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IX.—*On the Rendering Visible of Ultra-Microscopic Particles and of Ultra-Microscopic Bacteria.*

By H. SIEDENTOPF, Ph.D.

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THE theoretical discussions concerning the capabilities of the Microscope have, following the lines of Abbe and Helmholtz, in the main related to the resolving power of objectives, having established as a limit that structural elements up to a fineness of a quarter μ (μ = a thousandth of a millimetre) can be resolved. This question of the resolution of structure is for most microscopic research of material importance. It is the aim in microscopy, not only to determine that there *is* a structure in an object under investigation, but above all it is desirable to know what that structure is like. Resolution of structures more minute than those indicated above has not been possible because the light is diffracted by the elements of which the structure is composed.

But the question of the resolvability of a structure is not the only one that can be applied to microscopic observations. There may be cases in which we may have to be satisfied with the simple evidence of the existence of a structure, just as in astronomical research we do not confine ourselves to the observation of the details of the planets, but also seek to render clearly visible very faint, or ordinarily invisible, fixed stars.

Now gold ruby glasses may be said to represent for microscopic research that which the heavens with the fixed stars do for astronomical investigations. These glasses appear perfectly clear and homogeneous to the naked eye, and when tested by the usual microscopic methods show no trace of turbidity. Gold ruby glass is not the only object suitable for these investigations, but all

turbid or colloidal solutions, fixed or fluid, are similarly suitable, provided that the average distance of the single particles is no smaller than half a wave-length.

Let us suppose that the dimension in every direction of these small particles is less than half a wave-length. In that case it is clear that their microscopical images will only be diffraction discs. Now such, for simplicity's sake, will be called "ultra-microscopic" particles; for the expression will at the same time indicate that the resolution of detail in the structure of these particles lies beyond the resolving power of the Microscope.

It might be suggested that mere evidence afforded by such diffraction discs does not sufficiently differentiate the respective particles, and therefore such investigations as the present ones might be treated as superfluous. But I believe that the experiments with gold ruby glasses which I was able to make, at the instigation of, and together with Dr. Zsigmondy, have afforded an optical proof that distribution of gold in these glasses is discontinuous, and have also demonstrated that there are a number of phenomena characteristic of diffraction discs such as colour, order of position, condition of polarisation and brightness, and in fluids also kinds of movement. So many properties seem to warrant a careful diagnosis.

Now, microscopic investigations relating to ultra-microscopic particles cannot be effected by the usual methods. The coloured ruby glasses, in which the distribution of the various particles was demonstrated, showed no sign of their existence when examined in the ordinary way, or even when examined by dark-ground illumination of the usual kind. Under such conditions the glasses appeared perfectly homogeneous. One might almost have expected that these gold ruby glasses in thin slices would have given some indication of heterogeneity, because they might be supposed to be somewhat analogous to stained bacterial preparations.

It was therefore necessary to devise a new method which would permit these small particles to become visible by direct observation as far as possible. The main feature of this method depends upon the regulation and arrangement of the illumination, which, as will be observed, differs materially from that hitherto employed. As in general particles to be optically imaged are not self-luminous in themselves (or where they might be slightly self-luminous the light would be so weak as not to be of any service), we are from the outset compelled to rely upon an artificial light-source of great specific intensity, such as the electric arc or brilliant sunlight.

When this is made to impinge upon the particles they become visible by the cone of rays which they diffract. But the intensity of the illuminating rays is naturally very much higher than that of the rays diffracted by the particles. In order to make smaller

particles visible, therefore, by this diffracted light the illumination must be arranged in such a way that none of the illuminating rays are permitted directly to enter the eye — in other words, all light, except that which is diffracted by the little particles, must be scrupulously excluded. Ordinary dark-ground illumination would seem to be suitable for this purpose; but it is important to note that with the usual dark-ground arrangements, when used with arc or sunlight, innumerable reflections occur at the several lens surfaces of the condensers, and there are besides many inconvenient reflections in the preparation itself (as will be explained more fully further on), so that this kind of illumination will not be suitable for the purpose in question.

If, however, matters are arranged in such a way that the axis of the illuminating cone is at right angles to the axis of the cone diffracted upwards into the Microscope, and if the cones are of such a dimension that no part of the one overlies any part of the other, then all reflections in the condenser are made harmless, and no stray light can now enter the objective. This method is therefore a further evolution of the so-called dark-ground illumination, and permits us to use the brightest sources of light.

Another illustration may be mentioned to make this clear. It is well known that small particles of dust floating in the air become visible as soon as a beam of sunlight is allowed to enter through a hole in a dark room, provided the observer's eye be approximately at right angles to the beam.

If now the illumination over a small area is increased by focussing a sunbeam by means of a condenser, and if the particles in this area are observed by a Microscope, then we have the principle of this simple method.

Optical images of ultra-microscopic particles are polarised diffraction discs, in other respects they are subject to the same condition as images of stars in telescopes.

It is not difficult to explain why this device enables particles in gold ruby glasses to become visible, while ordinary methods do not. Let me remind you that a high power objective only reproduces a sharp image of an exceedingly thin layer of an object. Now, with ordinary methods of illumination a great number of layers above and below a focussed layer receive light, and numberless particles lying in all these layers diffract light up into the objective. As these particles are out of focus they appear in the image-plane as discs of diffused light. As these diffusion discs overlap one another in the image plane they form a veil of light sufficiently powerful to completely eclipse the small diffraction discs representing the particles in the layers actually in focus.

It is therefore of vital importance to illuminate only those *particles which are to be made visible*, and the method of doing this is by focussing the arc light upon a small spectroscopic slit, the

light from this slit being focussed by a condenser upon those particles which are to be made visible. The size of the slit can be precisely controlled, and, with a knowledge of its width and of the condensing system employed, the exact thickness of the layer of illuminated particles can be regulated to a nicety. It will be found convenient to adjust the thickness of this illuminated layer to about 1 or 3 μ, so that it may correspond with the depth of focus of the objective.

We will now examine the limit of the smallest size of particles which it is possible to render visible by this method. The following considerations will help us to solve this question, at least approximately. It is known that radiation from a surface depends on three main factors—first, on the specific intensity of radiation; secondly, on the area of the radiating surface; thirdly, on the solid angle at which the radiation is emitted from the surface. This amount of energy can be expressed in terms of candle-power, and the limit of sensitiveness of the human eye for light is also known. From these two quantities, namely, the limit of least sensitiveness of the eye, and the limit of the greatest radiation which can be obtained by diffraction from the particles, we are in a position to determine the limit for the smallest dimensions which can be made directly visible. Within the scope of practical experiments this limit approximately works out at forty square millionths of a millimetre, which therefore corresponds to a circle of a radius of about $\frac{4}{1000000}$ mm.* It is of particular interest to note that the result of these practical observations appears to approach very nearly to the theoretical limit of visibility of the minutest particles.

Now, it may be taken for granted that with no artificial illumination, however intense, will it be possible to discern with the human eye dimensions so small as those attributed to medium sized molecules (about $0 \cdot 6$ $\mu\mu$). Even if we were to succeed in making the molecules self-luminous by any conceivable process, the specific intensity of the luminosity would have to considerably exceed the power of the sun's rays, a feat decidedly improbable.

Permit me here to mention that I particularly wish to guard against any over-estimation of the capabilities of the methods exhibited to-night. In particular I would wish to repeat that the procedure in question does not give any optical solution of the *true shape and size* of the small particles. Whatever their form may be you will always obtain a small diffraction disc as the image. Only when an ultra-microscopic particle is so much enlarged that one of its dimensions exceeds half a wave-length (in other words when it in part passes out of what may be called

* H. Siedentopf und R. Zsigmondy, 'Über Sichtbarmachung und Grössenbestimmung ultramikroskopischer Teilchen mit besonderer Anwendung auf Goldrubingläser.' Ann. d. Physik, x. (1903) pp. 1-39. Diam. = ·008 μ.

the ultra-microscopic condition) can we differentiate it under the Microscope as a rod, a thread, or an elliptical disc.

Diffraction discs of various particles show according to their size and formation great differences in brightness and colour.

I may further mention that we were able to demonstrate small particles in gold, silver and copper because the refractive indices of these metals were essentially different from the medium in which they were imbedded. As regards the oxides of organic bodies, such as are contained in colloidal solutions of silicic acid (SiO_2), oxide of alumina (Al_2O_3), and albumen, our method is not as yet applicable, doubtless because the refractive indices of these bodies do not differ sufficiently from those of the medium in which they are contained.

No doubt the question will present itself to your minds, whether this method of illumination can be applied with advantage to the investigation of cellular tissues, &c. Up to the present, so far as time has permitted for experiment in this direction, I must own that the result is a negative one; this, however, by no means precludes the possibility of something being done in the future. But experiments with ultra-microscopic bacteria have been more promising, and although at present I cannot say for certain that such *ultra-microscopic bacteria* have actually been viewed, I think I may say that *my experiments point to the perfect feasibility of making them visible, so that bacteriologists may actually discover germs which have been suspected to exist.*

I will therefore give you a short description of the special device which I have designed for this purpose, and which, whilst differing in application from the method previously described, carries out the principle of dark-ground illumination in another manner.

Bacteria are made visible solely by the light they diffract: and they appear as luminous discs on a dark ground because the direct illuminating rays are stopped out.

In the arrangement for this purpose the axis of the illuminating cone of light, and that of the rays diffracted by the object, are in a straight line, and not at right angles to each other, as in the other methods. Preparations of bacteria can therefore be mounted in the usual way. The direct illuminating rays are stopped out by a method, suggested by Abbe, viz. by grinding flat and blackening a small central portion of the curved surface of the front lens of the objective. The portion ground away is exactly calculated to suit the aperture of the illuminating objective. The technical execution of this method requires very great precision, but special advantages are secured thereby. In the first place, reflections can no longer occur between the lenses; secondly, the tedious centring for dark-ground illumination is obviated; thirdly, a stop made like

this cannot be decentred; and lastly, the objective remains available also for observation in the ordinary way without dark-ground illumination; one may even say that it must give better images than it did before the central portion had been stopped out, because according to the laws of the diffraction theory, a diffraction disc produced by an annular opening of *suitable* dimensions is even somewhat smaller than that formed by the full aperture under similar conditions.

An alteration has been made in the mount of the condenser which enables a convenient and rapid change to be effected from an optical system of 1·4 N.A., as used for the usual illumination of bacteria (after Dr. Koch), to another optical system consisting of an objective specially corrected and stopped down to a small aperture by which a dark-ground illumination is obtained, and which allows sunlight or arc light to be directly employed.

On the table here I have a preparation of ordinary cholera bacillus shown by this method. It will be noticed how thick and pronounced the appearance of the exceedingly fine flagella has become. But it is not this to which I would draw your attention so much as to a number of bright discs representing something which lies in the same thin layer on which the objective is focussed, but which cannot or can scarcely be seen by ordinary methods of observation. Ultra-microscopic bacteria might be expected to look something like this, though of course, I do not intend to suggest that there are any in the preparation on view.

In conclusion I must point out that these investigations have been materially assisted by the liberal manner in which all the necessary means were placed at our disposal by the firm Carl Zeiss of Jena.