

THE PRESENT POSITION AND THE FUTURE OF THE MICROSCOPE—A GENERAL SURVEY.

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Mr. J. E. Barnard, President of the Royal Microscopical Society, then delivered an address, of which the following is a condensed report, in which he indicated future lines of development in microscope design and in microscopy.

On behalf of the Royal Microscopical Society, I trust I may be allowed to convey to Sir Robert Hadfield the expression of my great appreciation of the efforts he has made, resulting in the holding of this Symposium. The subject is one that is in need of discussion; but, had it not been for Sir Robert's scientific insight and energy, it is unquestionable that the meeting would never have taken place. As the time that is allotted to me is of necessity short, it will be impossible to give anything like a full survey of the subject of microscopy. I shall, therefore, be compelled to limit myself to such points as appear to me to be of interest, although I admit that I am not always selecting the ones of greatest importance.

An examination of the programme of this Symposium might lead to the conclusion that the subject of metallography was the most important branch of microscopical research. In point of fact this is not so. Although the importance of the subject is admitted, yet the amount of attention given to it is not anything like so great as that devoted to biological researches. It is therefore probably quite true that ninety per cent. of the microscopes in use at the present time, whether in this or any other country, are in the hands of those who are working at biological subjects. Even of this class, the science of medicine will absorb the greater portion; and it is therefore unfortunate that the medical side of the subject is treated so lightly—at least, if we may judge from the programme. It is, I am afraid, only in accordance with the accustomed attitude in medical circles for little interest to be taken in pure microscopy, although in diagnostic work the importance of the microscope has never assumed a larger place.

In view of the paucity of contributions on the biological side, I shall, therefore, direct more attention to this than I should otherwise have done, and the few remarks I make will be more particularly in relation to the microscope as used for biological research.

A consideration of the microscope resolves itself of necessity into two parts, the mechanical and the optical. From the mechanical standpoint there are two designs in general use—those referred to as the Continental and the English form of microscope. In the Continental type it has usually been customary to have what is known as the horseshoe foot, mainly, I imagine, because of its ease of construction by mechanical engineering methods; whereas the English design of microscope, which has hitherto been mainly made by hand, is of a more steady type, and the points of support are so distributed as to give more stability to the instrument in any position.

The essential parts of the instruments are a coarse adjustment, to give the body tube a quick motion in the direction of the optic axis, and a fine adjustment, which gives it a much slower motion in the same direction. The tube is adjustable in length, to enable correction to be made for varying thicknesses of cover glass, although a large number of workers appear to regard it as a ready method of obtaining greater or less magnification, with disastrous effects on the resulting image.

There is only one fixed part of a microscope which is used for biological purposes, and that is the stage. But metallographers require that the stage shall also be adjustable in the direction of the optic axis. The body tube itself should be made so that it can be closed to a length of 140 millimetres, including any objective changing device that may be on the nose-piece; and it should be possible to lengthen it to at least 200 millimetres or 250 millimetres if long-tube objectives are used.

All these adjustments are in the direction of the optic axis of the instrument. Two others are usually provided, which are at right angles to this direction—that is, a mechanical stage for actuating the object, and in certain of the best class instruments an arrangement for centering the sub-stage condenser to the axis of the objective. While there are many points which might be raised on the mechanical side, there are only one or two that I have time to mention. The main points about most microscopes appears to be that they are unstable. I have a considerable number in my own possession, but I do not think I have one, even now, which, if I centre an object on the stage with the instrument in a vertical position, still maintains its centration accurately if the instrument is put into the horizontal. The probability is, therefore, that there are few microscopes made at the present time that exactly fulfil the conditions necessary for high-class photomicrographic work, or for observational microscopic work of an exacting order. I trust, however, that an instrument exhibited at this Symposium will embody the necessary improvements to rectify this matter.

Some misapprehension appears to me to exist also as to the relative purpose of the coarse and the fine adjustments. The coarse adjustment should be sufficiently well made, and if the user is sufficiently expert, to enable him to bring into view any object, whether it is being observed with a low or a high power objective. The fine adjustment is then used for accurate focussing and for getting a conception of the object in depth. In biological work, at any rate, this is very rarely the state of affairs as carried out. In using

an oil immersion objective, for instance, a common method is to immerse the objective and then lower it so that it all but touches the top surface of the cover glass. The objective is then raised by means of the fine adjustment until the object comes into view. While this may act fairly well with very thin cover glasses, it is a haphazard method when cover glasses of varying thicknesses are used. It should be realised that when microscope users are sufficiently educated, they will be able to tell how far they are from the actual image by the appearance of the light in the field of view, that is, if the object is illuminated with reasonable accuracy.

Mechanical stages also appear to me to need some consideration. The stages which will on actuation cause no shift of the object other than in the direction intended, or any alteration of focus, are rare. Further, those in which the screws project over the side for a considerable distance with the result that any slight jar or knock causes them to be displaced, and, it may be, actually bent, are objectionable when used under laboratory conditions.

There is, I think, much to be said for the type of stage which has either co-axial milled heads on a vertical axis, or, if inconvenient to make, milled heads which are on separate axes. This method of construction, I think, of necessity results in a much stiffer and more stable stage. There is, in fact, a general lack of stability going through nearly all parts of a microscope. But it is significant that, even so long ago as the beginning of last century, the instrument as then designed had much greater attention paid to this point. The microscope, an illustration of which I show on the screen, is to my mind an embodiment of a principle that should receive attention. So soon as English makers are in a position to consider the production of an instrument of a special type, it is my intention to have one made. In this the general principle is that all the optical parts are carried on a bar which is, in effect, an optical bench, and that this is strutted in such a way as to give stiffness to the instrument as a whole. The only effort that I am aware of that has been made in this direction is in the microscope designed by Dr. Rosenhain, particularly for metallography, but which is adaptable for ordinary work. This instrument, to my mind, is such an improvement on any other type of stand that I am at a loss to understand why metallographers have not more generally taken it up. It might appear that I am exaggerating the importance of stability in the stand. But it should be realised that any want of centration in the optical parts, or want of alignment in the optic axes of these parts, results in more serious deterioration of the resulting microscopic image than any other single factor. The optical parts of a microscope are the objective, for obtaining the primary magnified image of the object; the ocular, for further enlarging that image and transmitting it to the eye; and the sub-stage condenser, for illuminating the object with a larger or smaller cone of light. The limitations of time will prevent me from doing more than refer very briefly to some properties of the optical parts.

It is generally assumed that magnification is the primary function of an objective. But in point of fact the main point is not magnification but resolution. By resolution is meant the power the

objective has of separating and forming correct images of fine detail. That known as the Abbe Diffraction Theory, is the theory on which modern optical calculations are based, and it is safe to say that it was never more fully accepted and never rested on a surer basis than at the present time. There has been much discussion in this country of that theory, and probably a good deal of misconception has arisen from its inapt designation; for the term "Diffraction Theory" is perhaps somewhat unfortunate. I cannot do better than quote the late Lord Rayleigh in reference to this matter. He said: "The special theory initiated by Professor Abbe is usually called the Diffraction Theory, a nomenclature against which it is necessary to protest. Whatever may be the view taken, any theory of resolving power of optical instruments must be a diffraction theory in a certain sense, so that the name is not distinctive. Diffraction is more naturally regarded as the obstacle to fine definition, and not, as with some exponents of Professor Abbe's theory, the machinery by which good definition is brought about."

This very clearly and accurately sums up the position. The Abbe theory tells us that there are two main factors determining resolution: that is, the numerical aperture of the objective used, and the wave-length of the light. Numerical aperture is determined for us by the optician, and it is well known that, with an oil-immersion objective, a numerical aperture of 1.4 is at the present time the practical limit. Metallographers are in a somewhat stronger position, as a mono-bromide of naphthaline immersion objective was, and presumably still is, made by Zeiss, which had a numerical aperture of 1.6. This represents the absolute limit at the present time, and there is no indication that numerical aperture will be increased in this sense by present methods.

The other factor governing resolution is the wave-length of light, and in this connection it must be borne in mind that to resolve a regularly marked structure, the distance between the markings must be more than half a wave-length. Under ordinary conditions of illumination we cannot go very far in the direction of increased resolution unless we have resort to an illuminant such as a mercury vapour lamp, which is rich in blue and violet radiations. There is much room for investigation in this direction, as the ideal illuminant for microscopic work has yet to be found. But I do not know of any one that approaches so nearly to it as the one I have mentioned—the mercury vapour lamp. It suffers only from one disadvantage that I can see, and that is that the differentiation due to staining is not so clearly brought out as when ordinary light is used. But as staining is itself an artificial process, and is simply done to differentiate structures, it only means a certain amount of education to enable us to appreciate the differences, even under the light from this lamp. The only stains which it does not show quite well, or, rather, in which the colour-tint is altered, are those in which red predominates. Any other colour is shown perfectly and in reasonable gradation. The advantages of this illuminant are that it is even and uniform. It has a fairly large area, and can be used therefore for any class of work. Its intensity can be varied within considerable limits by having a resistance in series, so that the current density is altered to suit the particular

work under observation. Further, it is possible, by interposing neutral screens, to vary the light intensity if the electrical method is inconvenient. Owing to its possessing practically no red radiations, its mean wave-length is shorter, and by using suitable screens light which is truly monochromatic, green, blue or violet, can be obtained at will. These lamps are made both in glass and quartz, but the quartz ones are preferable, because they admit of the use of heavier current, with greater luminosity; and further, they have a much longer life. I have exhibited two of these lamps, because I regard them as far in advance of any other form of light available to the microscopist at the present time, whether he is a biologist or a metallographer.

The whole subject of illumination, so far as the illuminant is concerned, needs investigation also, because there is, I think, little doubt that a modification in the intensity of the illumination of any particular object enables us to use a larger light cone than we could do under ordinary circumstances. That is, variation of the intensity is an alternative to the use of the iris diaphragm in the sub-stage of the microscope. But it is in the direction of using invisible radiations in the ultra-violet, or, it may be, radiations which are still shorter than the ultra-violet, that developments in microscopic work are, in my opinion, likely to occur.

There are two other points, which I can only refer to, but which, I trust, may be dealt with more fully in the succeeding papers. One is that, while the resolution limits are so inflexible, that does not by any means apply to mere visibility. By illuminating small particles by means of an annular cone of rays, that is, what is ordinarily known as dark ground illumination, or by illuminating them at right angles to the optic axis of the microscope—what is known as the ultra-microscopic method—particles of a very much smaller order of size can be made visible. But we cannot tell anything about their form, nor can we accurately tell their size. We are only conscious of their mere existence.

Another point to remember is that magnification is definitely limited to something like 750 diameters with microscopes under ordinary conditions, if we want to get the best optical effect. We may, as a matter of convenience, have still higher magnifications, because it is not given to everybody to appreciate fine detail unless an image is somewhat enlarged. But it must be appreciated that any increase beyond 750 or 800 diameters does not result in us seeing anything more. It simply allows us to see the object on a somewhat larger scale. We may therefore summarise as follows:—An object which is much smaller in size than the resolution limit can be rendered visible, providing the light with which it is illuminated is of sufficient intensity, and it is sufficiently different in refractivity from the medium in which it lies. To resolve a series of equi-distant points or lines in an object, their distance apart must exceed half a wave-length of light in the medium in which the object is immersed. Johnstone Stoney has shown that a pair of lines or objects can be separated when their distance apart is rather smaller than the resolution limit required for a number of points or lines in a row. But it should be borne in mind even here that the resolution limits apply if a definite standard of definition is required. An isolated object, or pair of objects, are not so well

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defined if they exceed the resolution limits as laid down for recurring structures. It cannot be too fully appreciated that illumination is the keynote of all sound microscopic work, and this applies whether the illumination is by means of visible radiation under ordinary conditions of work, or whether it is in experimental work in which the use of invisible radiations are concerned.

There is much room for research in this direction, and it is to be hoped that this is one of the points which will be seriously taken up. Apart from any question of research, the education of the user is perhaps of vital importance. It is little use for opticians to make great efforts to turn out a satisfactory instrument if the user is incapable of taking advantage of the quality of the optical or other parts. I trust, therefore, that this Symposium will give an impetus in this direction, and that it will help microscope users to realise how much remains to be done.