

Letters to the Editor.

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Markings on Diatoms and Resolving Power of Microscopes.

AT various times there has been discussion as to the actual nature of the markings on the valves of diatoms—whether pits, projections, or perforations.

Any image formed by a microscope of objects the dimensions of which are not large compared to the

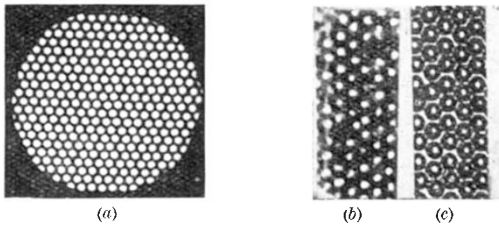


FIG. 1.—(a) Diatom (from New Zealand), diameter 0.004 inch, magnification 150; photograph taken with $\frac{1}{2}$ immersion objective; (b) part of a enlarged, magnification 900; (c) similar enlargement of a negative taken under the same conditions as a, but with the objective $\frac{1}{2}$ in. closer to the diatom.

wave-length of light, should be considered rather as phenomena the meaning of which has to be interpreted than mere magnified copies of the objects in the field. This is true even when the objects are quite thin and flat, but when the thickness is variable, the appearance of the image changes very rapidly as the focal plane is made to approach or recede from the uneven surface.

This is well illustrated in Fig. 1, a, b, and c; a is a photograph of a New Zealand diatom, magnification 150, taken with a $\frac{1}{2}$ immersion objective. The diameter of the disc is 0.004 in. and the dots are separated by $\frac{1}{2000}$ in.; b is an enlargement of part of a (magnification 900); c a similar enlargement from a

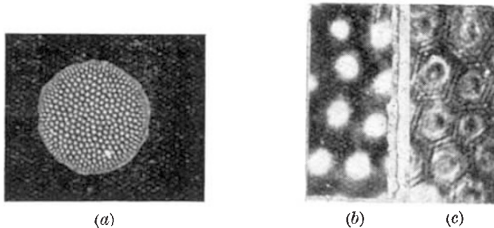


FIG. 2.—(a) Photograph of a piece of perforated zinc; (b) photograph from an enlarged copy of a on a thick bichromate film of gelatine, the focal plane of the enlarging lens being about $\frac{1}{4}$ in. from the summit of the gelatine bosses; (c) similar enlargement of gelatine print, the focal plane of the enlarging lens being coincident with the floor of the depression between the bosses.

negative taken in the same condition as a, but with the focal plane $\frac{1}{2000}$ in. closer to the slide. In Fig. 1, b, the dots appear as bright circular patches, while in Fig. 1, c, the dots are dark and are separated by well-marked hexagonal boundaries.

These appearances can be explained if the surface of the valve is supported or covered with convex bosses separated by troughs with somewhat rounded cross sections. The bosses act as condensers, and when the focal plane of the objective approaches that of the bosses, the latter show bright images of the source of light.

Of course, no very good image can be produced by a lens the diameter of which is only two or three wave-lengths, but the bosses do produce some con-

centration of the rays passing through them, and the size of the bright patch focused by the microscope varies with the distance between the focal plane of the objective and the approximate focus of the bosses.

In Fig. 1, c, the focal plane of the microscope coincides with the floor of the valleys separating the bosses, and what is photographed is the virtual image formed by the concave trough with some of the dependent diffraction bands.

For the sake of comparison, a surface similar to that assumed for the diatom valve, but on a large scale, was prepared by photographing a piece of perforated zinc (Fig. 2, a) and printing this on a thick film of bichromated gelatine. The bichromate was removed by soaking in cold water, and the plate was then placed for some time in a fairly strong solution of glycerine, and allowed to drain. In this way the unaltered swollen gelatine remained as convex bosses, which, though rather flat on top, yielded results very similar to that obtained from the diatom. Photographs corresponding to b and c of Fig. 1 are given in b and c of Fig. 2. The marking on such diatoms as *P. Angulatum* or *A. pellucida* differ probably from the coarser form only in scale.

I remember seeing at one of the Royal Society's soirées a photograph of *A. pellucida* taken by ultra-violet light with quartz lenses, which might very well have been supposed to represent a piece of perforated zinc. In 1879, Messrs. Powell and Lealand showed me *A. pellucida* under a $\frac{1}{2}$ immersion lens, and here the valve appeared covered with parallel lines so well defined that it seemed that several more lines might have been inserted between them. A small alteration of adjustment, however, changed the direction of the apparent lines, and in one condition the two series were both visible, their intersection suggesting dots.

The late Lord Rayleigh was, I believe, the first to point out that the resolving power of a lens (which may be defined as the least distance which must separate two objects if their images are also to appear separated) depends on the difference of the optical length of the rays from the objects to their respective images. If this difference does not exceed a quarter of a wave-length at least, there will be no real separation; and for complete separation a difference of not less than a half wave-length is requisite. This statement applies to all optical instruments, but the appearances in the field depend in a great measure on the illumination; whether, for example, the objects are self-luminous like stars, or obtain the light by which they are seen from a common source, in which case there is a phasic relation between the waves in each ray.

Microscopists seem to have an exaggerated idea of the resolving power of their lenses. With an object consisting of alternate opaque and transparent lines on a plane film very thin compared with the wave-length,¹ no objective (no matter what its numerous apertures might be, or what form of illumination was employed) could separate the lines if their spacing was much less than a whole wave-length. In *A. pellucida* the spacing is about half a wave-length, and the fact that their resolution is readily effected in ordinary light shows that the surface of the valve is uneven. A difference of elevation of a two-hundred-thousandth of an inch between the hills and valleys would allow ridges or dots to be distinguished, while if the surface were plane they would be quite unrecognisable.

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¹ The only test plates in which, so far as I know, these conditions are approached, are those which I ruled on films of methyl violet. The film in question varies from a tenth to a thirtieth of a wave-length in thickness.