

On a METHOD of ILLUMINATING OPAQUE OBJECTS under the
Highest Powers of the MICROSCOPE. By F. H. WENHAM.

(Read March 26th, 1856.)

REPEATED experiments have shown, that it is a matter of extreme practical difficulty to contrive any method of condensing light directly down upon an object, when viewed under an eighth or twelfth object-glass of large aperture. In the first place, the close proximity of the front lens and its setting, will only allow a thin conical disc of light, to find a passage towards the object, at an angle of seldom less than 100° , or at an obliquity far too great to be practically useful; and secondly, when the object is covered with thin glass, considerably more than half the light will be lost by the reflection from the surfaces, the rays from which enter the microscope, and occasion an amount of glare and fog sufficient to obscure the object; for these reasons I think that there is but little chance of obtaining any useful result in this direction.*

The methods that I now bring before the Society are based upon an entirely different principle, which is not applicable to *dry* objects, but only to those mounted either in Canada balsam, fluid, or any other refractive medium. An experience of nine months warrants me in the assurance of its complete success, as a means of investigation—objects being brilliantly illuminated in a jet-black field, with an objective of 170° of aperture or more.

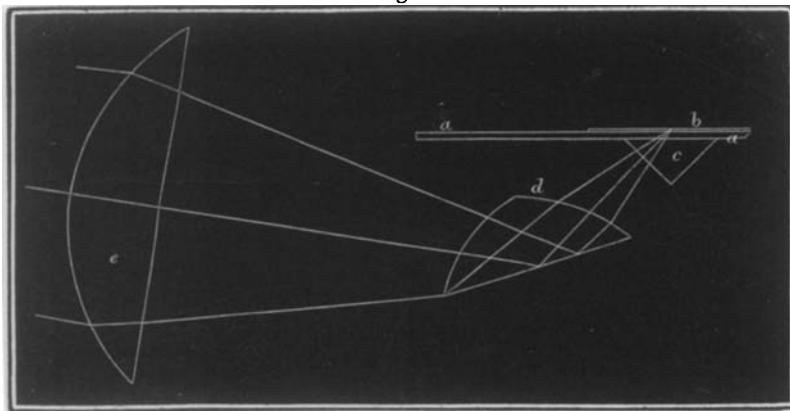
The principle of operation consists, in causing rays of light to pass through the under side of the glass slip upon which the object is mounted, at the proper angle for causing *total internal reflection from the upper surface of the thin cover*, which is thus made to act the part of a speculum, for throwing the light down upon the under-lying objects, immersed in the balsam or fluid.

As there will be no total reflection from the planes of a parallel plate of a refractive material, it is necessary to adopt some method for allowing the rays to enter the medium at such an angle as to cause total reflection from the upper surface. There are many methods of effecting this; those which I now describe I have found to be the most practicable and useful: *a*, *a*, fig. 1, is a glass slide containing objects mounted in balsam; *b*, thin glass cover; *c*, is a right-angled prism

* Since the above, Mr. Ross has shown me his ingeniously-contrived Leiberkuhn, applied to the highest powers for illuminating *uncovered* opaque objects, and which performs most admirably; to my mind undoubtedly proving the fact, that the minute scales from the wings of butterflies, &c., are perfect *cellular* structures.

cemented on to the under surface of the slide with Canada balsam; *d*, is an Amici prism for condensing and directing the rays into the prism *c*; *e*, is a large bull's-eye condenser placed with its convex side towards the lamp.

Fig. 1.

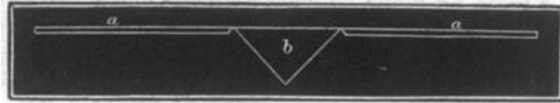


Making ample allowance for all possible differences of refraction in the slide, balsam, and cover, the angle of total reflection for the mean refrangible ray, will vary from 40° to 45° from the perpendicular—at any rate it will never exceed the latter degree; consequently for this reason I consider the right-angle prism the most convenient for most purposes, as the rays may be passed perpendicularly through its surfaces without any trouble arising from refraction.

The mode of action illustrated by the diagram is simply as follows: the rays from the luminous source are first collected and converged by the large bull's-eye lens *e*, and then further condensed and directed upwards by the Amici prism *d*; they next enter the surface of the right angle prism *c*, and pass directly onwards till they reach the upper side of the thin cover, from whence they are totally reflected down again, forming a brilliant surface of light, which will of course illuminate any small bodies immersed in the balsam just below. If the cover is clean and free from scratches, not the smallest portion of light from the luminous source will find its way through. The view of the objective will be unimpeded, and the field perfectly black. Another way of causing the light to enter the prism, is by means of a parabolic condenser, adjusted as under ordinary circumstances; the light will in this case enter the two faces of the prism at the same time, which is some advantage; it must be sufficiently small to have some

play in the cavity at the apex of the paraboloid; if the right-angled faces are one quarter of an inch square, it will perform very well. The objection to the plan just described is the necessity of having a separate prism for every object, which, though of advantage in some remarkable and peculiar cases, is not necessary for all. Fig. 2 is more universal in its applications; *aa* is a thin plate of brass, *b*, a right angle prism let in exactly flush with the upper surface; any small objects

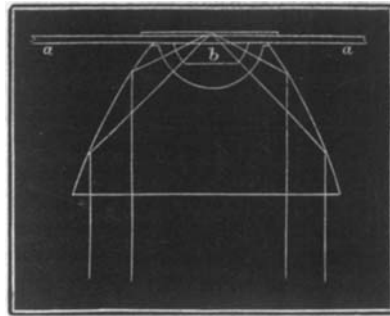
Fig. 2.



such as animalcules, *Diatomaceæ*, pollen, &c., must be laid upon the prism with water, and covered with thin glass; total reflection will then occur from the uppermost surface, in the same way as in fig. 1, and illuminate the objects in the fluid. Any ordinary plane slide containing objects mounted in balsam may be placed upon the plate and prism, first interposing a drop of water. It is almost unnecessary to remark that if this, or some other fluid is not interposed, the rays will all be reflected from the back of the prism itself, instead of passing onwards into the slide.

Fig. 3 is another method; *aa* is a glass slide—under this is cemented with Canada balsam a lens, *b*, nearly hemispherical, with a segment removed so as to leave the thickness equal to about one-third the diameter of the sphere. The flat facet of the lens is blackened. The radius of curvature should be about two-tenths of an inch: the use of the blackened facet is to exclude all rays below the incident angle of total reflection. This lens is intended to be used in conjunction

Fig. 3.

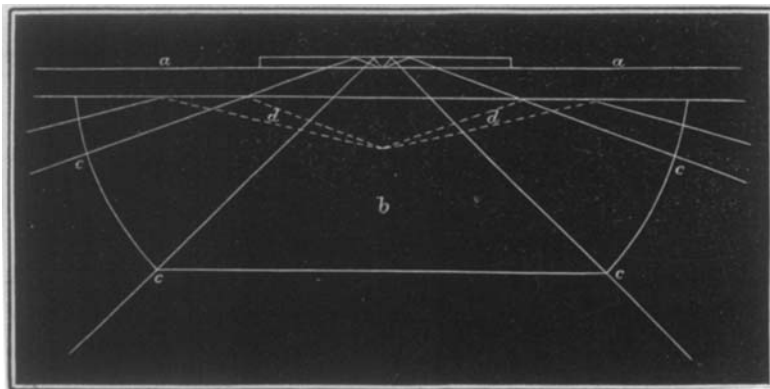


with the parabolic condenser, in the manner represented by the figure. The rays from the parabola pass through the surface of the lens in a radial direction without refraction, and proceed till they reach the upper surface of the thin glass cover, where they are totally reflected and converge upon the object; the

cover in this instance acts precisely the part of a Leiberkuhn, with the advantage of more perfect reflection.

A lens of this description may be let into a thin plate of brass as in fig. 2, and used in the same way as an aquatic holder, the parabolic condenser always being used for concentrating the light. When a slide containing balsam-mounted objects is placed above the lens, instead of using water, it is preferable to employ turpentine, or oil of cloves; the refractive index of the latter being nearly the same as crown glass. The reason for introducing this agent is because light impinging upon the polished plane between a greater and a less refractive medium, will always suffer total reflection at the surface of the former, at a given angle dependent upon the relative refrangibilities. If water is used, the angle of the illuminating pencil will be limited to about 160° ; above this, all rays will be reflected down again by the flat surface of the lens, and lost, as shown by fig. 4; *aa* represents the glass slide, with

Fig. 4.

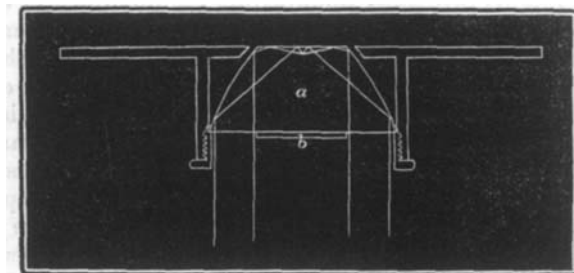


objects in balsam; *b* is a hemispherical lens placed underneath the slide, with water interposed; *cc*, rays which pass onwards to the top plane of the thin glass cover, to be reflected down again upon the object: the dotted lines, *dd*, are the portions of the illuminating pencil, that will be lost by being reflected from the flat surface of the *lens*—of course if a medium of nearly the same refractive power as the glass is used, such as oil of cloves, all this light will be transmitted and rendered available.

Another variation in this principle of illuminating opaque objects, is that illustrated by fig. 5: *a* is a small paraboloid of solid glass with a flat top. A black stop, *b*, of the same diameter as the apex, is fixed at the base of the parabola, for

the purpose of stopping out direct rays. This paraboloid is set in a ring, which is screwed underneath a flat brass plate,

Fig. 5.



so as to bring the upper plane surface of the glass exactly level with that of the plate in the manner shown by the figure. The parabola must be sufficiently short to prevent any rays from passing *within* the angle of total reflection relative to the flat top—or the paraboloid may be cut off at the point in the curve intersected by an angle of 45° drawn from the focus.

If a powerful series of parallel rays be sent into the base of this paraboloid, not any of the light will find its way through the upper flat surface. The whole will be reflected down again into the body of the glass. If now a piece of thin glass is placed on the top, with a drop of water, the greater portion of the illuminating pencil will be transmitted to the upper surface of the cover, and from thence totally reflected, illuminating any small objects contained in the fluid. Glass slides containing balsam objects may be placed on the apex of the paraboloid, using an intermedium of turpentine, camphine, or oil of cloves, in preference to water. This same reasoning also applies when small objects are viewed directly in fluid, by being laid on the flat top of the paraboloid, and covered with thin glass. When the nature of the substances will admit of it, for the purpose of obtaining greater intensity of illumination, they should be placed in turpentine or oil of cloves; in this case the whole of the light will be reflected from the top surface of the cover—no separate reflection taking place from the upper plane of the paraboloid, as with water. In using this instrument, all that is required is to throw direct light into the parabola, by means of the concave mirror.

Having now described some modifications of this principle of illuminating opaque objects, as most especially adapted for the highest powers, numerous experiments will justify me in saying a few words as to the effect. The light may be obtained of any required degree of intensity, and the field per-

fectly black, with objectives of the most extreme aperture; some *Diatomaceæ* mounted in balsam, are shown with a degree of beauty and delicacy, that I have never seen equalled, and from the lights brilliantly illuminating the prominences on the surface, many of them wear an entirely different appearance to the same objects seen as transparencies, and from the absence of all irregular refraction and colour, and the purity of the vision, the mind is impressed with the fact, that we are viewing them under their true features, as cellular structures, and in some instances displaying such a singular arrangement and configuration of markings, in cases where I had not even suspected them to exist, that I shall on a future occasion give some illustrations of them. It must not, however, be expected that all the *Diatomaceæ* can be seen by these methods, for some of them, when mounted in balsam, are so exceedingly translucent, that they will not hold a sufficient quantity of light, to be viewed as strictly *opaque* objects.

For this method of illumination, the greatest nicety is required in the adjustment of the object-glass, the slightest defect in this causing milkiness and indistinctness of vision—indeed so particular is the care required in this respect, that a different adjustment is sometimes necessary for various parts of the same object, in a case where it lies in an inclined position in the balsam.

With regard to the relative merits of the three methods that I have mentioned; for those who are already possessed of a parabolic condenser, the preference is most decidedly to be given to the hemispherical lens, fig. 4, set in a very thin plate of brass, but the truncated paraboloid, fig. 5, is by itself a most convenient piece of apparatus, readily applied and easily managed, forming a most useful adjunct to the other.

On the VEGETABLE CELL. By F. H. WENHAM.

(Read May 28th, 1856.)

IN the 'Annals of Natural History' for May, 1856, there is a notice, by Professor Henfrey, relating to my paper on 'Cell Development,' published in the 'Quarterly Journal of Microscopical Science' for Jan. 1856. I prefer making my reply through the medium of the same Journal, which is accessible to all whom the subject may concern.

The notice commences by saying:—"The essay contains internal evidence of the author's want of familiarity with the subject treated." It does, in all probability, contain irregularities and omissions which may possibly be excused in an inexperienced writer on these particular subjects. I pretend