# VIII.—A COMPARISON ULTRA-MICROSCOPE.

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Eight Text-Figures.

Microscopic objects may be divided into two classes, those that are seen by transmitted light and those that can be made self-luminous. The former are seen as the result of partial or of selective light absorption—that is, they may be semi-opaque, the elements of structure absorbing more or less light, or they may be seen in colour as the result of selective absorption. For an object to be self-luminous it must be illuminated in such a manner that no direct light reaches the micro-objective, but only that which is refracted or scattered by the object itself. Under these conditions an object is seen as a bright image on a dark background, a condition of affairs analogous to the observation of stars in the sky at night. The main purpose in all microscopical observations is to obtain the greatest resolution, and the term "limits of resolution" is applied to that state of affairs in which no further separation of elements of structure can be obtained. Only late in the last century was it definitely recognized that there are theoretical limits beyond which the microscope as ordinarily employed cannot go. If two points in an object are within a certain limiting distance of one another they cannot be separated by any known means. Further, if an isolated element of structure is smaller than a certain limit the image seen does not represent its exact form or size. Magnification as such does not help as, however much the image may be enlarged, there is no resulting increase of resolution. The main factors governing resolution are the numerical aperture of the objective used and the wave-length of the light employed. The best results are therefore to be expected when numerical aperture (N.A.) is large and the light used of the shortest wave-length practicable. While this power of delineating fine detail is of primary importance in all observations, another factor comes in with self-luminous objects which is hardly less so, and that is visibility. The term "ultra-microscope" is often inaccurately applied to all illuminating appliances that render an object self-luminous, but it should in fact only be used to describe apparatus which renders visible particles smaller in all dimensions than the resolution

limits. In actual practice this limit is reached with isolated objects or elements of structure that are less than 0·2 micron in diameter. When objects larger in any dimension than this limit are being so observed the method may be described as "dark-ground" illumination, whereas when the objects are smaller "ultra-microscopy" is the correct term. Neither dark-ground illumination nor the ultra-microscope can be regarded as a means of increasing resolution; their sole purpose is to obtain greater visibility. In theory there is no limit to the smallness of an object that can be made visible if a sufficiently powerful illuminant is used. In practice, however, the limits are governed by the following conditions:—

(1) That the particles under observation can themselves scatter enough light to enable them to be seen.

(2) That the particles are separated from one another by intervals that are within the limits of resolution of the microscope objective used.

(3) That there is sufficient difference of refractive index between the particles and the medium in which they are immersed. (For reasons that cannot be set out here two elements of structure that are as close together as 0·17 to 0·18 $\mu$ can be resolved by transmitted light, although a row of such elements would not be so resolved unless they were at least 0·2 $\mu$ apart.) The diffraction image obtained of an ultra-microscopic object is a small disc of light the intensity of which depends on the factors already enumerated. The visual image bears no definite relationship to the form or size of the particle; it is merely an indication that some such particle is there. Particles very small in relation to the wave-length of light used can be seen; in fact Lord Rayleigh regards the blue colour of the sky as due to diffraction by molecules of air.

The elementary principles of ultra-microscopic vision were appreciated by Faraday and by Tyndall. Faraday satisfied himself that the colour of ruby glass was due to minute inclusions of metallic gold by passing a narrow beam of sunlight through a piece of such glass. He observed the effect of scattering and suggested the cause, but his microscopic methods were not sufficiently good to enable him to see the individual particles. The apparatus designed by Siedentopf and Zsigmondy depended on the principle of illuminating an object at right angles to the direction of observation. The elementary principle is indicated by fig. 1, in which A is the source of light, D a lens throwing an image of illuminant on to the object B, C the observing microscope. The object B can therefore only be seen by the microscope C when it is rendered self-luminous. The apparatus, although introduced nearly twenty years ago, still remains practically in its original form, except that some improvements have been introduced in the

containers for holding fluid under observation. It is shown in section in fig. 2, and consists of the following parts :—

A. Source of light, preferably either the electric arc or sunlight. If the former, the arc should be of the type illustrated, with

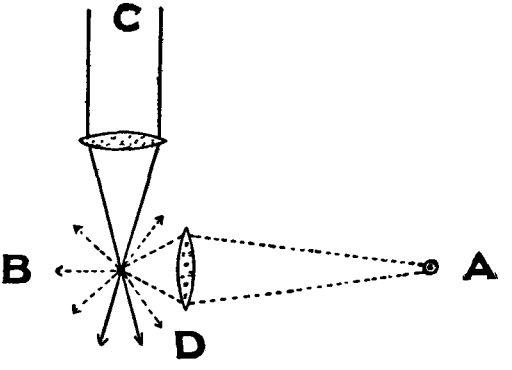


FIG. 1.

the positive carbon horizontal to ensure accuracy and constancy of alignment.

B. A projection lens of 80 mm. focus, chromatically and

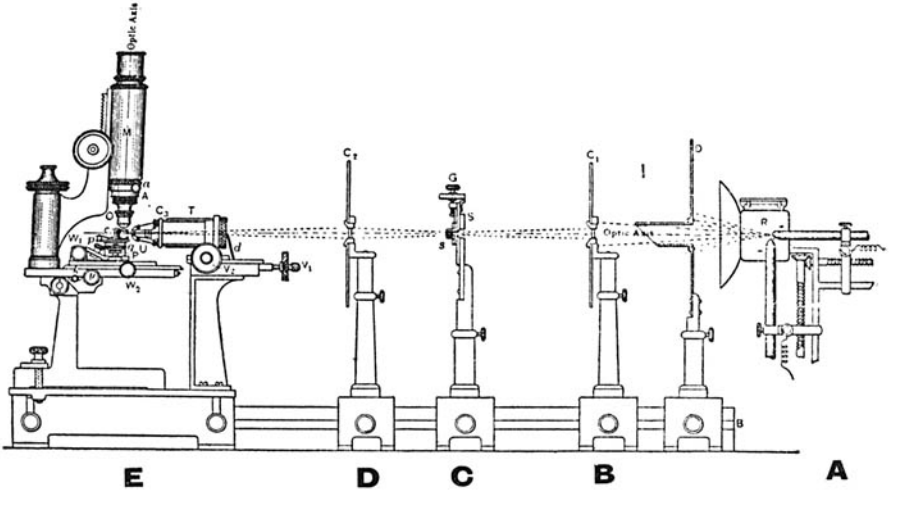


FIG. 2.

spherically corrected. Such a lens is essential, as the light has to subsequently pass through a narrow slit.

C. A precision slit (fig. 3), with adjustments for accurately

altering both its width and length. The slit is so placed that an image of the radiant A is projected on to it by the lens B. The entire slit head can be rotated through 90°, so that both length and breadth of the field of view can be seen in the microscope and suitably controlled. By this means the exact volume illuminated can be accurately determined.

D. A corrected lens similar to B, but of 55 mm. focal length, projecting an image of the slit into the image plane of the illuminating objective on the microscope E.

E. The observing microscope, with an objective placed at right angles to the direction of observation to project an image of the

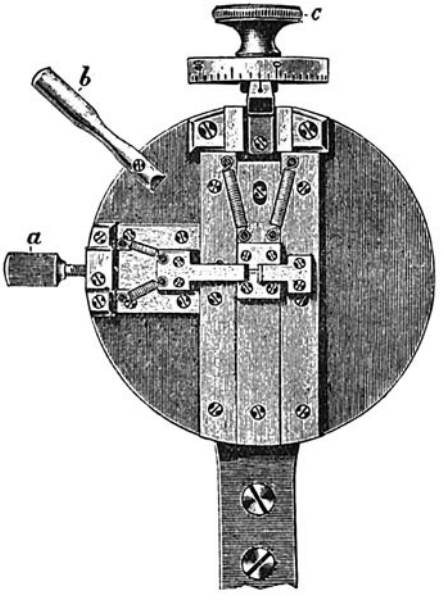


FIG. 3.

slit into the object suitably arranged on the stage. The stage must be provided with a vertical adjustment to enable the centre of the field of view to be brought to the axis of the illuminating objective. This objective must be supported so that it can be adjusted laterally, and also in the direction of the illuminating beam.

These are the essential points in the apparatus, and are substantially the same as described by Siedentopf in his original communication to this Society (Journal R.M.S., 1903, p. 573). It is sometimes a great advantage to be able to observe two preparations at the same time, particularly when changes which

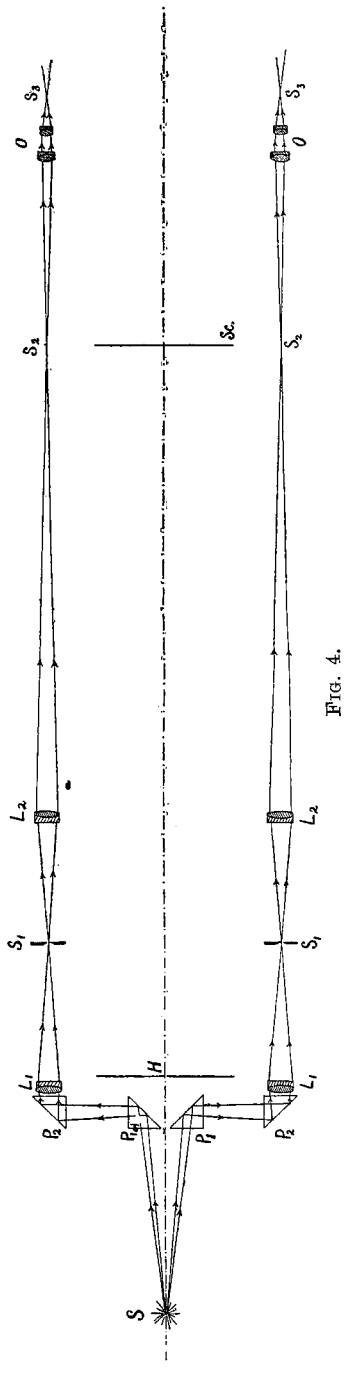


FIG. 4.

take place slowly are expected to appear in one of them. Such an apparatus was designed and made at the National Institute for Medical Research to determine the alterations in the colloidal contents of blood serum when exposed to particular wave-lengths in the ultra-violet region of the spectrum.

With such an apparatus it is possible to observe the unaltered serum as a control, and in a specially designed chamber, to be described, to watch the effect of any ultra-violet radiation. It might appear that the provision of two ultra-microscopes, side by side, and using two similar light sources would meet the case; but this is not so. The accuracy of any estimation of relative size of particles depends on a constant light source, visibility, as already explained, being mainly dependent on light intensity. It therefore became evident that the two systems must have one common illuminant, so that any variation would be equal on both sides. This has been accomplished by the method shown in fig. 4. S is the source of light; $P_1$ $P_1$ two right-angled totally reflecting prisms placed as shown with a small space between them; $P_2$ $P_2$ are two similar prisms placed to deflect the split beams on each side in the direction of the axes of the two ultra-microscope systems. $L_1$ $L_2$ are the lenses already referred to in fig. 2, with the adjustable slit (fig. 3) $S_1$ $S_1$ between. The image of the slit is therefore ultimately seen in the field of view of each microscope in the manner described earlier in this paper. The only additional point is the provision at the point H of a pin-hole in a black metal plate which projects an image of the arc on a translucent screen

S–*c*. This screen is provided with reference lines, so that the observer can, while looking into either microscope, become at once aware of any change in position or condition of the arc light. Further, in setting up the whole apparatus, this provides a most valuable common standard of reference, without which it would be difficult to obtain accuracy of alignment in both systems. The first step is therefore to get the light source, pin-hole and screen centre in accurate alignment equidistant from the centre of the reflecting faces of prisms $P_2$ $P_2$. The latter are adjustable on

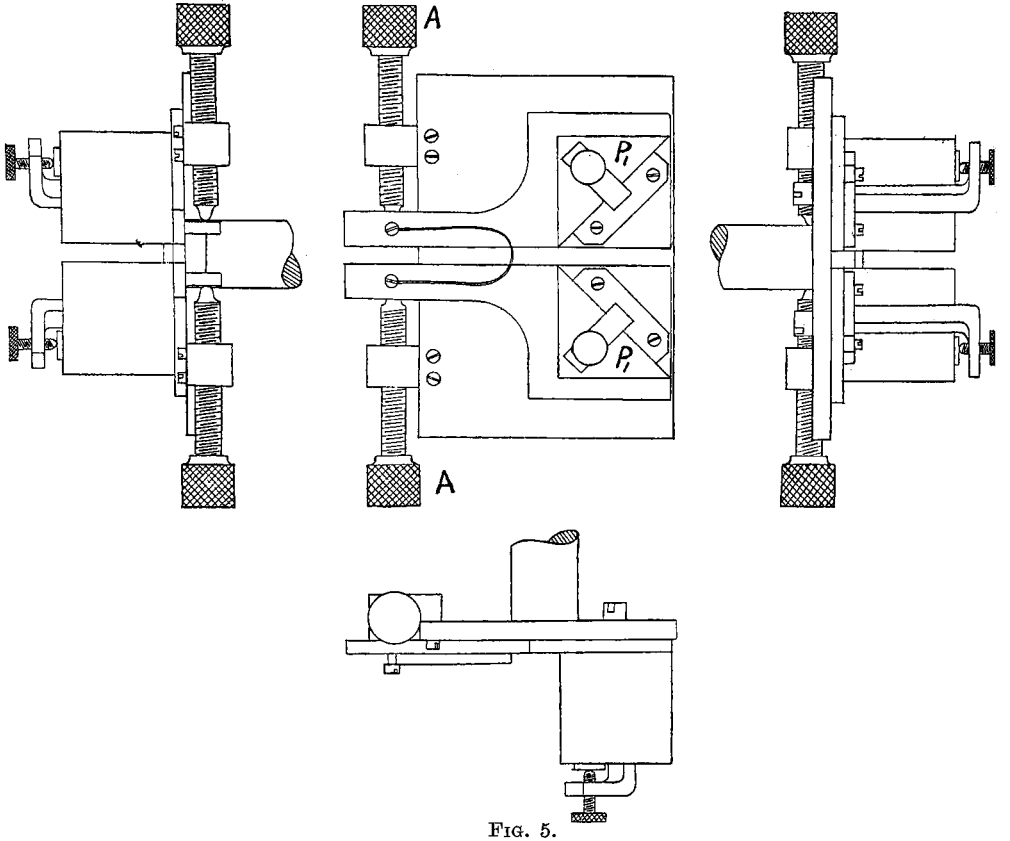


FIG. 5.

vertical axes passing through each reflecting face (see fig. 5), the adjustment being controlled by the screws A in the manner indicated by this figure. This adjustment must be of some delicacy, the screws being of fine pitch and well fitted. Once these adjustments are made no further attention should be necessary, any alteration in the light being corrected entirely by the pin-hole image. This method has enabled the whole apparatus to be kept in perfect adjustment for any period, and provides means whereby the light can be

restored to its central position even after the renewal of carbons. To provide means of easily observing small quantities of fluid a new form of chamber has been devised which is convenient to handle and can easily be cleaned.

It is seen in plan, section and side elevation in fig. 6, A, B and C. The small containing chambers, four in number, are seen at S. The supporting base plate P is of 1/8 in. brass 3 in. by 1 in. in size, the same as an ordinary microscopic slide, so that it will go on any microscope stage. On this brass slide another brass strip K is mounted which carries the glass slips forming the sides of the chambers. A narrow strip of brass $K_1$ of the same dimensions is placed over the glass strips, and is screwed in the positions

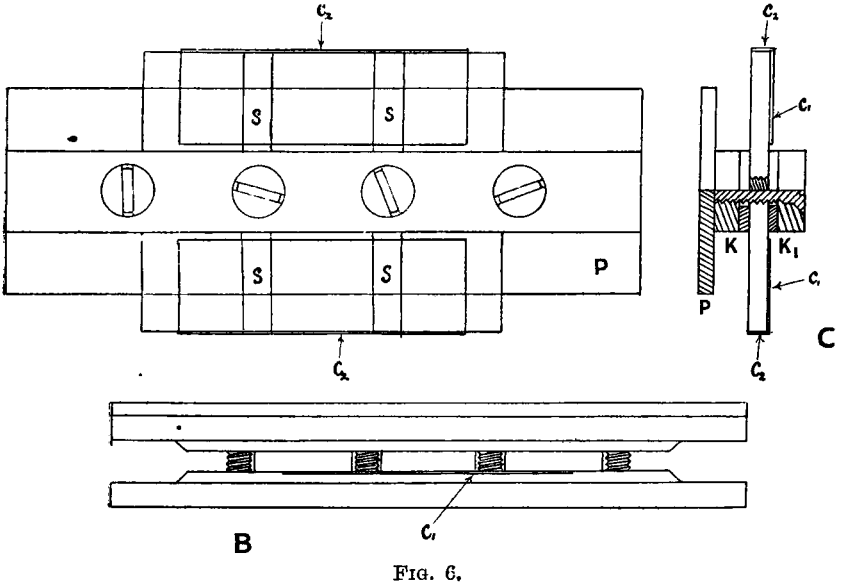


FIG. 6.

shown through to the brass base plate. On the end of the glass strips thin cover glasses $C_1$ $C_2$ are cemented with gold size, so that the thicker glass strips and the cover glasses on the top surface and front edge form chambers which are open on the under side. The surface $C_1$ is uppermost when the container is in action, and it is therefore easy to put a drop of any fluid to be examined in the angle formed between the cover glasses $C_1$ $C_2$. The fluid is kept there by capillarity in whatever position the slide may be. The direction of observation is at right angles to the surface $C_1$, illumination being at right angles to the surface $C_2$. The position of the fluid is seen in fig. 7, except that the liquid does not completely fill a tube in the manner there shown in section, but is held in the upper right-hand corner by capillarity. The particular

form of cell used when observations are being made to ascertain the effect of invisible radiations is seen in fig. 8. It is the same in general construction as that already described except that it is built up only of glass and quartz. It is seen in section in A, the

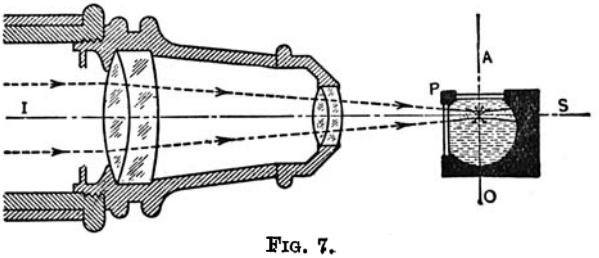


FIG. 7.

top cover glass $C_1$ allowing of the observation of the fluid as shown, while cover glass $C_2$ admits the illuminating beam. In plan B the sides of the cell A are of quartz, so that any irradiating beam, such as ultra-violet light, may be thrown in from either

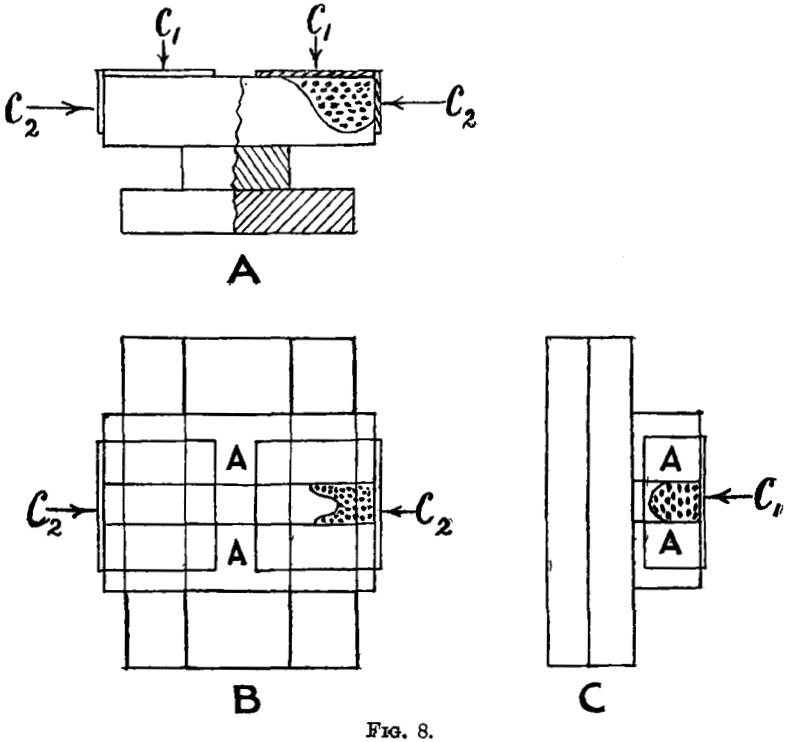


FIG. 8.

side without interfering with either illumination or observation. It is also seen in side elevation C, any further description not being needed. It is hoped to publish some of the results obtained with this apparatus in due course.