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Equipment for genitourinary medicine

The choice of microscopes for use in genitourinary medicine clinics

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Abstract

The current wide choice of microscopes and the use of the microscope as a diagnostic tool is discussed. The formation of images and the aberrations which occur with lenses and the ways in which these are corrected are described. The functions of eye pieces and illuminating systems are presented. The requirements for microscopes in every day use, research microscopes and portable microscopes are described, and suggestions made as to how these requirements may be fulfilled. Brief comments on the care and maintenance of microscopes are made, and a list of manufacturers and suppliers appended.

There is now such a dazzling array of microscopes available to microscopists that, except in the case of those who need very particular properties in the apparatus, choosing an instrument suitable for everyday diagnostic purposes has become increasingly difficult.

The conventional microscope has become instantly recognisable to practically everybody. This is largely due to its constant appearance in all forms of advertising and its use as a kind of symbol of scientific research, and to impart to the lay observer a feeling of academic high-mindedness, sometimes recognisably to the more knowledgeable in the most bizarre of settings and the most inappropriate circumstances. The fact of the matter is that quite apart from its more esoteric and romantic connotations, microscopy, in one form or another, plays a part in almost all manufacturing processes. Thus microscopes appear in many shapes and forms, according to their functions, the most important criterion being that the apparatus chosen should fulfil its function as a tool. As such it should be capable of performing accurately all of the tasks for which it is designed, it should be as simple as possible to use, within the bounds of such simplicity it should be versatile and capable of adaptation, it should be as easy as possible to maintain, and obviously should not be prohibitively expensive. Even with the wide choice now available, to find one instrument fulfilling all of these criteria is not easy.

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Forming an image

The microscope is an instrument for rendering fine detail visible. In so doing it will be necessary for the image to be magnified perhaps many times, but it is not the ability to make an instrument magnify that is technically difficult. The real problem lies in making things larger and at the same time keeping the image clear and distinct, and the magnified objects optically separated from those adjacent to them. These properties are governed by the resolution and resolving powers of an instrument, and these are dictated by the construction of the microscope, and in particular the lens systems and light source used.

Anything which is to be seen has to be intrinsically visible and must be large enough to be seen. These two essential requirements are easily confused, but they are quite distinct from one another. An object which is relatively large will remain invisible if it does not in some way interfere with the light which reaches the eye from it. Thus *Treponema pallidum* in an unstained preparation is invisible by ordinary brightfield microscopy because the organisms are so fine as to be virtually non-refractile. Tiny motes of dust, on the other hand, are quite visible as they move in a beam of sunlight in an otherwise dark room because they scatter light and the effect becomes visible. Special techniques such as dark-ground and phase-contrast microscopy can be used to enhance inherent very small changes in refractivity and phase to render such objects visible.

Intrinsic problems with lenses

It is consequently quite easy to render tiny refractile objects visible under the microscope, especially when they have been artificially stained, but when even the best optical microscopes reach a magnification of about 1000 to 1200 a state of "empty" magnification is reached. After this it is still possible to make objects appear larger but they become progressively indistinct. This loss of image clarity is due to two main problems with even the best of lenses. Because of the materials with which lenses are made, and because of the shape of the lenses two forms of "aberration" occur. Firstly, because lenses are thicker in the middle than at the edge greater refraction occurs in the centre and it becomes impossible to have the entire field in crisp focus at the same time. Similarly, because of the shape and construction of lenses there tends to be a prismatic effect in which white light is broken up to some degree or another into its component colours. In a less well-corrected microscope lens system this will result in observed objects in the field of view being surrounded by a coloured halo which makes resolution very difficult. These aberrations are corrected by making compound lenses composed of a number of smaller lenses differing in shape, and sometimes in the materials from which they are made using substances of different refractive indices.

The first corrected lenses were largely corrected only in the red-green areas of the spectrum and are called achromatic lenses or achromats. When a flat-field correction is also introduced they become plan-achromats. When further colour corrections are applied the lenses become apochromatic, and when further corrected to give a focused flat-field they become plan-apochromats. Plan-apochromatic lenses are the most highly corrected and consequently the most expensive.

Eye pieces

Microscope eye-pieces function basically to observe and further magnify the image projected into "space" by the objectives. The higher the magnification of the eye-pieces the more any aberrations occurring in the system are amplified and the worse the resolution of the image becomes. High magnification factors in eye-pieces are therefore seldom necessary, and in most cases are counter productive. In a few cases where the objectives are constructed of particular materials the eye-pieces will need to be specifically corrected. In the great majority of cases simple Huyghenian eye-pieces are quite sufficient and should by and large be of the lowest magnification required to give a distinct image. The limiting factor is the fact that the eye has an angular power of separation of 2′, which seen at a distance of 250 mm corresponds to 0·15 mm. It is therefore essential that the eye-piece enlarges the image given by the objective in such a way that two details of the object appear separated by at least this value.

Illumination systems

In my experience, it is most often in the sub-stage area of the microscope, that is to say the means by which the microscope object is illuminated and the condenser which properly focuses that illumination, that improper use and neglect occurs, and that this is probably the prime cause of sub-optimal images. With microscopes that still have a simple light-collecting mirror and Abbe condenser the achievement of "critical illumination" by the adjustment of these two components is essential. More modern microscopes usually have built in "Kohler" illumination, where the light source is focused onto a mirror or half-silvered prism and thence onto the condenser, and is contained within the construction of the instrument so that the whole is an integrated unit and can be moved from place to place in one piece. Improper use of the condenser usually occurs when the user wishes to decrease the amount of illumination falling upon the object. It is quite common for the condenser to be lowered out of focus to achieve this end which clearly produces a worse image. The light should be decreased by simply turning down the rheostat controlling the brightness of the light source, or by closing one of the diaphragms although this is not ideal. The purpose of the condenser is to focus light upon the object being inspected in such a way that the maximum amount of light is captured by the objective in use. In an ideal situation, therefore, the condenser needs to be adjustable for the focal length and numerical aperture of each objective. This may be achieved by almost infinite adjustment such as is incorporated in the excellent "pancreatic" condenser, but is usually provided by a simple turret system or rod or lever which simply switches in a lens which is superimposed on the condenser for high-power work. In my experience, a condenser which requires adjustment for each and every objective, desirable as this is to the purist microscopist, is usually a waste of time and money in the everyday situation since very few laboratory workers who use the microscope constantly will be bothered to adjust the condenser when required. It seems, therefore, that a condenser with just a switch-in lens for high power objectives is the best that can be done in these circumstances.

Choice of microscope

Having been through the components that dictate the quality of the image given by a particular instrument there appear to me to be three fairly distinct possible requirements in the Genitourinary Medicine Clinic. Firstly, there is the kind of instrument which is needed for diagnostic purposes, which will be in constant use every day. Secondly, there may well be a need for a "research" instrument of higher optical quality, and with alternative systems such as phase-contrast and fluorescence available, and with a good built-in photo-micrography system. Thirdly, there will be some who will need a system which can be used in "the field", especially in places where mains electricity is not available or is unreliable.

In terms of the every-day instrument, these will clearly be used from time to time by those who are not experienced microscopists, and indeed for teaching purposes. Again, in my experience, there has been for many years a feeling that such people require only poor quality apparatus giving poor quality images, and that the main criterion for their choice is that of price. In my view, the total opposite is true. The less experienced the observers the better the image required to enable them to recognise what they are seeing and to be able to do so again when they are on their own. Good quality apochromatic lenses with an easy to operate compensating condenser should be the minimum requirement for these instruments. Plan-apochromatic lenses, giving a flat-field, are clearly always desirable, but it would be difficult to sustain an argument that they are absolutely necessary in this case. They should always be purchased when affordable, however. Objectives of nominal magnifications × 10, × 40, and × 100 oil immersion should be provided. A built-in facility for dark-ground illumination, or a very simple exchange mechanism for a dark-ground condenser, is essential. The cardioid type of condenser, which is used dry, is preferable to the annular type which requires immersion oil, and can be messy and prone to sub-optimal adjustments especially in inexperienced hands. Kohler illumination provided by a bright light source such as a quartz-halogen lamp, is essential particularly when dark-ground illumination is to be used. When dark-ground is available the oil-immersion lens will need a built-in stop or an adjustable diaphragm capable of reducing its numerical aperture to less than 1·0. In general terms, the better the quality of optics, the simpler the method of operation, and the fewer the adjustable knobs and switches available for experimental "fiddling", the better and more recognisable the resultant images are likely to be.

In the case of a "research" quality microscope the specification should be somewhat more ambitious. Plan-apochromatic lenses are a minimum requirement for such an instrument. As many illumination systems as possible should be built-in, but in my opinion fluorescence is essential in a genitourinary clinic. There are constant developments in immunofluorescent techniques, and also fluorescent staining techniques, such as that used for trichomonas, appear regularly. Adaptability is important as the user may well wish at some stage to add teaching heads, a viewing screen, television monitoring, a projection head or similar embellishments. Such a microscope will also need a first class photographic system. In my view, the main requirement is that this should be as automated as possible, leaving as little scope for operator error as is achievable. Built-in filter options are also essential, especially if optimal results are to be obtained with the photographic system.

The third type of microscope is for use in daylight or using external light sources which are battery driven or possibly even using artificial light created by non-electric lamps. This requirement can be met by those firms which still manufacture microscopes with a sub-stage system containing a mirror which is adjustable for reflecting light through the condenser. Such a microscope can, in theory, be used wherever there is a light source bright enough to form an image through the lens system. Such microscopes, however, are usually manufactured only in the "student" class, and as a rule usually have fairly poor quality lenses as it is obviously not worth while fitting very expensive objectives to a system which has only a primitive light collecting mechanism. The advantage of such instruments clearly is that they are completely portable.

The secret of obtaining the best instrument for use in a given situation is to know what you want it to do. When you are unsure about this and about what is available advice is readily available from any of the major manufacturers. I think prospective purchasers need to be careful not to be persuaded to purchase instruments which have a specification way beyond that which is actually required in the work situation. It may well seem highly desirable to have the all-singing, all-dancing version of a particular model, but when this is to be used every day in a busy clinic solely for inspecting wet films for trichomonads and Gram stains the acquisition of a highly sophisticated instrument with easily adjustable components is usually counter-productive. It is my experience that those things that can be "adjusted" very often will be, and a very expensive, highly sophisticated, maladjusted microscope will give a much poorer image than that provided by a simpler less manipulable model which remains fairly constantly in alignment.

Care of the instrument

The best instrument in the world will never perform well if it is not well looked after. Careful treatment and daily cleaning of any microscope is essential if good results are to be obtained. Microscopy in the genitourinary clinic is obviously an extremely important diagnostic tool. The best and most experienced microscopist, however, will not produce consistently accurate observations with instruments which are inadequate for the purpose, badly adjusted or badly maintained.

In conclusion, my advice is to decide carefully your requirements, and then to put your specification to any of the major manufacturers for their suggestions as to how they can fulfil your wishes and at what price. Most of the major firms will be able to satisfy your needs.

Good microscopy is one of the most satisfying and rewarding of diagnostic techniques. A little more attention to the selection of the instrument required, and its subsequent care and maintenance, will ensure that the operator has the best chance of achieving, and maintaining, the very high standards that diagnostic microscopy demands of us.

I thank the following companies who responded so readily to my request for information about their specific products:
Carl Zeiss (Oberkochen), PO Box 78, Woodfield Road, Welwyn Garden City, Herts AL7 1LU; Olympus Optical Co, 2–8 Honduras Street, London EC1 Y0TX; CZ Scientific Instruments Ltd, 2 Elstree Way, Borehamwood, Herts, WD6 1NH; Prior Scientific Instruments Ltd, Unit 4 Wilbraham Road, Fulbourn, Cambridge, CB1 5ET; Zenith Microscopes and Instruments, Zenith House, 69 Lawrence Road, Tottenham, London, N15 4TG; BDH Ltd, Apparatus Division, PO Box 8, Dagenham, Essex, RM8 1RY; Cambridge Instruments, Viking Way, Bar Hill, Cambridge, CB3 8EL; Astell Scientific, Powerscroft Road, Sidcup, Kent DA14 5EF; James Turner (Liverpool) Ltd, Fleming Road, Speke, Liverpool, L24 9LS; Wild Leitz, 48 Park Street, Luton, LU1 3HP.
Any of the above, and other suppliers and manufacturers, will be able to give further advice on providing instruments to specification.

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Suggested further reading:

1 Cremer AWF. Elements of Microscopy. *Bull Inst Technicians Venereol* 1969;**8**:77.
2 Hartley WG. *Hartley's Microscopy*. Charlbury, UK. Senecio Publishing 1979.
3 Locquin MV, Langeron M. *Handbook of Microscopy*. London Butterworth's 1983.