams are misleading. It would appear that the condition at (c) would produce most light, because as compared with (d) it collects a larger angle of light from the source and brings it all to a point on the screen, but this is not correct.

The size of the source of light must be considered, and if a new set of diagrams are drawn in which not only the light from the central point but that from a point at each extreme edge of the source is shown, the size of the picture of the source of light which

is thrown upon the screen will be ascertained, and a more correct idea will be obtained of what is happening.

Fig. 2 (*b*) shows that the so-called parallel light emitted by a condenser when the light is in its focus, as shown in fig. 1 (*b*), is far from being parallel if the light source has any finite size, and that when it meets the screen it illuminates a large area. In the cases (*c*) and (*d*) in which the light is focussed to form an image of the source, the size of the image is much larger in the case of (*c*) than in that of (*d*), so that although the lens in the case (*c*)

FIG. 1.

collects more light, it spreads it over so much larger an area that the brilliancy on the screen will be far less.

There is no arrangement of lenses or reflectors by which the brightness of a source of light can be increased. All that can be done is to concentrate upon an object a larger number of rays than it would otherwise receive. If daylight falls upon an object in all directions from an unobscured sky, no arrangement of lenses or reflectors can collect more, and, therefore, a bull's-eye will not increase the brightness of an object illuminated by an unobscured sky. It is only of use in daylight in a room into which the light enters from a window which forms a small source of light, where light which would pass into other portions of the room can be

concentrated upon the object. In this case daylight having only an intrinsic brightness of 2 to 4 candle-power per square inch is seldom sufficiently intense to give the brilliancy that is required.

The case will be considered, therefore, of a small brilliant artificial source of light.

FIG. 2.

Supposing a source of light (s) has an area of S^2 . Suppose each unit of light has a brightness of (i). A point of light with a brightness of (i) will spread out the whole of its light over an imaginary sphere with a radius of curvature equal to the distance (d) from the light point to the object. The area of this sphere is $4 \pi d^2$, and the light received per unit area of the sphere from each point of a source of light is

$$\frac{i}{4 \pi d^2}$$

or from the total area of the source of light

$$\frac{i}{4 \pi} \frac{S^2}{d^2}.$$

The expression $\frac{i}{4\pi}$ will be the same in all the investigations, and may be called K , in which case the illumination received per unit area is

$$\frac{KS^2}{d^2}$$

or by an object (O)

$$\frac{KS^2 O^2}{d^2}.$$

If $S = 10 \times 10 \text{ mm.} = 100 \text{ square mm.}$
 $d = 400 \text{ mm.}$ $d^2 = 160,000 \text{ mm.}$

FIG. 3.

Then the illumination received by an object from a source of light is

$$\frac{K 100}{160,000} = \frac{K}{1600} \text{ per unit area}$$

and this forms a basis for comparison when no bull's-eye is used.

Now interpose a bull's-eye between the source of light and the object.

There are two positions for every lens where an image of a source of light can be produced on a screen.

Fig. 3 (1) shows the bull's-eye of a focal length of 75 mm. at a distance $x = 100 \text{ mm.}$ from the source (S), and $x' = 300 \text{ mm.}$ from the screen (O).

Fig. 3 (2) shows the bull's-eye at a distance $x = 300$ mm. from source. The axis of each bundle of light passes through the centre of the lens, and thus from similar triangles the relative diameters of the source and its image are [in Fig. 3 (1)] 1 to 3 and [Fig. 3 (2)] 1 to 1/3.

These are the two positions—namely, when the lens is at 100 mm. and 300 mm. from the source of light—where with a lens of 75 mm. focal length images can be formed in the given total distance of 400 mm. To ascertain the brilliancy the diameter of the bull's-eye must be considered, and we may take this as $L = 58$ mm. As the lens is circular its area is 2642 mm., and for convenience we may call this L'^2 .

Firstly, when the bull's-eye is at a distance of 100 mm. from the source of light, it receives from the source a total quantity of light, as if it were the screen in the previous case, of

$$\frac{K S^2 L'^2}{x^2} = \frac{K 100 \times 2642}{10,000} = 26.42 K.$$

The whole of this light is transferred to the object, but is spread out over an area which is the size of the image of the source of light. The area of this image is $\frac{S^2 x'^2}{x^2}$, and the total quantity of light must be divided by this area to obtain the intensity of unit area—namely,

$$\frac{K S^2 L'^2}{x^2} \div \frac{S^2 x'^2}{x^2} = \frac{K L'^2}{x'^2} = \frac{K \cdot 2642}{90,000} = \frac{K}{32}.$$

The formula shows that the brilliancy is only dependent upon the intrinsic brilliancy of the source K , the area of the bull's-eye L'^2 , and the distance of the bull's-eye from the object x' . In the case under consideration the brilliancy of the object $\frac{K}{32}$ is 50 times

as great as when illuminated by the direct beams $\frac{K}{1600}$. This may appear a large increase, but to obtain a correct impression of what is required in a microscope it must be remembered that magnifying power is not reckoned by the increased area of an object, but by the increased length, and an object magnified 7 diameters has an area 49 times as large as the original. Therefore, to obtain sufficiently increased illumination to allow of the use of a microscope magnifying 7 diameters without loss of light, we require 49 times

the illumination per unit area. In this case, therefore, if the illumination is sufficient without a bull's-eye to enable an object to be seen clearly with the naked eye, the use of a bull's-eye in the manner here described allows the object to be equally well seen with a microscope magnifying about 7 diameters.

Secondly, when the bull's-eye is at a distance of 300 mm. from the source of light and 100 mm. from the object, the same formula gives

$$\frac{K L'^2}{x'^2} = \frac{K \cdot 2642}{10,000} = \frac{J}{3 \cdot 8}.$$

Here the brilliancy of the object is increased from $\frac{J}{1600}$ to $\frac{J}{3 \cdot 8}$ about 420 times, and will allow of the use of a microscope magnifying about 20 diameters.

A substage condenser of a focal length of 8 mm. used without a bull's-eye produces a sharp image at a distance of 8.16 mm. from the object. If the source of light be as before at a distance of 400 mm. from the object, the substage condenser acts exactly as a bull's-eye, and the illumination becomes

$$\frac{K L'^2}{x'^2} = \frac{K L'^2}{(8 \cdot 16)^2}.$$

We are, however, not illuminating an opaque object scattering light in all directions. We are passing light through a transparent object, and use a microscope object glass which can only receive light of a definite angle from the object. Suppose an object glass to have a focal length of 8 mm. which can only receive light at an angle of 0.5 N.A. or 60°. The substage condenser may be of a larger size than is required to admit 60°, but if so the light that is thrown through the object at a greater angle than this will not be collected by the object glass, and need not be considered; therefore, for the purpose of the investigation the substage condenser may be considered to have a diameter of 8 mm. and an area of 50.26 mm. The light received by the object that will enter the object glass will be

$$\frac{K \cdot 50 \cdot 26}{(8 \cdot 16)^2} \quad \frac{K}{1 \cdot 3}$$

an increase of about 1230.

Now interpose a bull's-eye 75 mm. focal length 58 mm. at 75 mm. from the light.

The bull's-eye will direct beams of parallel light from each point of the source of light upon the substage condenser which

are 58 mm. diameter, but only beams 8 mm. diameter will pass through the substage condenser into the object glass; therefore, the bull's-eye is only employing an aperture of 8 mm., and the useful light received by the bull's-eye will be

$$\frac{K S^2 L'^2}{x^2}.$$

The whole of this light is passed through the object spread out over an area which is the size of the image of the source of light. The area of this image is $\frac{S^2 x'^2}{x^2}$, and the intensity of unit area will be as before

$$\frac{K S^2 L'^2}{x^2} \div \frac{S^2 x'^2}{x^2} = \frac{K L'^2}{x^2} = \frac{K 50 \cdot 26}{8^2} = \frac{K}{1 \cdot 27}.$$

The effect of using a bull's-eye with a substage condenser is quite inconsiderable, and has thus only increased the intensity from $(8)^2$ to $(8 \cdot 16)^2$ in the ratio of the slight alteration in the focus of the substage condenser caused by using parallel light instead of light coming from a distance of 392 mm.

What has actually taken place due to the use of a bull's-eye is that the size of the area of the object which is illuminated is larger without loss of illumination. The relative diameters of the image of the source of light in the two cases with and without the use of a bull's-eye are approximately in the ratio of the focal length of the bull's-eye to the distance of the light from the condenser, in this case 75 to 392. The use of the bull-eye increases the diameter of the illuminated area approximately five times, but does not increase the intensity.

An experiment with a photometric eye-piece shows a small but noticeable decrease in the light due to the loss by reflection at the surfaces, and to the absorption of the bull's-eye, which loss has been neglected in this discussion.

But on applying the theory to the modern form of dark-ground high-power illuminator it does not appear to agree with practice.

The dark-ground illuminator referred to is of the type with two reflecting concentric surfaces. It forms a thoroughly good image of the illuminant, it has a focal length of about 3 mm., and is almost free from aberration, and there is no theoretical reason why it should not behave as any other well-corrected condenser. The following experiments show that it gives a far more intense light with a bull's-eye than when it is used alone:—

Experiment 1.—A piece of paper placed in immersion contact with a 3 by 1 slip with cedar-wood oil—

- (a) With dark-ground illuminator alone, gave an intensity 1.
- (b) With dark-ground illuminator and bulls'-eye focussed to give parallel light, intensity 3.45.
- (c) With dark-ground illuminator and bull's-eye focussed to form image $2\frac{1}{2}$ in. from illuminator, intensity 15.

Experiment 2.—A specimen of *Coscinodiscus* mounted in an aqueous solution—

- (a) With dark-ground illuminator alone, intensity 1.
- (b) With dark-ground illuminator and bull's-eye focussed to give parallel light, intensity 3.
- (c) With dark-ground illuminator and bull's-eye focussed to give image $2\frac{1}{2}$ in. from illuminator, intensity 7.

The increase in intensity is very marked and requires explanation.

The question as to whether this illuminator delivered a larger angular cone upon the object under the different conditions was considered when the image of the light was focussed to a distance only $2\frac{1}{2}$ in. from the illuminator. It was still about 30 times the focal distance away, the light from it was not far from being parallel compared with the reflecting power of the curves, and it should not affect the optical effect of the apparatus. Careful measurement showed no appreciable increase in the angular size of the cone of light delivered upon the object.

The nature of the image of the illuminant formed by this illuminator did not give any serious distortion.

The question as to whether such an increase in light could be occasioned by reflection from surfaces of the slide, cover glass, object glass, or the object itself, seemed improbable on account of the large amount of such increase, but the following experiments appear to show that such is the case:—

Experiment 3.—An isolated malaria parasite which filled a red corpuscle, stained and mounted in balsam, was illuminated by a dark-ground illuminator, and the light intensity reduced by means of a pair of neutral glass wedges until the object was only just visible. The use of a bull's-eye exactly as in Experiments 1 and 2 did not render it more visible.

Experiment 4.—A stained anthrax bacillus, mounted in balsam, gave the same result.

Experiment 5.—A specimen of stained blood, mounted in balsam, viewed with a low power and put slightly out of focus so as to give a uniform matt surface of light, also gave the same result as Experiment 3.

Experiment 6.—A stained section of tissue, mounted in balsam, gave an appreciable increase in brilliancy when the bull's-eye was used, but not as great as in Experiments 1 or 2.

It appeared, therefore, that specimens mounted in balsam,

where the refraction is almost the same as the cover glass and slip, are not increased in brilliancy when a bull's-eye is used, and that such increase must be due to reflection from the cover glass and slip.

That this may be so may be illustrated by the following diagrams.

If, as in fig. 4, the image formed by the illuminator of the source of light is very small, such light as is reflected by the

FIG. 4.

cover glass does not come back to the object and increase its brilliancy; but if, as in fig. 5, the image formed by the illuminator is large, then all light from distances a , b and c from the centre will be partially reflected by the cover glass in such a manner that they fall upon the object in the centre. The effect of the bull's-eye is to produce a large instead of a small image of the source of light

FIG. 5.

on the object, and it would appear that this may be the reason for the increase in light observed in the case of a bull's-eye on such specimens as living bacteria in water. The film is very thin, and many sets of reflections as indicated in fig. 5 might take place.

If an object such as paper, or even a section of tissue, is of itself a diffusing or reflecting material, each particle that is illuminated reflects light in all directions, and it is easy to understand

that the larger the amount of the material that is illuminated, the greater will be the brilliancy of any individual point.

The photometry of bull's-eyes and illuminators may appear in itself to be a matter of small consequence, but the investigation of the dark-ground illuminator points to results as regards reflection and its surprising influence on illumination, that suggest considerations concerning the cause of glare and mistiness in ordinary observation which may lead to important modification in our ideas.