Microscopy with Ultra-violet Light.

By J. E. BARNARD.

THE microscope is now so widely used in all branches of science and in industry that it is not surprising to find an increasing demand for greater optical efficiency. It must be admitted that in comparatively few cases is the instrument used under such conditions as to secure the best possible result; but this is due to lack of appreciation of the principles involved, and will be remedied only by a wide educational effort. Even when the greatest optical efficiency is secured, the limitations are soon felt. The chief need is for increased resolution, that factor on which the delineation of minute structure depends. Advances of great value have been made in methods of rendering visible minute objects, but it must be clearly realised that, while this greater visibility can be secured, no information as to the form or structure of objects which are below the resolution limits is to be obtained by this means. Increased magnification is by some workers still regarded as desirable, but unless this is accompanied by proportionally increased resolution, the results are worse than useless, and can lead only to serious errors of interpretation.

Two factors mainly govern resolution—namely, the numerical aperture of the objective, and the mean wave-length of the illuminant. No increase of numerical aperture has been obtained since the classic researches of Abbe, resulting in the production of apochromatic objectives; and in the present state of knowledge there appears little likelihood of any substantial advance in this direction. By using light of short wave-length, a promising field of research is at once opened up. An increase of resolution is obtained even with visible light if the violet or blue end of the spectrum is utilised, but the increase is much more definite if ultra-violet light is used, although the image is no longer a visual one.

The computation of microscope objectives for use with ultra-violet light presents considerable difficulties, as only two substances sufficiently transparent to these radiations are available—quartz and fluorite. So long ago as 1860 Spencer in America used fluorite for this purpose, and at a much later date Boys in this country suggested the possibility of using fused quartz. In 1904 Kohler, of Jena, succeeded in computing objectives entirely of fused quartz, some earlier ones which were fluorite-quartz combinations being thereby superseded. Ultra-violet light, therefore, became available for microscopic work, but the practical difficulties in the use of the apparatus are so considerable, calling for almost more knowledge of physical than of microscopical methods, that it has been used by few.

The results obtained, particularly in biological work, are in many cases of great interest, as, in addition to the advantages already indicated, there is the further important point that organisms are dealt with and photographs obtained of them in the living state. The classic researches of Hartley showed that organic substances which are perfectly transparent to ordinary light have very definite absorption regions or bands in the ultra-violet, and that their absorption is, in many instances, so characteristic that it constitutes an accurate method of identification. To a considerable extent, this fact is of value when using ultra-violet in microscopy. Objects that show little or no structure by transmitted light are seen to be highly organised when examined by ultra-violet radiations, and the structure seen is in part dependent on the wave-length of the light used. Objects for examination by this method must be dealt with in the living state, or at least in such a manner that no change takes place in their constitution. It follows that none of the ordinary methods of mounting such things as micro-organisms, in which staining, hardening, fixing, drying, or heating is resorted to, can be employed. The method is, in fact, its own staining process, differentiation of structure depending on the difference of absorption in ultra-violet, and not on complex staining processes, which are in some cases causing appearances not associated with the organism itself. Further, apart from the alteration that may take place in the tissues themselves as the result of such processes, their employment in the method under consideration would render them opaque to the radiations used, and, therefore, useless for the purpose. The organisms or tissues are simply mounted in any suitable fluid, such as water, normal saline, Ringer's solution, etc., which is transparent to ultra-violet light and the photograph is taken at once. The result is an image that, whether it shows more or less than a stained preparation, is a representation of the object in the living state, and with greater resolution than can be obtained in the microscope by any other process.

Such a method is obviously one to be tried to its utmost whatever practical difficulties may be involved, and there is little doubt that in time it will be recognised as what it really is—the only great advance in microscopic technique for a generation. The apparatus as used by the present writer is in its essentials the same as that devised by Dr. Kohler (Fig. 1), although in many points of detail improvements have been devised. The quartz objectives are three in number, their equivalent focal lengths being 6 mm., 2·5 mm., and 1·7 mm., their effective numerical aperture being respectively 2·50, 1·7, and 0·7. It will at once be appreciated that in cases where the full aperture can be utilised the two higher powers are of much greater N.A. when used with light of 275 $\mu\mu$ wave-length than any objective available for use with ordinary light. These two are glycerine immersion combinations, the refractive index of the immersion fluid being 1·447. As these systems are not homogeneous, the cover glasses are optically worked fused quartz of uniform thickness.

The slides are also of fused quartz, fitted into a carrier of a special type, which ensures that the surface of the slide is a constant distance from the objective, a point that in practice is of considerable importance. The quartz oculars are five in number, and range from an initial magnification of 5 to 20, giving camera magnifications of from 200 to 3600 diameters. The latter is a good deal too high for satisfactory results with most objects—in fact, it is doubtful, on theoretical grounds, whether such a magnification is justified. The quartz sub-stage condenser is made with a duplex top, so that a combination is available for each objective to ensure that a suitable cone of illumination is used in each case. This is used as a glycerine immersion system with the two higher-power objectives, and as a dry system with the lowest one.

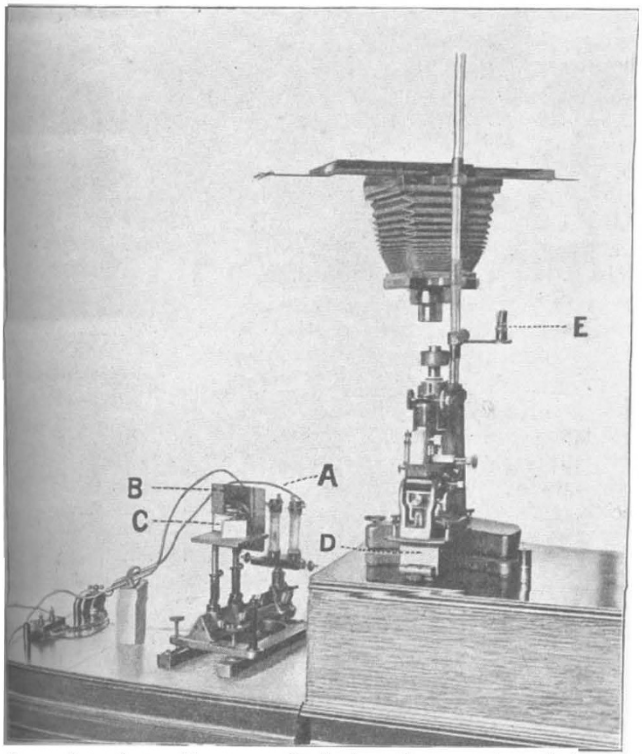

FIG. 1.—A, spark gap; B, quartz condensing lens; C, quartz prisms; D, box containing reflecting quartz prism; E, fluorescent ocular. The position of the other parts described will be evident to any microscopist.

The source of light is produced by a high-tension discharge in air between metal electrodes, usually cadmium or magnesium, although other metals may be used if they produce a suitable line spectrum. There are obvious limitations in this respect, as the character of the spectrum emitted must be such that the principal lines in the ultra-violet region are sufficiently separated and of considerable intrinsic brilliancy. The spectrum of iron, for instance, is excluded, as, although it is rich in bright lines, these are so numerous and therefore so close together that the isolation of one line is impossible under the conditions realised in this method.

The spark is produced by means of an induction coil of special design giving a heavy discharge of relatively low potential, the equivalent spark-gap being about 5 cm. This is further reduced by placing a condenser immersed in oil in parallel with the spark-gap. The interrupter may be either an electrolytic one or a mercury break, the latter appearing to be more satisfactory. Special arrangements are made for accurately adjusting both the length of the spark and its position in relation to the optic axis of the microscope. The image of the spark is projected by means of a quartz lens, so that, after passing through a pair of quartz prisms of opposite rotation, an image of the spark in one wave-length is obtained approximately at the position of the iris diaphragm below the sub-stage condenser. To facilitate adjustment, a disc of uranium glass is placed at the latter position so that an image of the spark can be observed and focussed as required, after which the uranium glass in its carrier is swung aside. The direction of the illuminating beam is at right angles to the optic axis of the microscope; it has, therefore, to be reflected by a right-angled quartz prism along the axis in the same way that the mirror operates in an ordinary microscope.

The preparation being placed on the stage, the light adjusted, and the condenser accurately focussed on the object, the actual focussing of the image has to be carried out. This is effected by means of a fluorescent searcher eye-piece which is mounted above the quartz ocular, and by the use of which an image is seen on a fluorescent screen and focussed by means of an auxiliary magnifier. This operation is one of considerable difficulty, and only after long practice can success be assured. Its difficulty varies, too, according to the wave-length used; in some cases the fluorescent image is bright, but in others it is much more difficult to see. Some objects themselves fluoresce, with the result that a sharp visual image cannot be obtained. The method now largely adopted by the writer is to observe the object by monochromatic light as emitted by a quartz mercury vapour lamp. This illuminant has bright lines in the violet, blue, green, and orange regions, and by means of suitable screens any one of these can be transmitted.

The image having been focussed visually in one of these lines, the fine adjustment of the microscope is moved by a predetermined amount so that the image is in focus for any desired wave-length in the ultra-violet. This method is quite practicable provided that the fine adjustment of the microscope is of sufficient accuracy (the searcher eye-piece is not used in this case except to confirm the accuracy of the process). The focussing having been performed, the searcher eye-piece is removed, the camera placed in position, and the exposure made. The image is projected for a certain distance, so that it is in focus at the plane of the plate with a known length of camera. The exposures required are as short as two seconds under favourable conditions, even at high magnifications.

FIG. 2.—*Saccharomyces Pastorianus* (yeast). × 1700. Illuminated by means of concentric dark-ground illuminator.

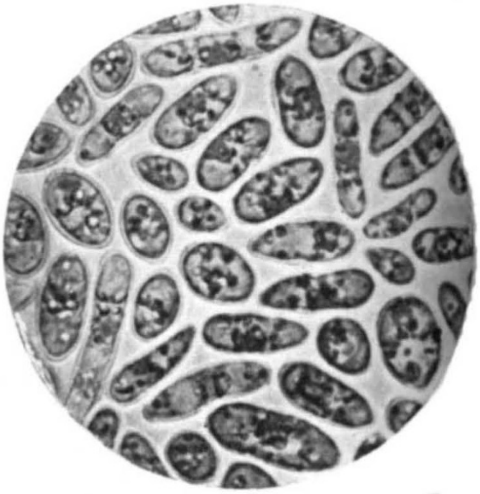

FIG. 3.—*Saccharomyces Pastorianus* (yeast). × 1700. Illuminated by ultra-violet light.

FIG. 4.—*Bacillus anthracis.* ×1500. Dark-ground illumination.

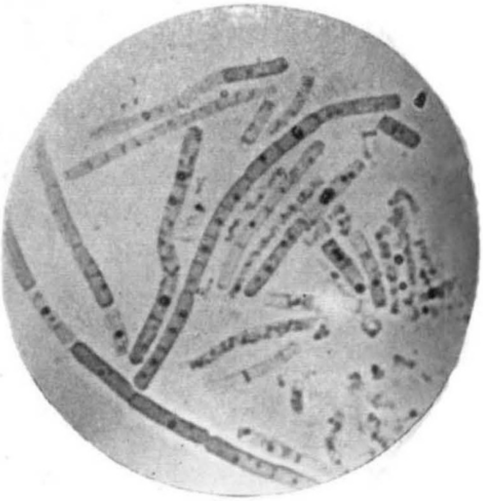

FIG. 5.—*Bacillus anthracis.* ×1700. Ultra-violet light.

There was considerable difficulty in obtaining a suitable photographic plate, as one was required of fine grain and with the smallest possible quantity of gelatine on its surface. Gelatine is itself opaque to ultra-violet light, so that the photographic action is confined to the surface of the gelatine, little or no penetration in depth taking place as with ordinary light. The result is that plates must be prepared with the smallest possible quantity of gelatine, but with the maximum quantity of sensitive silver salts that the gelatine can hold together. Such a plate has been prepared by the Kodak Co., and has proved satisfactory. Plates as prepared by Schumann for work in the far ultra-violet have also been experimented with, but for various reasons have not proved so satisfactory. The resulting negatives are at first glance somewhat disappointing if judged by ordinary photographic standards. They are always thin and lacking in violent contrasts, owing to the superficial action of the light, but the detail and fineness of lines due to the shorter wave-lengths used are evident to anyone having any knowledge of photomicrography. Whether the utmost resolution that theory demands can be achieved is at present unproved, not because the method has failed, but because of the difficulty of finding an object that can be regarded as a satisfactory test.

The accompanying illustrations give some idea of the comparative results obtained with living organisms. Figs. 2 and 4 are illuminated by a concentric dark-ground illuminator, the most satisfactory method available for observing living organisms by ordinary light, and Figs. 3 and 5 by a solid cone of ultra-violet light. The apparatus as figured is now in operation in the microscopical department at the National Institute for Medical Research.

Industrial Research Associations.

II.—BRITISH NON-FERROUS METALS RESEARCH ASSOCIATION.

By ERNEST A. SMITH.

THE Research Association for the non-ferrous metals industry had its inception during the recent conflict, when manufacturers, under the stern necessity of war demands, began to realise the need for fuller knowledge respecting the metals and alloys with which they had to deal. Preliminary meetings were held at the end of 1918 and during 1919, the attendance being well representative of the non-ferrous metals and allied industries, and included smelters, founders, metal rollers, tube manufacturers, wire drawers, and makers of every class of industrial alloys, including the precious metals. After some unforeseen delay the association was formally incorporated in January, 1920, with headquarters in Birmingham, the centre of the British non-ferrous metals industry.

The association seeks the membership not only of firms engaged solely in the non-ferrous metals industries, but also of firms which are substantial users or workers of non-ferrous metals and alloys, such as engineers, shipbuilders, railway companies, etc. It will be obvious that if there is to be effective development of research in this important national industry, the whole-hearted co-operation of every manufacturer and user of non-ferrous metals is essential.

Whilst the main object of the association is to carry on research, it also seeks to disseminate technical and scientific information relative to the production, treatment, manufacture, and uses of the non-ferrous metals and alloys. To this end a bureau of information has been started, thus supplying a long-felt need experienced by many manufacturers in this industry. Attempts are being made to make the bureau as comprehensive as possible, and already good work has been done.

With regard to the scientific aspects of the research work to be undertaken, no definite programme of research has yet been arranged, owing to the comparatively recent date of incorporation, but the council is dealing fully with the matter in the near future. Technical committees, representative of the various sections of the non-ferrous industry, have been appointed to review the field of research in each particular sphere and to report to a full council in due course.

It may be well, however, to indicate briefly in which directions intensive research appears to be most necessary for the future development of the industry. It is now generally recognised that, important and necessary as improvements in smelting and other processes of metal production undoubtedly are, the most marked technical advance in the immediate future may be expected from a more complete study of the properties of metals and alloys, as influenced by thermal or mechanical treatment, and by the presence of foreign matter. This being so, the first duty of the association will be to initiate researches into the fundamental principles which underlie the working of metals and alloys. Whilst it is true that the past few decades have seen considerable progress in non-ferrous metallurgical research, a careful review of this work reveals the fact that existing knowledge respecting non-ferrous metals and alloys is far less exact and complete than that which is available in the case of iron and steel. This lack of more extensive knowledge was brought home to manufacturers by the claims made upon the industry during the war period, and has helped to emphasise the importance of systematic research to provide that new knowledge without which an industry cannot make progress.

More exact data are required in connection with the physical constants of most of the industrial metals, and the vexed problem of the cause of hardening under mechanical treatment still requires elucidation; also problems concerning the quality of hardness and methods of testing such.

Apart from the conduct of research into the constitution and properties of metals and alloys, the most pressing problems that await solution appear to be those connected with the melting, casting, and working of metals in works practice. Each stage of manufacture, from the raw material to the finished product, presents its own individual problems. Realising the importance of starting with a sound ingot, the Brass and Copper Tube Association, in co-operation with the Research Department, initiated in 1918 a research into the production of sound brass and copper castings, under the direction of Prof. Thos. Turner. This research, which was established in a temporary laboratory in Birmingham, has now been handed over to the British Non-Ferrous Metals Research Association. As the result of two years' work, results of practical value have been obtained, and a report will be issued to members shortly. Attention has been directed mainly to the important question of the inclusion of gases, a subject which has been brought more into prominence since the introduction of gas and oil melting furnaces.

The laboratory experiments have been repeated