The principles and practice of electron microscopy

IAN M. WATT

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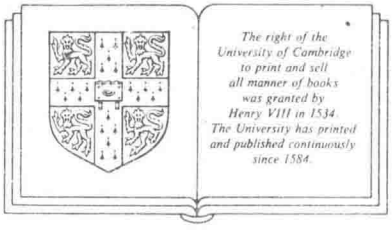
Johnson Matthey Research Centre



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Preface

Electron microscopes have been available commercially for 45 years now, and several thousands are in use throughout the world, operating in a wide range of fields. Although they started out as transmission instruments, with consequent limitations for certain types of examination, the introduction of the scanning microscope only 20 years ago revolutionised electron microscopy and broadened its scope very considerably. More recently the availability of a range of analytical techniques and the development of both hybrid and more specialised models of microscope have made it necessary to consider which member of the e.m. family is most appropriate to a particular examination, and how it can best be employed. This book has been written for anyone whose work or study encounters the results of electron microscopy, whether in biology, medicine or technology. Its purpose is to describe in simple terms the range of instruments and techniques now available and to illustrate how their capabilities are employed. The book mentions some of the factors which have to be considered and optimised by the microscopist, in the hope that the user of the results of microscopy will feel he understands more of what is going on, even though he may never see an electron microscope in operation. If this results in a closer collaboration between the 'customer' and the microscopist it will be to the advantage of both.

In attempting to give an easily understood survey of the broad field of electron microscopy the author has been helped by his background as a general-purpose

microscopist providing a service in electron microscopy to 'customers' from a range of disciplines; initially at Sira (formerly the British Scientific Instrument Research Association) and more recently at the Research Centre of Johnson Matthey p.l.c. He was already immersed in microscopy when the scanning microscope was introduced (initially as a technique looking for applications – how the scene has changed!) and, having had the use of both types of microscope, is able to appreciate the strong and weak points of both. This balanced viewpoint was helped by regular association over a number of years with other practising microscopists who formed the steering committee of a research group on electron microscope techniques, based on the EM Unit at Sira.

It is only possible to mention a few of the many people to whom the author is grateful for support and stimulation in his first 25 years in electron microscopy. Without the forethought of the late Dr John Thomson and Dr K. M. Greenland, who arranged for an unwanted electron microscope to be transferred to Sira in 1958 and encouraged the author to become an expert in using it, the episode would not have begun. A series of colleagues in the EM Unit at Sira, notably Ann Allnutt and the late Noel Wraight, shared and nourished his enthusiasm for the subject. The supporters of the Electron Microscope Techniques Group have already been mentioned. Thanks are due to the present Management of Sira Ltd for permission to reproduce micrographs and results obtained at Chislehurst. Similarly, the author is grateful for the help and encouragement received at Johnson Matthey Research Centre from numerous colleagues, but particularly from the Group Research Director, Dr G. J. K. Acres and the Research Manager, Dr M. J. Cleare. The facilities and staff provided for word processing and copying have helped considerably in the preparation of the final version of this book.

Finally, I must apologise to my family for the disruption of family life which this book has caused over a number of years.

Ian M. Watt
Reading, 1983

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1
Microscopy with light and electrons

As the unaided human eye moves closer to an object it produces in the brain of the observer a progressively larger and more detailed image until the eye is about 25 cm from the object, at the so-called *near point* of the eye. The observer's eye cannot focus on objects closer than this and the image rapidly becomes blurred if the distance decreases further. What is now needed is a device to give the effect of moving closer but with the enlarged image still appearing no closer than the near point; that is, to help the human eye to see smaller detail than it could unaided. This device is a magnifying system or microscope which may take one of a variety of forms to be described in this book. It may produce an aerial image in space, in which case only one person at a time can see it, or it may project an image on to a screen or photographic material so that several people can view it together. Before looking into the types of microscope which are available we can consider the very wide range of objects which are to be examined, a range from postage stamps and fabrics down to the individual atoms of which matter is composed. This range of dimensions covers six orders of magnitude, requiring enlargements up to millions of times greater than lifesize. Such enlargements are beyond the capability of traditional microscopes and this book endeavours to explain the new technology which makes it possible to meet these requirements.

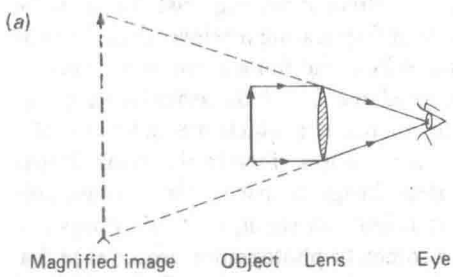
Microscope systems using light

The simple microscope

The simplest of all microscopes is the hand lens or magnifying glass which is a single biconvex lens of glass or plastic. An object placed close behind the lens appears to be bigger when seen through it, so that more detail can be seen in it than without the use of the lens. Figure 1.1 illustrates diagrammatically and photographically the enlargement produced by a simple hand lens. A wider field of view is seen when the eye is placed close to the lens.

The magnification produced by a single lens is approximately $25/f$ where f is the focal length of the lens in centimetres. To increase the magnification the focal length must be reduced, which in practice requires the lens to have more sharply curved surfaces. A × 10 magnifier is already a bulbous piece of glass and shows various defects in image formation. These occur in all lens systems and will be described in more detail in the next section.

Figure 1.1. The magnifying glass. (*a*) Ray diagram showing how the lens converges light from the object so that it appears to come from a larger 'image' which is sufficiently far away for the eye to focus it comfortably (*b*) Photograph through a magnifying glass.

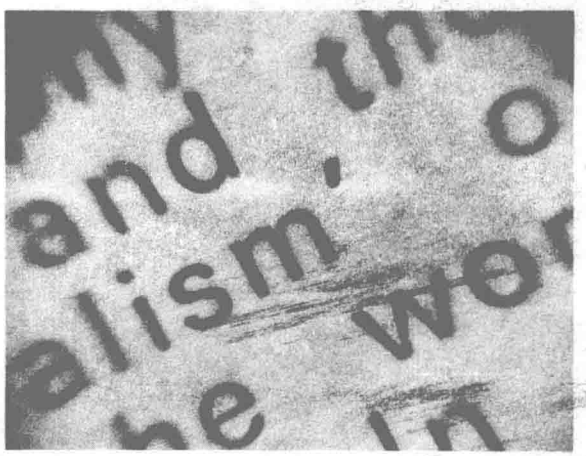


Defects of lenses

There are a number of image defects which result from the shape and size of the lens and one which depends on the material of which it is made. Let us first of all look at the last one. Figure 1.2 shows a magnified image of black printing on white paper as seen through the single biconvex lens of a × 5 watchmaker's eyeglass, photographed on panchromatic film. The original image was tinged with colour, particularly away from the centre of the field of view, and this has been recorded as an apparent smearing of the image. This is because the single lens suffers from *chromatic aberration* and results from the material of the lens having a different light-bending ability (refractive index) for light of different colours. The effect is the same one which enables us to split up white light into a spectrum of colours by passing it through a prism (Figure 1.3). In a simple lens the effect of chromatic aberration is to form a succession of coloured images in different planes, the blue one being closer to the lens than the red one. A screen placed at B will show a focused blue image ringed with red, and vice versa when placed at R. (There are of course the other spectral colours in between but only these two are illustrated, for clarity.)

Fortunately the degree of *dispersion* of coloured rays, i.e. the difference between the refractive index for the two extremes of the spectrum compared to the average refractive index, varies between different

Figure 1.2. Photograph of white light image of black printing on white paper seen through a single biconvex lens. Much of the smearing out of the letters away from the centre of the field is due to the image being coloured by the chromatic aberration of the lens.



types of glass. For example 'flint' glass has a higher dispersion than 'crown' glass. It is therefore possible to replace a single 'chromatic' lens by an *achromatic doublet* which has the same focal length for light of two different colours, e.g. red and blue (Figure 1.4). If a third glass type is used the correction can be made for three colours, and the lens combination is called *apochromatic*. A simpler way of avoiding chromatic aberration is to form the image using light of only one colour, i.e. monochromatic illumination, in which case only one focal length is involved. Figure 1.5 is the same as Figure 1.2 but illuminated with sodium yellow light. Chromatic aberration has now been eliminated and the image is cleaner but still imperfect. The remaining faults are caused by the shape and size of the lens and are: spherical aberration, distortion, field curvature, astigmatism and coma. For the present purpose it is not necessary to understand these in detail, but they will be described briefly in connection with our simple microscope.

Spherical aberration, perhaps more aptly described by the German term *öffnungsfehler* or aperture defect, becomes troublesome when image-forming rays make more than a small angle with the axis of the lens or, looking at it in another way, when light rays make large angles of incidence with the surfaces of the lens. The resultant effect is that axial rays are brought to a focus further from the lens than rays passing through the periphery of the lens. The degree of spherical aberration is measured by the axial separation of the foci for axial and marginal rays. A lens suffering from this defect will not have a unique focus, but only a 'best' focus or 'circle of least confusion' in the image. This is shown diagrammatically in Figure 1.6. It is this focus, not the ideal or *Gaussian* focus, which will be used in practice. One way of minimising the aberration is by inserting an opaque 'stop' so that only the central part of the lens is used; this is wasteful of light however and darkens the image. The aberration may also be minimised by splitting the refraction between several surfaces so that no large angles of refraction are required at any single surface. Fortunately it is possible to eliminate the defect completely by replacing a single lens by several with appropriate surface curvatures. If different glasses are used for the separate lenses the combination may be made achromatic at the same time.

A lens or lens combination which has been corrected for spherical and chromatic aberrations may still

Figure 1.3. Glass (and plastic) lenses and prisms have different refracting powers for the various colours of light. Blue (solid line) is bent more than red (broken line), hence white light images are a succession of coloured images spread out along the axis of the lens, blue being nearer to the lens than red. The actual separation between the colours depends on the type of glass and its power of dispersion.

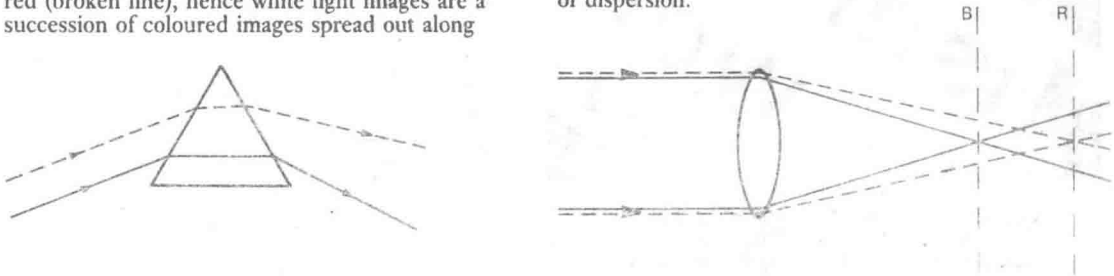
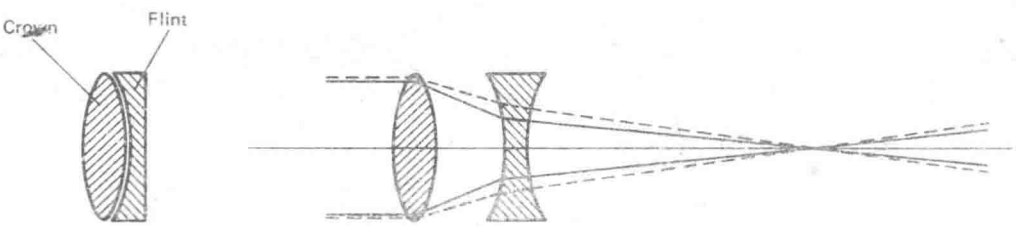


Figure 1.4. Achromatic doublet combination comprising a converging lens of crown glass and a weaker diverging lens of more highly dispersive flint glass. The combined effect is of a converging lens with equal focal lengths for two colours; red and blue correction is illustrated but it may be designed for other combinations of colours.



show *distortion*, a defect in which the magnification varies across the image field. Figure 1.7 shows the effects of the two types of distortion, a square object having its sides apparently sucked in by *pincushion distortion* and blown outwards by *barrel distortion*. It should be noted that in this particular defect the sharpness of the image is not affected, but only the local magnification at different points in the image.

Spherical and chromatic aberrations both influence the quality of image detail on the lens axis. Images of off-axis points are affected by astigmatism, field curvature and coma. In the first, illustrated in Figure 1.8, an off-axis point is imaged as a line in two different planes. Again there is only a 'best' image between the two line images, but the effect can be eliminated by careful design of the lens components. Field curvature is an effect by which a plane object is reproduced as a curved image. Lenses in which astigmatism and field curvature are both corrected are called *anastigmats*. Photographic lenses are examples of this type and the designs are frequently complex, involving four, five or six 'elements' of carefully calculated shape and separations to eliminate or at the least minimise the effects of the aberrations which have been mentioned. Because of the sometimes conflicting requirements of each defect the final design is a compromise to fit a particular set of imaging conditions. Hence, if the lens is used for a different purpose, e.g. a lens corrected for parallel light from distant objects used as a close-up lens with strongly divergent light, certain aberrations will no longer be eliminated and the image quality will deteriorate.

Figure 1.5. As Figure 1.2 but photographed using monochromatic light from a sodium vapour lamp. Colour fringing has now been eliminated and spherical aberration is now the major defect present.

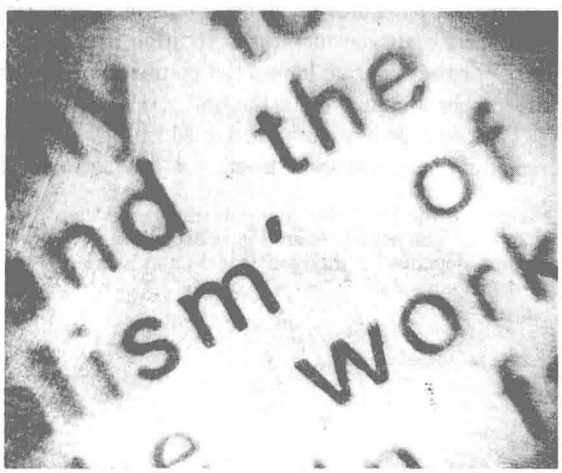


Figure 1.7. The image of a square object formed by a lens with (*a*) pincushion and (*b*) barrel distortion. The defect is one in which the magnification varies with the distance from the axis of the imaging system.

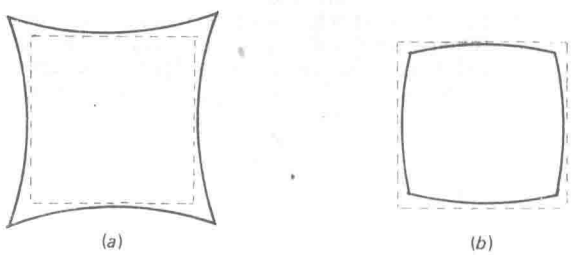
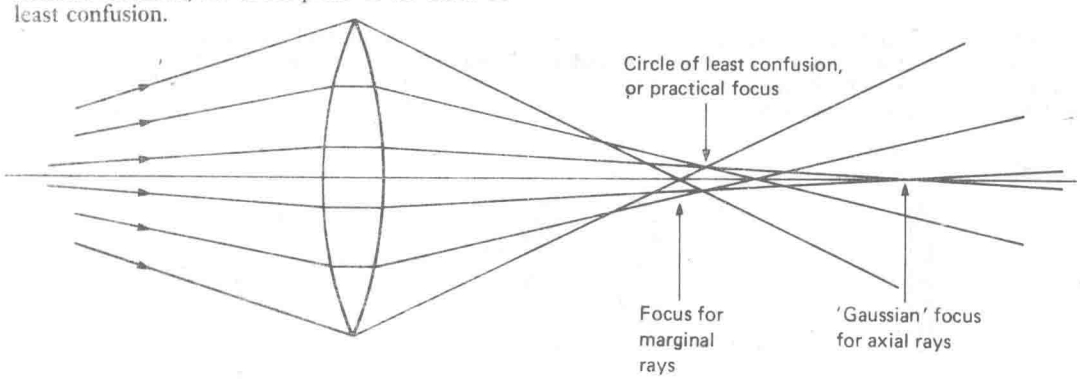


Figure 1.6. Imaging of a point object by a lens with spherical aberration. The practical focus will appear to be where the imaging rays occupy the smallest diameter, i.e. at the plane of the circle of least confusion.



Returning once more to our simple microscope, we can see that it is possible to improve the performance of a single lens by making it of several components, at the very least an achromatic doublet corrected for spherical aberration; better quality magnifying glasses are improved in this way. Even so, if magnifications of more than about 10 are required, and particularly if a magnified image is required on a screen or photographic plate, the simple microscope is preferably replaced by a two-lens system, the second magnifying the image formed by the first. This system is called a *compound microscope*, and will now be described.

Figure 1.8. Diagrammatic representation of the defect of astigmatism. This is a defect whereby the image of an off-axis point becomes two lines at right angles to one another, in different planes. The separation between the planes is a measure of the degree of astigmatism in the lens. The best circular rendering of the point is the circle of least confusion between the two line images.

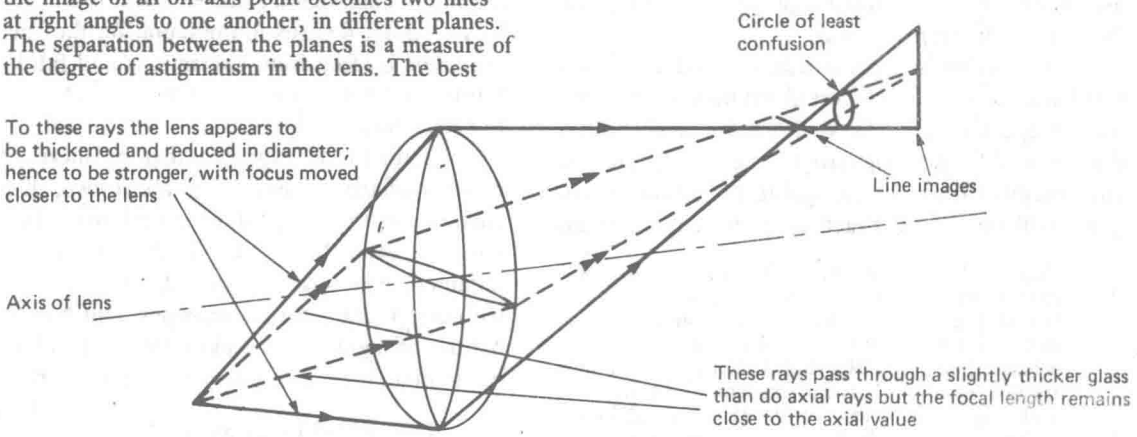
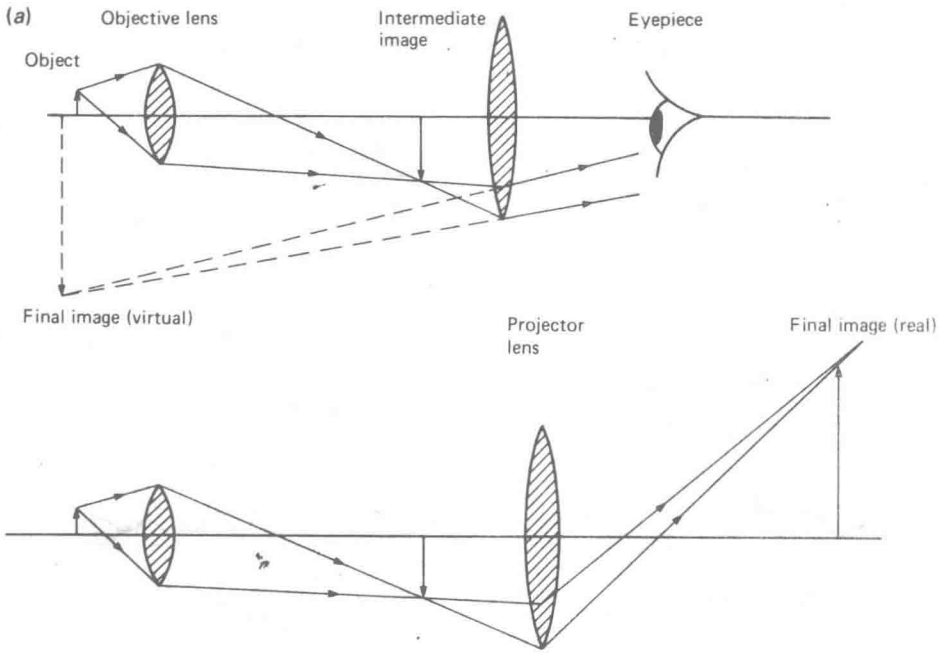


Figure 1.9. Diagrammatic representation of the principle of the compound microscope.
(*a*) Virtual image viewed directly by the observer.
(*b*) Real image projected onto a screen or photographic film or plate.



The compound microscope

Most simply (Figure 1.9) this comprises two convex lenses appropriately spaced from each other and the object. The object is placed just beyond the focus of the first or *objective* lens, which forms an enlarged real *intermediate* image between the two lenses. The second lens or *eyepiece* can be used as a magnifying glass to form a magnified virtual image of the intermediate image in a plane convenient for the observer to see. Alternatively the second lens can be used to project the second image onto a screen (and is then called the *projector* lens).

If the individual magnifications produced by the two lenses are ×10 the overall magnification of the microscope will be ×100. With a more powerful (i.e. shorter focal length) objective lens, e.g. ×40, and the same eyepiece the overall magnification of the microscope will be ×400. Practical microscope systems have a choice of highly corrected objective lenses, usually mounted on a revolving nosepiece so that the overall magnification can be rapidly changed between several values (e.g. ×50, ×100, ×400, and ×1000) by changing objectives. The eyepiece magnification can also be altered by substituting different lenses, but this should only be done with discretion (see later, *empty magnification*). A practical compound microscope will of course be a refinement of the simple two-lens theoretical system shown in Figure 1.9; an important component is the *condenser lens* or system of lenses which focuses illumination on the specimen; this must be free from aberrations and fulfil certain requirements to match the illumination to the objective lens being used.

It might be expected that by increasing the number of magnifying stages above two, or making each one more powerful, an overall magnification of tens or even hundreds of thousands might be obtained, enabling very fine detail to be examined with compound light microscopes. Unfortunately the nature of light itself makes this last achievement impossible, for reasons which will now be described.

The diffraction barrier

Light is a form of electromagnetic energy which is transmitted as a wave motion. We can visualise

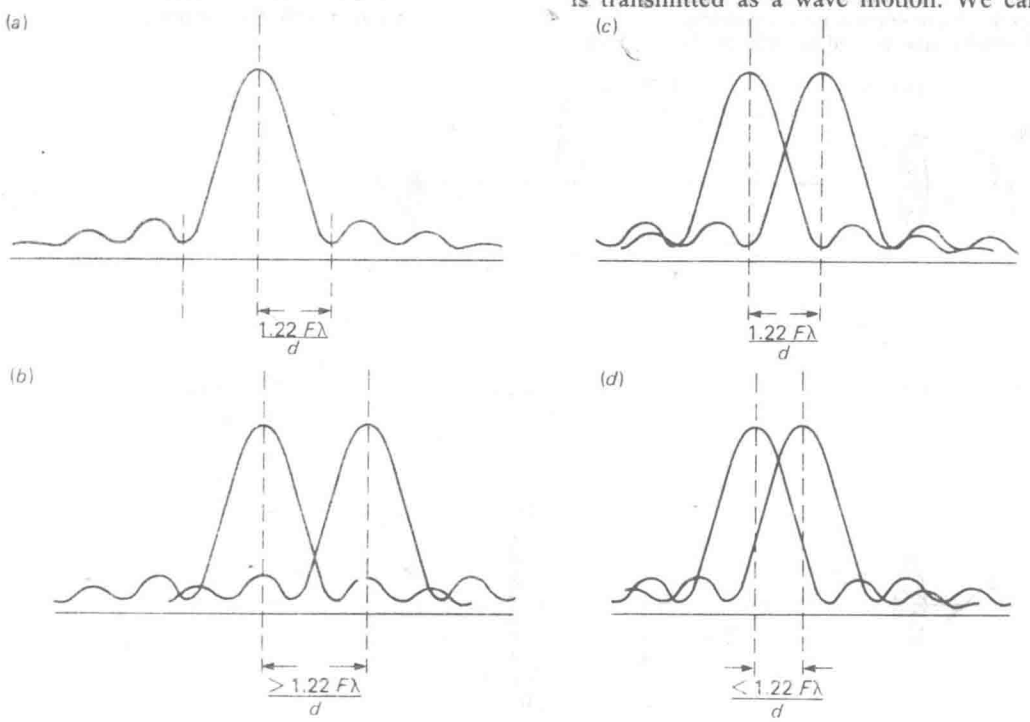


Figure 1.10 (*a*) Intensity distribution in image of point object formed by perfect lens. (*b*) Overlapping patterns of two point images sufficiently far apart to be recognised clearly as separate points. (*c*) 'Rayleigh Criterion': two points are just 'resolved' when the separation of the peaks equals the radius of the Airy disc. (*d*) Image points are not resolved when the separation of peak intensities is less than the radius of the Airy disc.

many of its effects if we picture it as a set of ripples moving away from its source, e.g. from a lamp filament. Whenever the waves impinge on an obstacle secondary wavelets are set up centred on the obstacle and these can interact or interfere with the main wave-front to produce local increases or decreases of illumination. Thus, passing light through a hole in an opaque screen results in a bright patch of light with fringe (diffraction) effects intruding into the geometrical shadow, because of the effect of the secondary wavelets from the edges of the hole.

The action of an optically denser medium is to slow down a wave-front of light. A biconvex lens converts a plane wave-front from a distant source into a contracting one which converges to the focus of the lens. The effect of diffraction, however, is to make the image of a point source not a second point but a small bright spot surrounded by less intense light and dark haloes. The spot is called the 'Airy disc' by opticians and it can be shown by calculation to contain 84% of the light in the image plane. The intensity distribution in and around the disc is shown approximately in Figure 1.10*a*. The radius of the disc or, more precisely, of the dark ring surrounding it is $1.22\ F\lambda/d$ where F is the focal length of the lens, d its diameter and λ the wavelength of light. It should be noted that this is the smallest point which can be projected by a perfect lens; the effect of lens aberrations on the image may be to make the central maximum wider than the value given above.

If two points are imaged by the lens each will be represented by an Airy disc and haloes. Moving the object points together will move the intensity patterns together until they overlap (*b*). As the overlap increases there will be a point at which the elongated patch of light can just be identified as coming from two points close together. According to Rayleigh this situation where the two individual points can just be discerned separately, or *resolved*, occurs when the intensity maximum of one pattern falls in the first intensity minimum of the other, i.e. the separation between the two peaks is $1.22\ F\lambda/d$ (*c*). Any lesser separation would result in formation of a continuous blur with no discernible minimum in it (*d*). The criterion of the resolving power of an imaging system as the separation between two object points where images can just be seen as separate is common to all microscopy, although a little difficult to apply in certain cases.

Resolving power of microscopes

In 1873 the German optician Ernst Abbe published his theory of the formation of images by microscopes. In it he related the diffraction limited resolving power, defined by the Rayleigh criterion described above, to the angle subtended by the objective lens at a point object. Abbe's theoretical relationship, borne out in practical observations, stated that the minimum resolvable separation d_0 in the object was given by

$$d_0 = \frac{0.61\lambda}{n\sin\alpha}$$

where λ = wavelength of the illuminant, α = half angle subtended by the objective at the object, and n = refractive index in the space between object and objective lens. This relationship can be used to work out the theoretical limit of resolution of any microscope system. The product $n\sin\alpha$ is called the *numerical aperture* (NA) of the objective lens, and is usually engraved by the manufacturer on the lens mount. Since α can never exceed 90° an objective in air cannot resolve distances smaller than 0.6λ; an oil immersion system will be limited to a resolving power a little less than 0.5λ. In round numbers the resolving power of the light microscope is limited by diffraction to about 0.3 μm for white light and about half this using U-V light and quartz lenses.

A typical laboratory microscope with × 40 objective of NA 0.7 or thereabouts has its resolution limit at about 0.5 μm with white light. The relationship between NA, angular aperture and resolving power is shown diagrammatically for several microscope objectives in Figure 1.11.

Useful and empty magnification

We saw earlier that the purpose of a microscope is to magnify specimen detail and present it at a distance where the eye can see it. The useful magnification of a microscope is therefore one which will increase the size of the minimum resolvable distance until it is large enough for the eye to see. The normal eye is just able to resolve two points separated by 0.1 mm at its nearest distance of distinct vision. The least magnification necessary to make use of a microscope's resolution d_0 is thus $100/d_0$ times (when d_0 is expressed in μm). For white light this figure approximates to 300 times the NA of the objective. In practice this figure should be increased, since it is

tiring to work at the extreme of visual resolution for any length of time, but a magnification of 1000 times the NA is the maximum which can usefully be employed. Higher enlargements than this, although permissible in large display micrographs which will not be viewed closely, only enlarge the unresolvable blurs in the image and do not introduce any further detail into the micrograph. This over-enlargement is said to introduce *empty magnification* into the image.

Taking as an example the laboratory microscope with × 40 objective of NA 0.7 this should be used with a stronger eyepiece than × 5 to enable its potential resolving power to be utilised; × 10 would be a suitable eyepiece, or × 15, but anything in excess of this will only contribute empty magnification.

Depth of field

We have seen how the diffraction of light places an upper limit of about × 1000 on the useful magnification of most light microscopes. A further impediment in the way of examining fine detail in practical three dimensional objects is the fact that this detail is only seen in a very shallow depth of the specimen. The *depth of field*, or thickness of specimen which will be seen acceptably sharply in the magnified image, may be only a fraction of a micrometre in some cases and comparable with the resolving power. Specimen detail outside this zone will be enlarged into an out-of-focus blur, which confuses the overall picture.

Figure 1.12 shows diagrammatically the concepts of depth of focus and depth of field in lens systems and illustrates how these depths are less for a wide aperture lens than for one with a narrower aperture. The microscopist can produce a mental impression of fine detail over a wider range than the depth of field of his instrument if he racks the focused plane up and down within the object. However, this impression of detail in depth cannot be recorded in a single photograph to show to another person.

Beating the wavelength restriction

Microscopy would have remained only half-way towards the goal of seeing matter on an atomic scale were it not for a combination of discoveries which in a period of three years showed the existence of suitable radiation with shorter wavelength and the necessary lens systems for focusing it.

Electronic illumination

A glow discharge in a gas and an incandescent metallic filament in a vacuum are both sources of electrons (or cathode rays as they were originally known), demonstrated by J. J. Thomson in 1897 to be negatively charged particles of very small mass. Electrons from such a source may be attracted to and through a hole in a positively charged plate, called the anode, and may be formed into a beam by suitable magnetic and electrical fields. The cathode ray tube,

Figure 1.11. Diagrammatic illustration of the relationship between angular and numerical apertures of microscope objectives and the diffraction limited resolving power for $\lambda = 550$ nm.

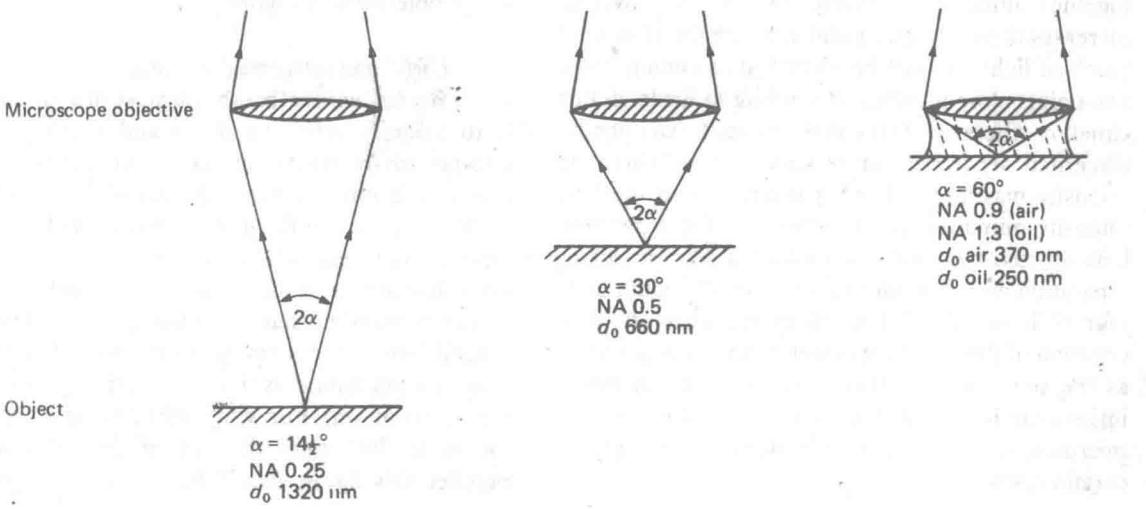
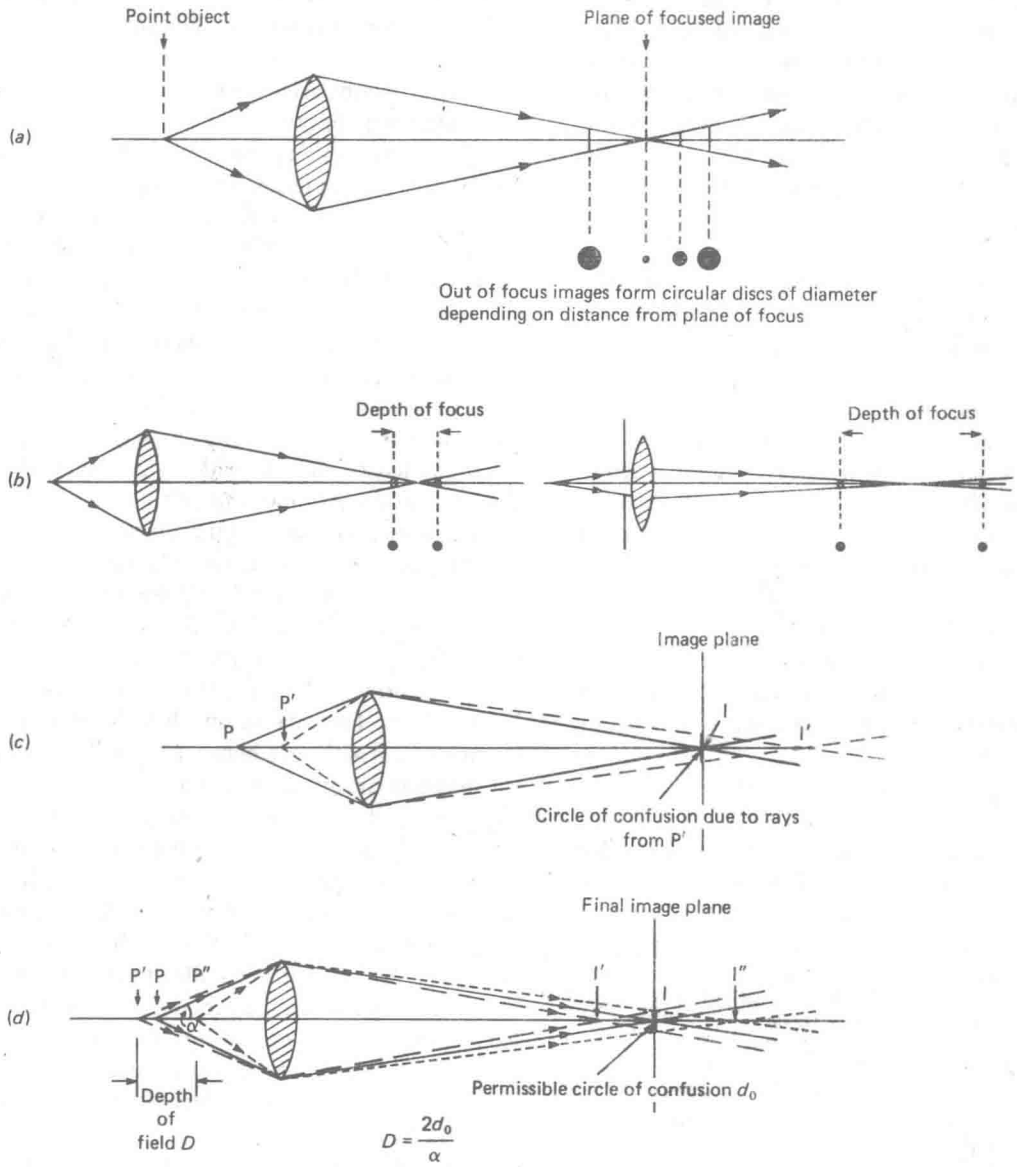


Figure 1.12. Diagrams illustrating depth of focus and depth of field in microscope systems. Depth of focus is the latitude in positioning the screen or photographic material before the image sharpness deteriorates beyond a chosen amount. The focused image of a point is a disc, because of diffraction, plus any aberration effects, so there is a finite depth of focus even at the highest resolving power of the system. The diagrams in (*b*) show how the depth of focus is increased by reducing the angular aperture of the lens.

Conversely, if the image plane is fixed it can be seen that the images of object points such as P′, situated closer and further than P along the lens axis, will be discs or circles of confusion whose size depends on the amount of defocus PP′ and the angular aperture of the imaging rays (*c*). The *depth of field* P′P″ is the axial distance in the object space between points whose discs in the final image plane have diameters up to a permissible maximum value (*d*).



fore-runner of today's television tubes, used these principles without, however, going beyond the basic knowledge given above. An electron which has been accelerated by an anode potential of V volts is called a 'V volt electron' and has an energy of V *electron volts* (eV); the higher its energy the faster it will be travelling and the heavier it becomes.

The utilisation of the electron to form the basis of a new optics – electron optics – was made possible by combining the discoveries of de Broglie (1924) and Busch (1926). De Broglie showed that a moving electron had a dual personality. It can be regarded either as a moving charged particle or as a radiation with associated wavelength. The key relationship deduced by de Broglie was expressed by

$$\lambda = h/mv$$

where λ is the de Broglie wavelength of the particle, h is a constant (equal to Planck's constant), and m, v are the mass and velocity of the electron. Working from this concept we see that a beam of moving electrons can be regarded as a beam of invisible radiation which, given the means of focusing it, could be used to form an image. The real advance is apparent when we put numbers into the equation and see that the electron wavelength is in fact many orders of magnitude shorter than that of light. Typical values are listed in Table 1.1.

Now, the kinetic energy $\frac{1}{2}mv^2$ of the moving electron is equal to Ve. Substituting for v in the de Broglie equation and putting in numerical values for h, e and m we arrive at a more convenient form of the de Broglie equation:

$$\lambda = 1.23/\sqrt{V}\ \text{nm}.$$

This shows that a 50 keV electron has a wavelength of 0.0055 nm. The significance of this in Abbe's resolving power relationship $d_0 = 0.6\lambda/n\sin\alpha$ is obvious. If an electron objective lens of NA 1.4 were available then a 50 kV electron microscope would be capable of resolving detail as fine as 0.0024 nm, or about a hundredth of the size of an atom. All this presupposes firstly that the electron lenses are available and can be used in a compound microscope, and secondly that the lenses can be made as perfect as glass lenses in light-optical microscopy. So let us now look at the nature and characteristics of electron lenses.

Table 1.1. *Variation of velocity, mass and wavelength of moving electrons with energy*

V (volts)	Velocity (km h^{-1})	Velocity relative to velocity of light	Mass, relative to rest mass	λ (nm)
1	593	0.0020	1.0000020	1.226
10	1876	0.0063	1.0000196	0.3878
10^2	5932	0.0198	1.0001957	0.1226
10^3	18730	0.0625	1.001957	0.03876
10^4	58460	0.1949	1.01957	0.01220
10^5	164400	0.5482	1.1957	0.00370 (3.7 pm)
10^6	282200	0.9411	2.9569	0.00087 (0.87 pm)
3.10^6	296700	0.989	6.870	0.00036 (0.36 pm)

Microscopy with electrons

Magnetic lenses

An electron moving in a magnetic field experiences a force tending to change its direction of motion except when it is travelling parallel to the magnetic lines of force. If the magnetic field is suitably shaped a beam of electrons diverging from a point at one end of the field will leave it as a beam converging to a second point; in other words the field has a focusing action on electrons in a similar way to a convex glass lens and light rays. There is, however, no magnetic equivalent of the diverging, concave, lens. Magnetic lenses can be made to give magnified or diminished images, and sequences of lenses can be used to form compound microscope systems. The focusing properties of magnetic fields were worked out in theory by Busch in 1926, although it remained for others, e.g. Knoll and Ruska, to exploit the principles by constructing practical lenses.

The principle of a magnetic lens is illustrated in Figure 1.13. This shows an electron entering the magnetic field in the gap between two hollow cylindrical magnets arranged with unlike poles facing one another. The focusing action, resulting in the electron path cutting the axis, is accompanied by a circumferential motion, illustrated in Figure 1.13*b*, which is a view back along the axis of the lens. In practice the complete figure would be rotated about the axis of symmetry xx' so that a parallel beam of electrons entering the lens would be focused to a point on the axis of the field. Electrons travelling along the axis would be moving parallel to the magnetic lines of force and would be undeflected in their passage through the lens field.

In practice a convenient way of producing a magnetic field of the form shown in Figure 1.13 is to pass an electric current through a coil of many turns of wire wound on a hollow cylindrical former. Such a