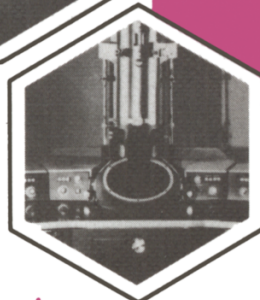
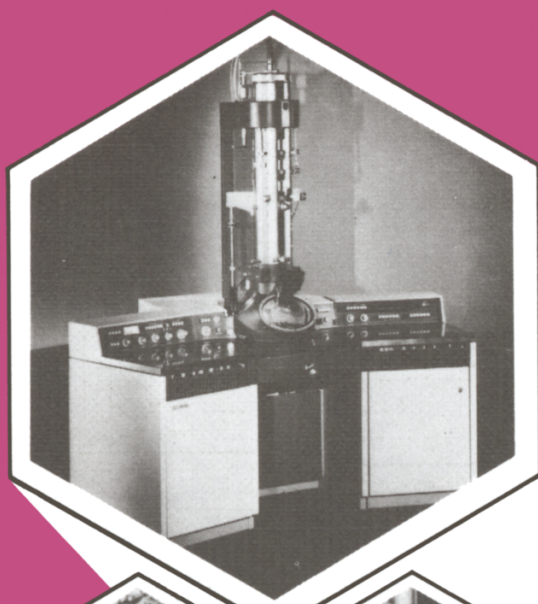


**PRACTICAL ELECTRON
MICROSCOPY
IN
MATERIALS SCIENCE**

**1 Monograph One
The operation and
calibration of the
electron
microscope**

J W Edington



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**THE OPERATION AND CALIBRATION
OF THE ELECTRON MICROSCOPE**

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*Monographs in Practical Electron Microscopy
in Materials Science*

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THE OPERATION AND CALIBRATION OF THE ELECTRON MICROSCOPE

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PREFACE

This is the first of a series of monographs on electron microscopy aimed at users of the equipment. They are written both as texts and sources of reference emphasising the applications of electron microscopy to the characterisation of materials. The flexible monograph format will enable both the student and the professional scientist to extract the required information easily. In this context the series should be useful in specialist undergraduate and postgraduate lecture courses and as an introduction to the technique for professional scientists.

In some places the author has referred the reader to material appearing in other monographs in this series. The following three monographs are in preparation:

2—*Electron Diffraction in the Electron Microscope*,

3—*Interpretation of Transmission Electron Micrographs* and

4—*Typical Electron Microscope Investigations*.

Subsequent monographs in the series will cover quantitative metallography, together with aspects of design of electron microscopes.

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1. THE OPERATION AND CALIBRATION OF THE ELECTRON MICROSCOPE

1.1 Introduction

An electron microscope uses a series of magnetic lenses to focus an electron beam that is accelerated, by a high potential, through the specimen *in vacuo*. The main features of a modern microscope are shown schematically in figure 1.1. An electrically heated tungsten filament, at a selected negative potential 20–100 kV, is mounted on a ceramic insulator behind a Wehnelt cap with a central hole. The electrons emitted by the filament are accelerated to earth and are focussed, via a double condenser lens system with field-limiting apertures, onto the specimen. An image or diffraction pattern from the specimen is obtained on a fluorescent screen via a three- or a four-lens (illustrated) magnification system. Image contrast may be enhanced by the use of an objective aperture, and the area for diffraction may be selected by a selected area aperture. The image is focussed with the objective lens and magnification is controlled by the excitation of the intermediate and/or diffraction lens. The depth of field of the instrument is such that the top and bottom of the specimen are simultaneously in focus while its depth of focus enables the plate or film camera to be situated up to 50 cm above or below the viewing screen.

There are limitations on the performance of an electron microscope because of lens defects. Nevertheless, in general the accuracy of operation and calibration of the instrument controls the quality of electron micrographs, and the precision with which they can be analysed quantitatively. In addition, the type of information required from the specimen controls the operation procedure employed, and therefore to some extent the details of the information obtained. It is our purpose here to describe the important lens defects and their influence on instrumental performance, together with the various operation modes and calibration requirements of the microscope. Some of the terminology used here is defined in subsequent sections. In particular, that associated with the diffraction pattern may be found in the early parts of section 2.

1.2 Lens Defects

Like glass lenses, all electromagnetic lenses suffer from defects such as coma, barrel or pincushion

distortion, astigmatism, chromatic and spherical aberration which are discussed in detail by Haine (1961). From the operator's standpoint the last three are the most important in relation to the objective lens because they determine the resolution of the electron microscope.

Spherical aberration is particularly important because there is no convenient way of correcting it. This defect is shown schematically in figure 1.2(a). Electrons leaving a point P on the optic axis of the specimen at the object are refocussed at P' instead of P'. Consequently, in the image plane the point is imaged as a disc; its radius Δr_s is given by

$$\Delta r_s = C_s \beta^3 \quad (1.1)$$

where C_s is the spherical aberration constant and is typically 1 or 2 mm for an objective lens. Thus the point resolution deteriorates rapidly with increasing β , that is lens aperture.

Chromatic aberration arises because of the energy, and therefore wavelength, spread of the electron beam. Instabilities in accelerating voltage or lens current could be contributory factors, but both are negligible because of the high stability of modern power supplies. Although there is a small energy spread ~ 3 eV in the electron beam leaving the tungsten filament in the electron gun, the major factor is the large energy loss $\Delta E = 5\text{--}50$ eV suffered by many electrons on passing through the specimen. In effect the focal length of the lens varies with the electron energy, see figure 1.2(b). A disc of confusion, radius Δr_c , is produced, given by

$$\Delta r_c = C_c \beta \frac{\Delta E}{E} \quad (1.2)$$

where C_c is the chromatic aberration constant of the lens and is approximately equal to its focal length.

Astigmatism occurs when the lens exhibits different focal lengths, depending upon the plane of the ray paths. Thus, in figure 1.2(c), the rays travelling in plane A are focussed at P_A while those in plane B focus at P_B. Again a point on the object is imaged as a disc of confusion, radius Δr_A , given by

$$\Delta r_A = \Delta f_A \beta \quad (1.3)$$

where Δf_A is the maximum difference in focal

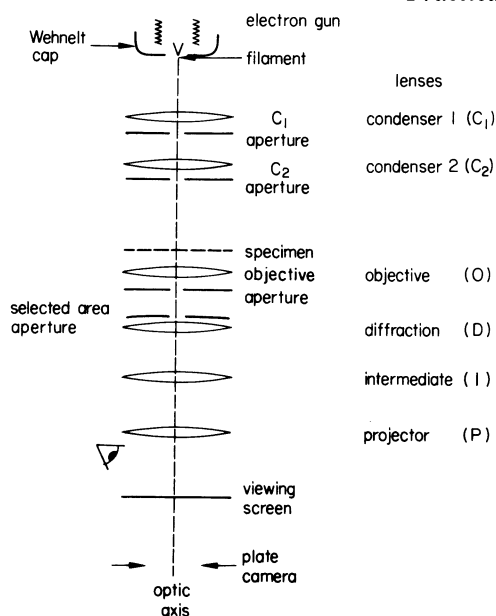


Figure 1.1 A schematic diagram showing the important components of the microscope

length arising from astigmatism. This defect may be corrected using electromagnetic astigmators that produce a small controllable magnetic field. Astigmatism of the condenser lens system is important because it reduces the coherence of the beam, while that of the objective lens is important because it can cause a serious loss in image resolution.

Condenser astigmatism is manifested by an

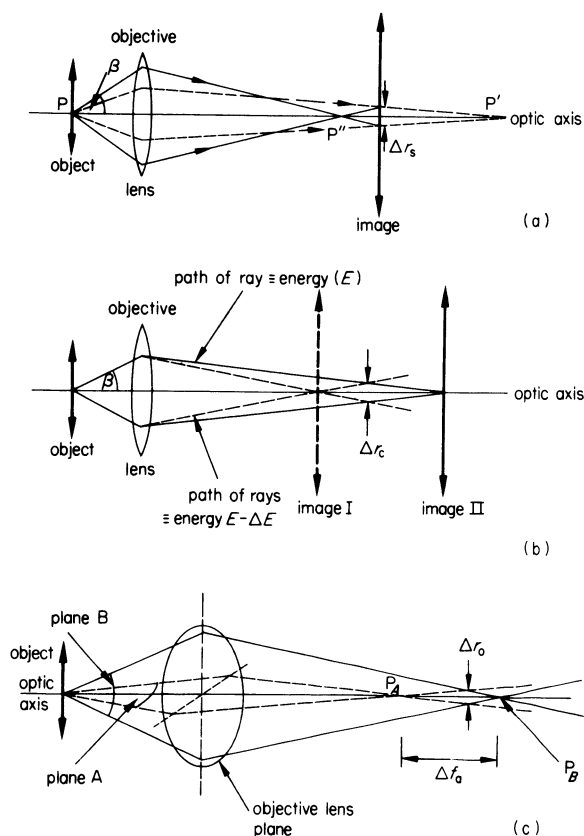


Figure 1.2 Objective lens aberrations: (a) spherical, (b) chromatic, (c) astigmatism

elliptical illumination spot when the condenser 2 is defocussed. The astigmator is adjusted to make the defocussed spot circular. Objective astigmatism is manifested by streaking in one direction of fine detail of the image, compare figures 1.3(a), (b), and may be corrected using the procedure outlined in section 1.4.2.

1.3 Resolution

There are two forms of resolution of interest in electron microscopy. The first is the instrument resolution, and the second is the effective resolution for a particular type of specimen.

Consider first instrument resolution, which is a function of the aberrations of the objective lens. Referring to equation (1.1) it is clear that spherical aberration demands the use of very small apertures to maximise resolution. However, if the aperture size is reduced too far, diffraction effects at the aperture become a limiting factor. In this context it was shown by Airy (1835) that diffraction by a lens aperture causes a point source to be imaged as a bright central disc, known as Airy's disc, surrounded by a number of fainter rings. Thus the intensity distribution in the image plane for two point sources in the object plane is that shown in figure 1.4(a) for a lens of angular aperture β . As S_1 and S_2 approach each other the disc images in the image plane overlap, see figure 1.4(b)–(d). It is shown in books on physical optics, for example, Jenkins and White (1951) that the closest distance of approach at which both discs can be resolved is given by the Rayleigh criterion that the central minimum, figure 1.4(e), has an intensity of 0.81 of the maximum. The point-to-point resolution limit is then

$$\Delta r_d = \frac{0.61\lambda}{\beta} \quad (1.4)$$

Since increasing the aperture size increases resolution according to equation (1.4) but decreases it according to equation (1.1), there is an optimum aperture size that minimises their sum, that is

$$\beta \approx \left(\frac{\lambda}{C_s} \right)^{1/4} \quad (1.5)$$

which gives a maximum resolution

$$\Delta r_{\min} \approx \lambda^{3/4} C_s^{1/4} \quad (1.6)$$

Using this approach typical values of optimum aperture diameter ($= 2\beta f$) may be calculated for the EM300 and these are given in table 1.1 for the high-resolution stage and pole pieces.

Consideration of the values of the optimum resolving power at 100 kV (see table 1.1) indicates that, before spherical aberration becomes the factor limiting resolution in the EM300, it is necessary to correct for astigmatism to the extent

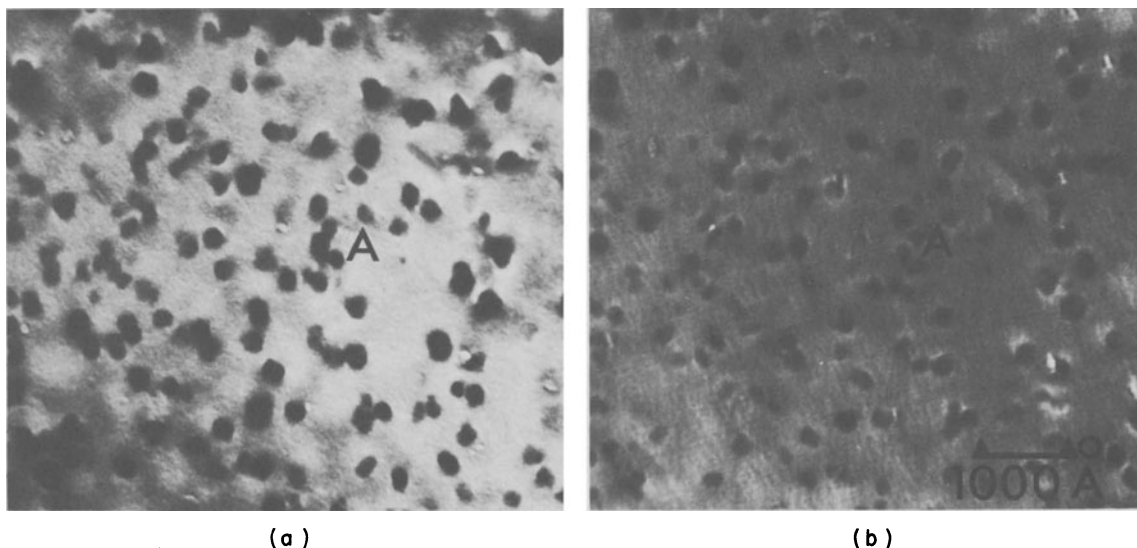


Figure 1.3 Bright field images of an aged Al-6% Zn-3% Mg alloy, showing η precipitates. Astigmatism (a) correctly, (b) incorrectly adjusted. The points marked A are the same in both figures

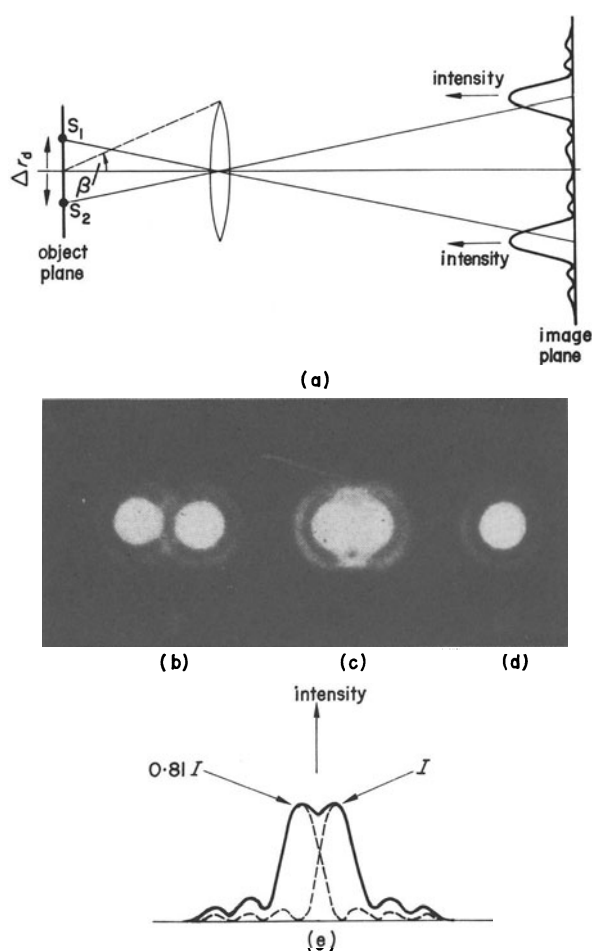


Figure 1.4 (a) The formation of two Airy disc images of point sources S_1 , S_2 through diffraction at the objective aperture (angle β), (b) clearly resolved Airy discs, (c) just resolved, (d) unresolved, (e) the situation corresponding to (c), that is Rayleigh criterion such that the intensity in the centre is 0.81 of the maximum

that $\Delta r_A = 2.3 \text{ \AA}$. In practice this means that one must be able to distinguish a variation in Fresnel fringe spacings of the order of $4 \text{ \AA}$ in $12 \text{ \AA}$, see section 1.4.2. This conclusion is derived for a resolving power of $\Delta r_{\min} = 2.3 \text{ \AA}$, $\beta = 10^{-3} \text{ rad}$ to obtain a value for Δf_a and then assuming $X_{\max} = 12 \text{ \AA}$ in equation (1.9). Clearly such accuracy of astigmatism correction will only be achieved by special efforts and a more realistic level of point-to-point resolving power for electron microscopes is $\approx 5 \text{ \AA}$.

The influence of the specimen is generally to degrade resolution through chromatic aberration effects arising because many electrons lose small amounts of energy in passing through the specimen. As an example consider the typical case of a thin metal foil of thickness approximately 50% of the maximum useful penetration. Perhaps up to 40% of the electrons included in the final image will have lost energy in amounts of up to 50 eV by plasma or single-electron* excitation in the specimen. Substituting in equation (1.2) values of $C_c = 3.3 \text{ mm}$ for the goniometer stage of the EM300, and E is 100 kV with $\beta = 5 \times 10^{-3} \text{ rad}$, r_c is 25 $\text{\AA}$ if the energy loss ΔE is only 15 eV. Thus, in a typical thin foil it is the specimen that limits resolution unless very thin regions are used to minimise energy losses.

Finally, it is important to note that, when attempting to produce high-resolution images, for example 5 $\text{\AA}$ point-to-point, objective aperture diffraction effects can be important. The diameter of the Airy disc produced by the objective aperture is $\sim 1.2\lambda f/D$, where f is the focal length of the lens and D is the aperture diameter. Typical values for a range of objective aperture sizes are given in table 1.2 for the EM300/301.

* For details of these processes see Raimes (1961).

Table 1.1 Optimum objective aperture diameters (D_{opt}) for maximum resolution, assuming spherical aberration is the controlling process for the high-resolution stage* of the EM300/301, $C_s = 1.6$ mm

Accelerating voltage (kV)	Wavelength (Å)	D_{opt} (μm)	Optimum resolving power (Å)	Theoretical resolving power for actual objective aperture diameters (μm)				
				30	25	20	15	10
20	0.086	33	4.3	4.8	5.8	7.2	9.6	14.5
40	0.060	30	3.3	3.3	4.0	5.0	6.7	10.2
60	0.048	29	2.8	2.8	3.2	4.0	5.3	8.1
80	0.042	28	2.5	2.5	2.7	3.5	4.6	7.1
100	0.037	27	2.3	2.3	2.5	3.1	4.1	6.3

* Multiply these values by 1.55 for the goniometer stage.

These values represent the practical resolution limit of the microscope for these aperture sizes and interpretation of image detail on a finer scale than the values in table 1.2 is quite uncertain. Thus the practice of using small objective apertures to reduce the inelastic scattering contribution to bright field images, or to exclude closely spaced spots where forming centred dark field images, can seriously reduce resolution and complicate interpretation of fine scale microstructural effects.

Resolution tests are discussed in section 1.12.

1.4 Important Aspects of Microscope Operation and Alignment

The following three factors are the most important in controlling image quality.

- (1) Alignment of the objective lens axis.
- (2) Adjustment of objective astigmatism.
- (3) Illumination conditions.

The additional following factor greatly eases operation of the instrument for materials science studies.

- (4) Axis-centred specimen tilting.

The principles behind (1)–(4) above are described in the following sections. It is important to realise that, although the specimen itself may reduce resolution through chromatic aberration (section 1.2), it is still necessary to align the instrument carefully, paying particular attention to (1)–(3) above because all factors are additive in determining final resolution.

Table 1.2 Values of the diameter of the Airy disc produced by diffraction from the objective aperture of the EM300/301

Objective aperture diameter (μm)	Diameter of Airy disc (Å)	
	High-resolution stage $f = 1.6$ mm	Goniometer stage $f = 4.1$ mm
40	1.8	4.5
30	2.4	6.1
20	3.5	9.1
10	7.1	18.2

1.4.1 Alignment of the Objective Lens Axis

Misalignment of this lens seriously reduces the resolving power of the instrument because of increased spherical aberration, and sensitivity to chromatic aberration. This alignment must therefore be performed extremely carefully. The influence of an off-axis beam entering the objective lens is shown in figure 1.5(a). Here the object ABC forms an image A'B'C' (solid ray paths) for a given objective lens setting. If, however, the objective lens setting is changed the image will be A''B''C'' (dashed ray paths) smaller than A'B'C' but central about the undeviated beam BB'. In addition, there will be a rotation of the image because of the helical path of the electrons through the lens. Essentially, the image rotates about a point (rotation centre) off the centre of the viewing screen when the objective lens current is changed, see figure 1.5(b). Depending upon the microscope, the beam may be made to travel down the optic axis of the lens by either tilting the incident illumination, figure 1.6(a), or adjusting the objective lens pole piece, that is the optic axis of the lens, figure 1.6(b), (c). This is achieved by adjusting either of these

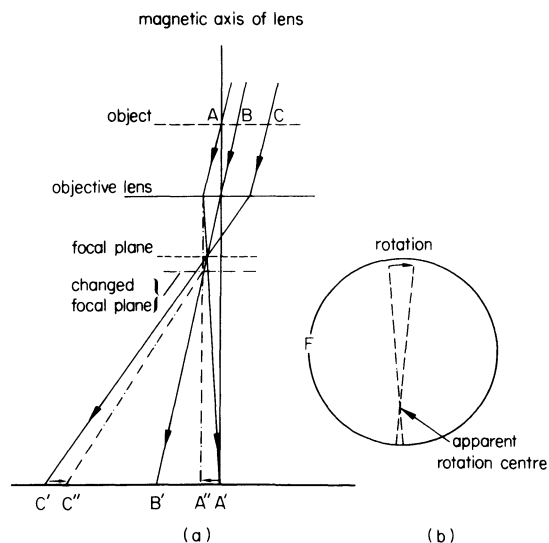


Figure 1.5 The effect of non-axial illumination of the objective lens: (a) showing the sweep of the image, (b) the combined effect of sweep and rotation of the image on the viewing screen

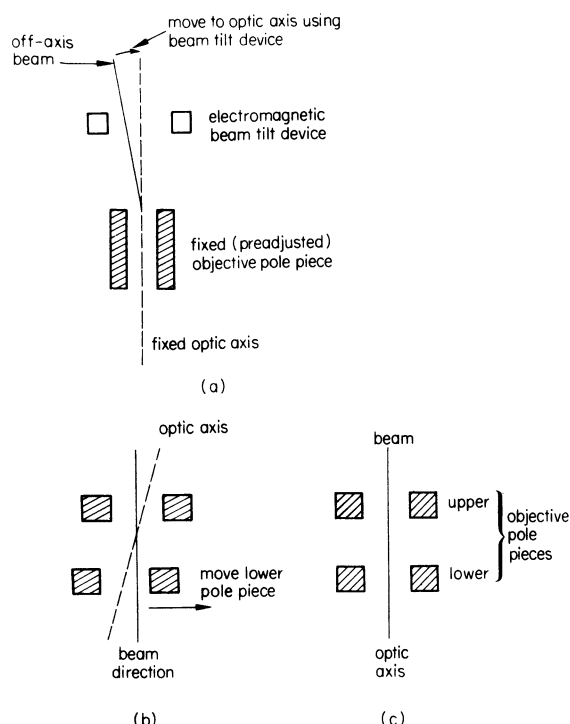


Figure 1.6 The correction of non-axial objective lens illumination by (a) electromagnetic beam tilt, (b), (c) movement of the lower objective lens pole piece for an immersion lens

controls to move the rotation centre to the centre of the viewing screen. The rotation centre may be defined by defocussing the objective lens and observing the centre of rotation of the image.

1.4.2 Objective Astigmatism Correction

Image resolution is very sensitive to objective astigmatism. Consequently, it is important to correct it carefully.

Conventionally, objective astigmatism is adjusted using the Fresnel fringes near the edge of a small hole viewed with the objective lens overfocussed.

Figure 1.7(a) shows schematically the occurrence of diffraction at the edge of a carbon film. Consider a plane wave scattered at A through an angle β . Interference between the wave and the undeviated wave at B is described by the path difference $AB - Z$, that is

$$X\beta = \frac{X^2}{Z} \quad (1.7)$$

which has a maximum when it is an integral number of wavelengths $n\lambda$, that is

$$X = (n\lambda D)^{1/2} \quad (1.8)$$

The contrast of the fringes is only appreciable for very small values of n because the amplitude of the scattered wave falls off as $1/AB$. If $n < 2$, the objective lens must be defocussed by $Z \approx 1 \mu\text{m}$ to observe a fringe spacing of $\sim 10 \text{ \AA}$. The width of

the first dark fringe decreases on approaching focus where it disappears ($Z = 0$). For an astigmatic lens with differences in focal length as shown in figure 1.2(c), focus will be different around a hole and the fringe will disappear at some points before others. Consequently the objective lens may be overfocussed* when viewing a small, round hole in a thin carbon film until a dark fringe is observed, as shown in figure 1.7(b), then the objective astigmator adjusted until the fringe is uniform around the hole and disappears uniformly on approaching focus. Figure 1.7(c) shows a correctly compensated image. This adjustment is always performed at the highest magnification possible ($> 100\,000\times$ on the screen) using the binoculars to view the image. The anticontaminator should be used to minimise contamination which increases the thickness of the film and makes observation of the fringe more difficult.

The divergence of the incident beam is an important factor which limits the observation of Fresnel fringes. If there are two beams at an angle β' the two fringe systems will be out of step by $Z\beta'$, the separation of the geometric shadow in the focus plane, see figure 1.7(f). In order to observe a given fringe system $Z\beta'$ must be $\ll$ fringe spacing. For $10 \text{ \AA}$ fringes $\beta' \ll 10/Z$ and if $D = 1 \mu\text{m}$ then $\beta' \ll 10^{-3}$ and if a narrower fringe is to be observed the divergence must be even smaller. Thus condenser 2 must be defocussed to decrease β' and to permit the observation of fringes.

Having obtained a photograph of the best corrected fringe, the residual astigmatism can be evaluated by measuring the maximum and minimum fringe width X_{max} and X_{min} . Then

$$\Delta f_a = \frac{1}{\lambda} (X_{\text{max}}^2 - X_{\text{min}}^2) \quad (1.9)$$

which may be used to evaluate the influence of the astigmatism on instrumental resolution.

When aiming for best resolution it is frequently necessary to correct for astigmatism on the specimen under study when a suitable hole may not be available. Such correction is required because the objective aperture itself can introduce additional astigmatism if it is not perfectly centred on the optic axis. In addition, astigmatism changes in the vicinity of specimen support grid bars. Under these circumstances it is best to correct astigmatism using the fine background image structure. This technique is illustrated in figure 1.7(g), (h). First the objective lens is focussed for minimum background contrast, figure 1.7(g). Any residual contrast then arises from variations in defocus of the objective lens from astigmatism which produces phase contrast as described in section 1.5.5. Then the objective astigmators are carefully adjusted for

* Overfocus is defined as an overexcited lens, that is focussed between the normal focal plane and the plane of the lens. Underfocus is an underexcited lens, that is focussed behind the normal plane of focus.

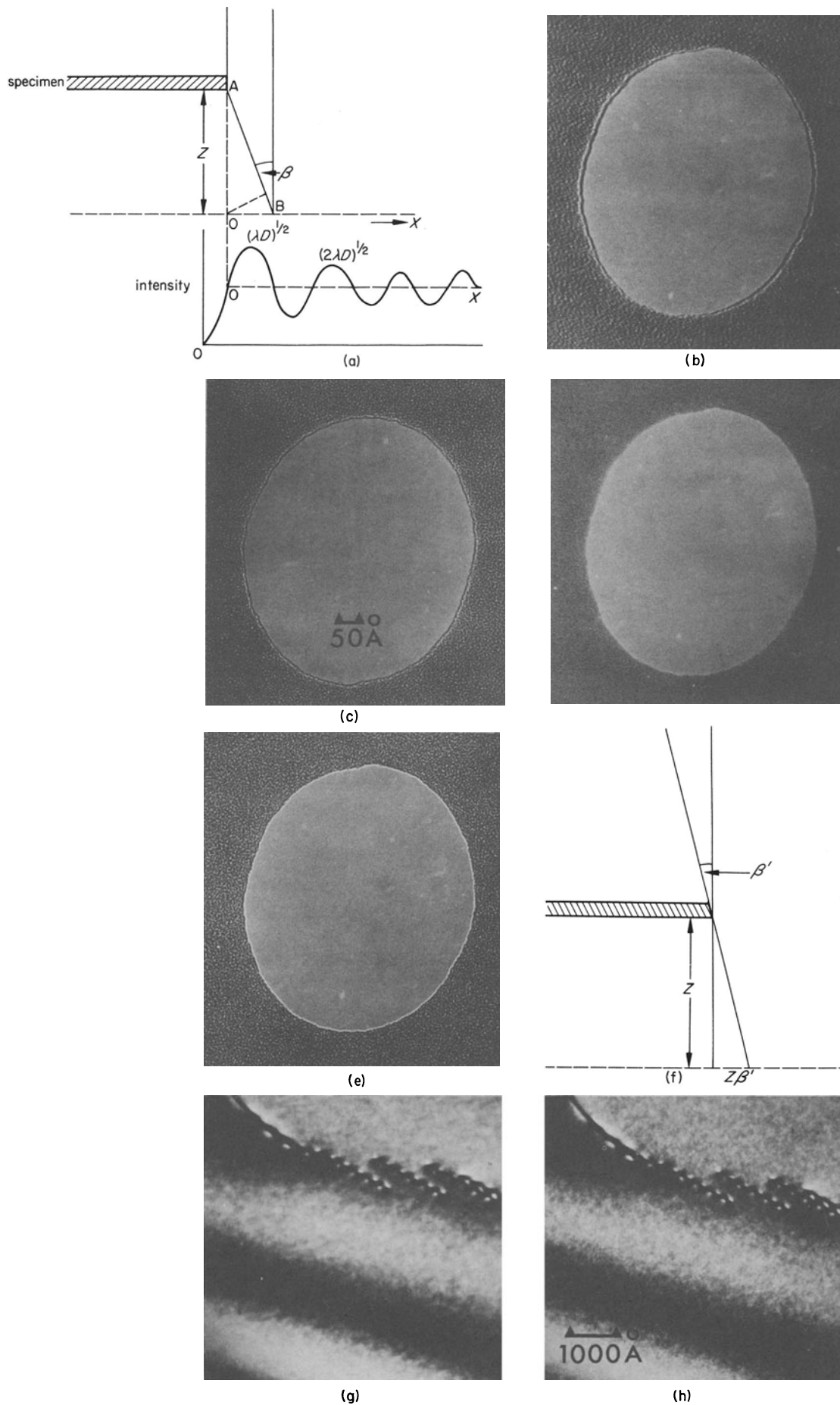


Figure 1.7 (a) The formation of Fresnel fringes at an edge, showing the intensity distribution at depth z below the specimen, (b) a hole in the carbon film showing the overfocus dark fringe for incorrectly and (c)–(e) overfocus, in-focus and underfocus images for correctly adjusted objective astigmatism, (f) the effect of incident beam convergence on Fresnel fringe formation, (g) in-focus image of molybdenum showing incorrectly adjusted objective astigmatism (this is an exaggerated example, significant maladjustment of astigmatism is present), (h) the same area with astigmatism correctly adjusted. Note the improvement in resolution on background.

minimum, but sharp, background contrast, figure 1.7(f), that is as uniform an objective focal length as possible.

This method is suitable for most crystalline specimens,* using the fine background structure of the image arising from the thin surface oxide layer usually present as for figure 1.7(g), (h). If image magnifications of $>100\,000\times$ on the screen are used the result is usually at least as good as the Fresnel fringe approach.

1.4.3 Illumination Conditions

The illumination conditions influence the quality of both images and diffraction patterns. As the beam intensity is increased, for example by weakening the excitation of condenser 1, the following effects occur.

- (1) Carbon contamination increases.
- (2) Local specimen heating increases, producing differential thermal expansion between specimen and support grid which causes more specimen drift.
- (3) Electrical charging of the specimen may increase.
- (4) The coherence of the illumination is reduced.

All of these effects reduce resolution in the image. Factors (1)–(3) above may be minimised by selecting minimum spot size using condenser 2 near focus, but this reduces the coherence of the illumination and therefore the resolution of the image. Consequently, choice of illumination conditions is a compromise. Thus the beam must be bright enough to keep exposure times $\lesssim 10\text{--}15\text{ s}$ to minimise loss in resolution from specimen drift. However, as far as possible, condenser 2 should be defocussed to two or three times the minimum spot size to ensure adequate coherence of illumination.

Diffraction patterns should be obtained with condenser 2 well defocussed (two coarse clicks of potentiometer) to maximise sharpness.

1.4.4 Axis-centred Specimen Tilt

In most electron microscopes the field of view changes when the specimen is tilted which necessitates frequent re-centring of the area of interest using the specimen traverse controls. Thus it is impossible to tilt the specimen in the most convenient way, that is while observing its orientation in the diffraction pattern, because the diffraction pattern comes from a continually changing area of the specimen. However, some modern electron microscopes, such as the EM300/301, have provision for preventing this movement so that the field of view remains constant during specimen

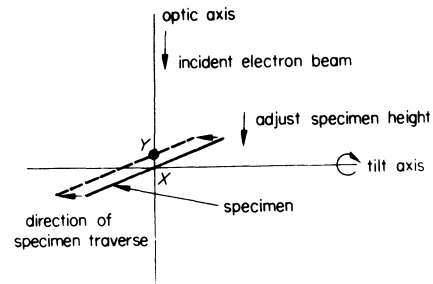


Figure 1.8 A schematic illustration of axis-centred tilt

tilts of $\pm 60^\circ$. To achieve this, the area of the specimen viewed must be positioned at the point defined by the intersection of the optic axis and the tilt axis, as shown schematically in figure 1.8. The solid line indicates the position of the specimen with the field of view X illuminated by an electron beam traversing the optic axis. If the specimen is now translated to the position defined by the dashed line the new field of view will be Y which is now above the intersection of the tilt and optic axes. However, Y may be brought to this point by adjustment of the specimen height. Clearly for a microscope with this adjustment the objective lens is operated close to a standard setting, that is focussed on X . This greatly simplifies the calibration procedures outlined in sections 1.11.1, 1.11.2 and 1.11.3.

In order to achieve axis-centred tilting it is necessary to be able to adjust and to fix the specimen tilt axis until it coincides with the optic axis, then to adjust separately the specimen height. Note that if the specimen is tilted while in the dashed position the field of view of interest will move in or out of the plane of the paper.

1.5 The Formation of Diffraction Patterns and Images

The microscope may be operated to produce either a diffraction pattern from a specific region of the specimen ($\gtrsim 0.5\text{ }\mu\text{m}$ in diameter) or one of several types of image. In all cases where quantitative information about the microstructure of the material is required, detailed correlation is made between the diffraction pattern and the image.

The essential features of the diffraction and image modes of operation can be explained in terms of a geometric optics treatment of the situation at the objective lens. Figure 1.9(a) shows an electron beam parallel to the optic axis and incident upon the specimen. The action of forming the image brings both the transmitted and the diffracted beam to a focus in the back focal plane of the objective lens. Thus a diffraction pattern is produced here.

* It may also be used for a carbon replica when it should be possible to obtain less contrast than is visible in figure 1.7(f).

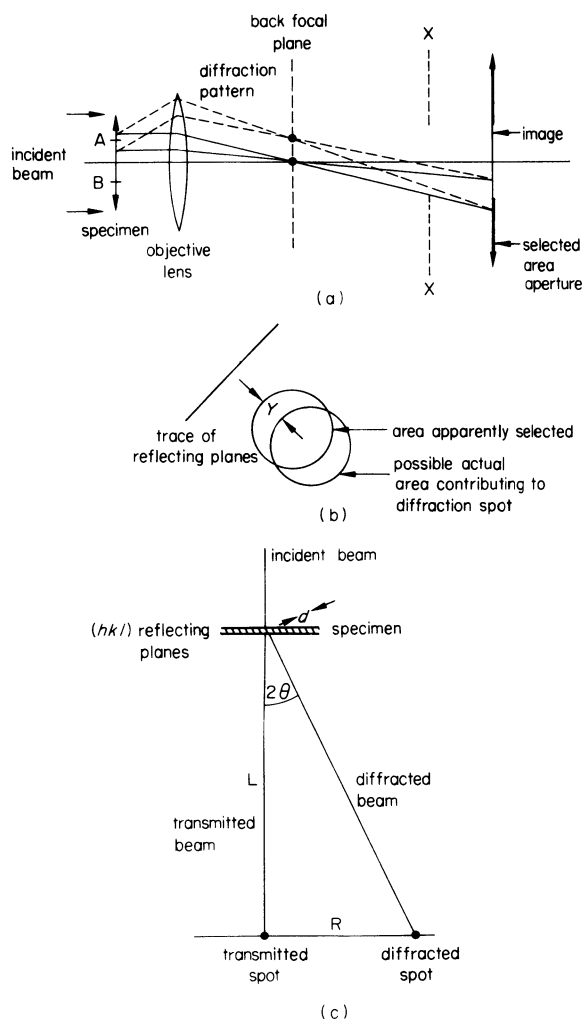


Figure 1.9 (a) Selected area diffraction using a selecting aperture in the plane of the image formed by the objective lens, (b) the influence of errors in microscope operation on the accuracy of area selection, (c) the diffraction camera construction to simulate the magnification of the diffraction pattern by the microscope lenses

Because both a diffraction pattern and an image of the specimen are always produced by the objective lens, a magnified image of either may be produced on the viewing screen by focussing the next lens in the magnification system on one or the other. The details of image and diffraction pattern formation are described below.

1.5.1 Selected Area Diffraction

It is essential to be able to form a diffraction pattern from a specific region of the specimen. The principles of this process, which is known as selected area diffraction (SAD), can be understood with reference to figure 1.9(a). Here there is an incident beam covering the specimen, and all transmitted and diffracted rays contribute to the diffraction pattern formed in the back focal plane of the objective lens,

as described above. However, if an aperture is inserted coplanar with the image, only the transmitted and diffracted rays that originate within the region AB of the specimen are allowed into the remaining magnification system. Those from outside are not. Consequently, although all electrons from the specimen contribute to the diffraction pattern formed in the back focal plane of the objective lens, only those from the region AB are allowed to contribute to the diffraction pattern formed on the viewing screen.

The procedure for performing this SAD has been described by Agar (1960) and Phillips (1960), being based on the approach of Le Poole (1947). First, the lens below the selector aperture is focussed on it to produce an in-focus aperture image on the viewing screen. In the EM300/301 the diffraction lens* is used for this purpose. This is easier with the objective aperture withdrawn. The objective lens is then used to focus the image of the specimen on the viewing screen. For easier focussing the objective aperture may be inserted to improve image contrast. Thus an image is formed at the selector aperture level by the objective lens because this aperture is already in focus on the final screen. The last step is simply to remove the objective aperture and to reduce the strength of the diffraction lens until a focussed (minimum spot size) diffraction pattern appears on the final screen, that is the diffraction lens is focussed on the back focal plane of the objective lens. The focus of the diffraction pattern is best judged using the viewing binocular and with condenser 2 overfocussed by 1–2 steps on the coarse control.

It is important to realise that there can be significant errors in the selection procedure. Thus, if the image and selector aperture are not coplanar, rays from outside the region selected will contribute to the diffraction pattern. Such a situation would occur if the selector aperture were in position XX, figure 1.9(a), relative to the image plane. Furthermore, spherical aberration produces a curved image because the focal length of the objective lens changes across its aperture. Thus the image and selector aperture cannot be coplanar. The influence of these two factors on the accuracy with which a specific area may be selected may be discussed in terms of the Bragg law (section 2.2.2.1) in which a diffracted spot arises by reflection from a set of crystal planes oriented at a specific Bragg angle θ relative to the incident beam. The area from which a specific diffracted beam originates is displaced by a distance Y , figure 1.9(b), perpendicular to the trace of the reflecting planes† and given by

$$Y = D(2\theta) + C_s(2\theta)^3 \quad (1.10)$$

* In a microscope with a three-lens magnification system the intermediate lens is used.

† That is parallel to the g vector (the line joining the transmitted and diffracted spot, see section 2.2.3).

where D is the distance by which the objective lens is out of focus. Thus, as the Bragg angle increases so does the inaccuracy of area selection. Consider a (220) reflection in a B [111] diffraction pattern. The value for $2\theta = 0.0297$ rad, and for a typical microscope $C_s = 3.5$ mm while the minimum focus step on the objective lens fine focus is $124 \text{ \AA}$. Then $Y = 3.7 + 917 \text{ \AA} \approx 921 \text{ \AA}$. In reality, however, it is only usual to focus the image using the medium control of the objective lens, which implies a value of $D \approx 3 \text{ }\mu\text{m}$ and a value of $Y = 1808 \text{ \AA}$.

From the above discussion it is clear that a reduction in the size of the selector aperture below that which selects an area $\lesssim 4000 \text{ \AA}$ in diameter is not particularly useful, because the error in area selection can easily be equal to its radius. Finally, for really accurate work* it is important to adjust the microscope accurately.

It is also very important to realise that the action of the objective lens rotates the image 180° relative to the diffraction pattern. It is pointed out in section 2.2.3 and figure 2.9 that a vector g may be defined that is normal to the reflecting planes in the crystal and points from the transmitted to the reflected beam. The directions of g in object, image and diffraction pattern are shown in figure 1.10(a). Clearly the objective lens rotates g by 180° in the image but not the diffraction pattern relative to the object. This is a basic property of the objective lens and leads to the requirement for a 180° rotation between recorded images and diffraction patterns in some instruments, see section 1.11.3.

In conventional SAD with a three-lens magnification system, the intermediate lens is focussed on the diffraction pattern in the back focal plane of the objective lens. Because this lens is also used to alter the magnification of the image, SAD must be performed at one specific image magnification which may be incompatible with the scale of the microstructure to be investigated. However, if the microscope has a four-lens magnification system, the diffraction lens may be used to focus on the back focal plane of the objective lens, while the intermediate lens is responsible for changes in image magnification. Then SAD may be performed over a wide range of image magnification.

In general for SAD the microscope is operated with standard lens settings, so that the magnification of the diffraction pattern on the final screen is constant, except for the small variations discussed in section 1.11.2. The magnification factor is usually discussed in terms of an equivalent camera length that would be necessary to produce the same magnification in a conventional diffraction

camera, that is without the lenses. This approach is shown schematically in figure 1.9(c) using the concept of reflection from crystal planes at the Bragg angle θ to generate the diffracted beam as described in section 2.2.2.1. If the distance from the transmitted to the diffracted spot on the plate is R , and the camera length is L , then $2\theta = R/L \approx 2 \sin \theta$ for small values of θ . Substituting in the Bragg relation equation (2.3) we have

$$Rd = \lambda L \quad (1.11)$$

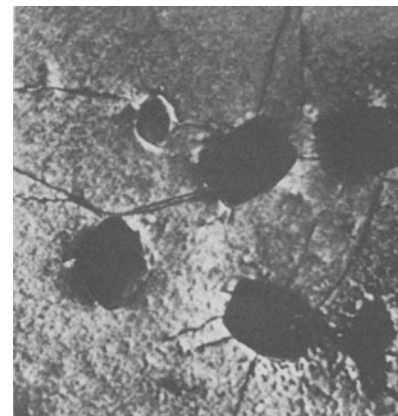
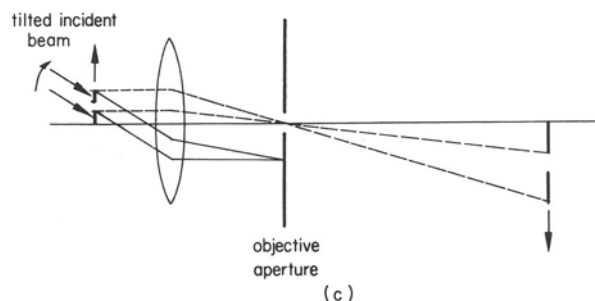
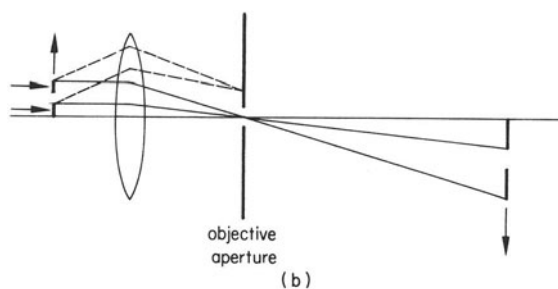
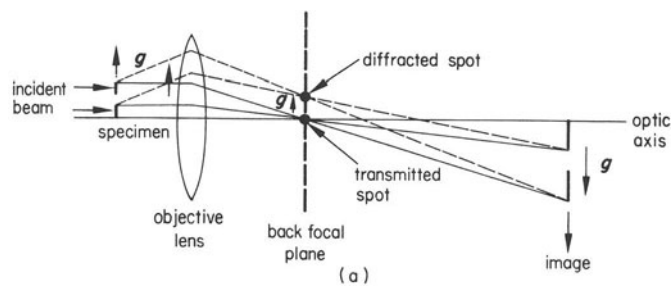
where λL is the camera constant which describes the magnification of the diffraction pattern, and may be calibrated as described in section 1.11.2.

For a three-lens magnification system, the magnification of the diffraction pattern may only be varied over a limited range by changing the projector lens excitation because the intermediate lens excitation is fixed by the need to focus on the back focal plane of the objective lens. However, in the four-lens magnification system, a wide range of camera lengths may be produced because only the excitation of the diffraction lens remains constant while the intermediate and projector lenses can be used to change the magnification of the diffraction pattern.

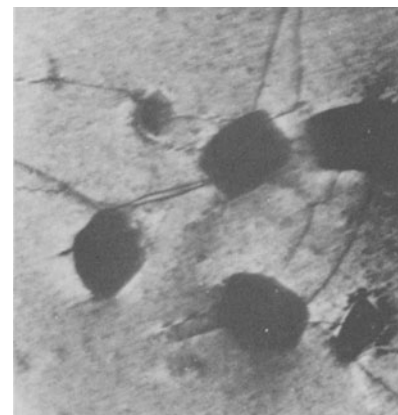
For most purposes electron microscopes are operated at fixed lens settings equivalent to a standard camera length, usually 50–70 cm at 100 kV. However, for some purposes it may be convenient to use a different camera length. A smaller camera length (10–30 cm) enables more of the diffraction pattern to be viewed on the screen. It is useful if small format film (for example 70 mm) is used: for producing a Kikuchi map as described in section 2.7.3.3 and appendix 5; to ascertain that stray high-order reflections are not present when tilting the specimen to produce exact (for example two-beam) diffracting conditions; to observe Laue zones (see section 2.7.2.2); or for weak beam images with high-order reflections (see section 3.17).

A larger camera length 1–5 metres is useful to investigate the fine structure of the diffraction pattern described in sections 2.16–2.19. As the magnification increases exact focussing becomes more difficult. Consequently, the camera length should only be increased to a value such that the required measurements can be made directly on the plate, or to render visible the details of the pattern on a photographic enlargement. At large camera lengths most diffracted beams will not fall on the viewing screen; consequently the centred dark field technique described in section 1.5.2 must be used to move the diffracted spot of

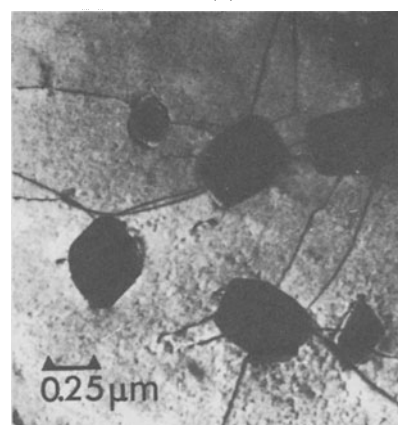
* Diffraction patterns from small areas down to $\sim 50 \text{ \AA}$ in diameter may now be obtained using scanning attachments that enable the incident beam diameter to be reduced to this value, for example Thompson (1973).



(d)



(e)



(f)

Figure 1.10 (a) The formation of a focussed diffraction pattern in the back focal plane of the objective lens, (b) the position of the objective aperture to form a bright field image, (c) tilted incident illumination to form a centred dark field image, (d) bright field, (e) displaced aperture dark field, (f) centred dark field of an aluminium grain in a Zn-40 wt % Al alloy. Dislocations and large zinc particles are visible [(d)–(f) Courtesy of A. Samuelsson]

interest to the optic axis. Under such circumstances the recommended procedure is to obtain centred dark field at the specific value of s^* required using the spot of interest before increasing the camera length. The values of s required for different types of fine structure are described in sections 2.16–2.19. Finally, it is important to realise that detection of fine structure depends upon obtaining a fully focussed diffraction pattern correctly exposed, see section 2.17.1 for details. Two important points are relevant here. First, overexposure will

* For definition see section 2.4, figure 2.16.

prevent detection of fine structure. Second, details of the diffraction pattern that are invisible on the screen will be recorded on the plate. Consequently, a series of exposures will often be necessary to obtain the maximum amount of information from the pattern.

1.5.2 Bright and Dark Field Images

Bright field (BF) and centred dark field (CDF) are the most commonly used imaging modes for

crystalline materials. Most of the analyses of crystal defects described in sections 3.4–3.9, 3.11–3.13, 3.22 involve comparison between, or detailed analysis of, BF and CDF images. Both types of image may be understood in terms of the image forming characteristics of the objective lens described above, figure 1.10(a).

A small aperture (5–70 μm diameter) may be inserted in the back focal plane of the objective lens to intercept the diffracted beam and only allow the transmitted beam to form an image. This situation is shown in figure 1.10(b) and is known as a BF image. Alternatively, the objective aperture could be displaced from the optic axis to intercept the transmitted beam and allow the diffracted beam to contribute to the image. This is known as a displaced aperture dark field, but a poor-quality image is produced because of the additional spherical aberration and astigmatism present when the electron path is not close to the optic axis. Compare figures 1.10(d), (e). In order to retain the

resolution of the BF mode, the illumination incident on the specimen is tilted so that the diffracted electrons travel along the optic axis, see figure 1.10(c) and compare figures 1.10(e), (f). This technique is known as centred dark field (CDF) and is performed in older microscopes by mechanically tilting the electron gun, or in more modern microscopes by using an electromagnetic beam tilt device. CDF should always be used whenever it is necessary to observe the detail of a dark field image.

The procedure for forming a CDF image may be understood with reference to figure 1.11. Here the specimen has been tilted to produce two-beam conditions, that is the transmitted and only one diffracted beam. As shown in section 2.2.2.1 the diffraction process may be thought of as reflection by a set of crystal planes oriented at a specific Bragg angle θ relative to the incident beam. In figure 1.11(a) both the incident and transmitted beams lie on the optic axis but the diffracted beam does not and is intercepted by the objective

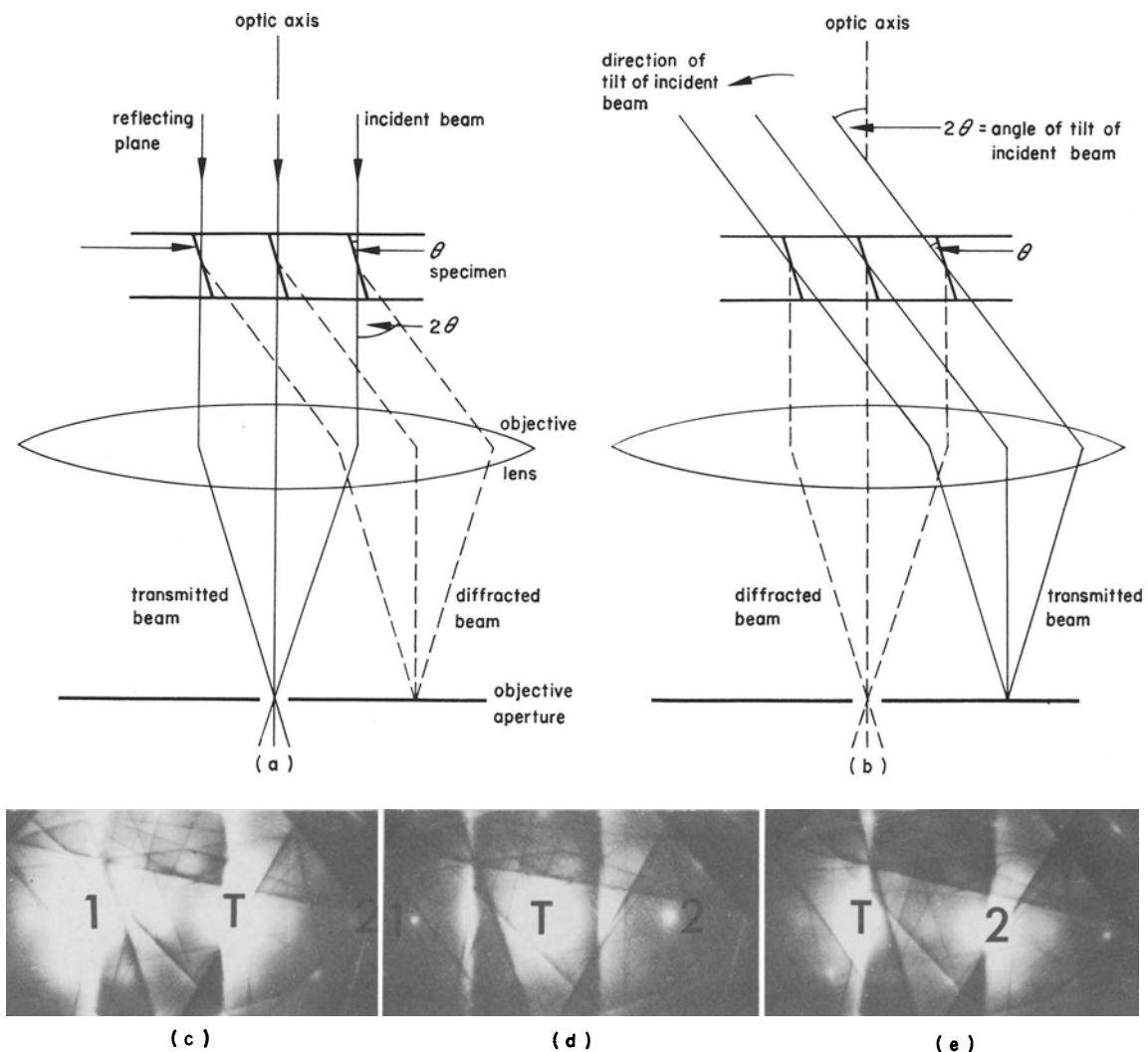


Figure 1.11 The relationship between the specimen reflecting planes and incident illumination for (a) BF and (b) CDF images, (c)–(e) the sequence of events occurring on the viewing screen during tilting of the incident illumination to form the CDF image in figure 1.10(f). The transmitted beam is labelled T and the diffracted beams 1 and 2 [(c)–(e) Courtesy of A. Samuelsson]

aperture (BF image). However, by tilting the beam through twice the Bragg angle (that is 2θ) reflection occurs from the other side of the same set of planes, figure 1.11(b). Thus the diffracted beam now lies on the optic axis while the transmitted beam follows the original path of the diffracted beam and is stopped by the objective aperture.

Remembering that the diffraction pattern on the viewing screen is simply a magnified image of that in the back focal plane of the objective lens, the sequence of events to perform CDF imaging is as follows.

(1) Produce a selected area diffraction pattern on the viewing screen, see section 1.5.1.

(2) Tilt the specimen until the spot of interest is bright (that is the Bragg law is satisfied), section 2.2.4.

(3) Operate the electromagnetic beam tilt controls (or the mechanical gun tilt if these are not available) to drive the transmitted spot into the position of the bright diffracted spot. This moves the weak diffracted spot opposite the original bright spot into the original position of the transmitted spot.* This point of coincidence may be judged by marking the original position of the transmitted beam using the beam stop.

(4) Refocus condenser 2 in the diffraction mode, centring the illumination where necessary, and return to the image mode.

(5) For maximum resolution the rotation centre of the objective lens may be adjusted in CDF image mode using the technique outlined previously in section 1.4.1 and the beam tilt controls employed to obtain CDF.

(6) If the microscope is provided with separate electromagnetic beam shift controls, for the beam tilt device, or, if the beam tilt controls can be independently aligned, it should be possible to switch directly from BF to CDF in the image mode and retain the images on the screen.

A typical sequence of events is shown in figure 1.11(c)–(e). Note that during beam tilting the original bright spot becomes steadily weaker while the opposite faint spot becomes brighter as the Bragg position is reached, see section 2.4. The BF and CDF images corresponding to figure 1.11(c) and (e) are shown in figures 3.2(d), (e). If the bright spot is moved to the centre it becomes progressively fainter because the Bragg angle is exceeded. Such a faint spot only produces a very faint image which will be almost invisible on the viewing screen. Thus it is necessary to tilt the specimen to make this spot bright again to produce a strong CDF image.

CDF imaging using specific diffraction spots is a very important and useful technique in analysing complicated diffraction patterns and in performing quantitative analysis of crystal defects. Each diffraction spot is produced by some diffraction process occurring at the specimen. Consequently the diffraction pattern contains all of the information concerning diffraction events from individual features of the specimen in the field of view. Therefore CDF imaging with each individual diffraction spot enables a direct correlation of the details of the image with those of the diffraction pattern.

Several examples of this approach are shown in sections 3.5.5, 3.6.2, 3.7.1, 3.8.1, 4.3.1, 4.9.2, 4.9.4, 4.11.1, 4.12.8, 4.13.2. The dark field analysis technique is also an important part of the techniques for quantitative analysis of crystal defects as discussed in sections 3.6, 3.8, 3.11–3.13.

1.5.3 Weak Beam Images

Weak beam (WB) images are formed in CDF using a faint diffraction spot, and have a higher resolution than conventional BF or CDF images. This technique has gained wide acceptance as a method of studying a variety of crystal defects, see section 3.17. It is essential to avoid any bright reflections in the diffraction pattern to simplify interpretation of the image. Under these circumstances resolution increases with increasing deviation from the Bragg reflecting position and it is known that high-resolution dislocation images require a value of $\dagger s \gtrsim 3 \times 10^{-2} \text{ \AA}^{-1}$. The maximum usable specimen thickness is determined by the amount of inelastic scattering which destroys the contrast. Inelastic scattering depends on both material and the particular reflection. In copper with a $\{220\}$ reflection the maximum useful thickness is $\sim 800 \text{ \AA}$ for maximum resolution. However, thicker regions may be used in silicon for $\{111\}$ reflections.

The simplest experimental method of obtaining a high-resolution WB image involves forming a CDF image with a first-order reflection while the bright Kikuchi line (section 2.6.3) lies well outside the third-order reflection. This may be achieved on any microscope but the procedure for one with electromagnetic beam tilt is shown below.

(1) Form a BF image of the region of interest and tilt the specimen to produce two beam reflecting conditions $\ddagger$ with the reflection 1 of interest, see figure 1.12(a) in which the position of the optic axis is indicated by the dashed outline of the objective aperture.

(2) Check that the objective centre of rotation is

* In microscopes for which the beam tilt controls cannot be separately aligned, it may be necessary to defocus condenser 2.

$\dagger s$ is the parameter for describing the deviation from the Bragg reflection, see section 2.4.

$\ddagger$ Only the diffracted spot of interest should be bright in the diffraction pattern.

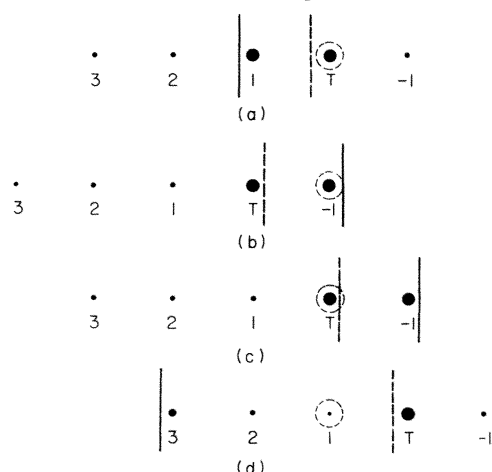


Figure 1.12 The sequence of events in the diffraction pattern on the viewing screen when forming weak beam images

correctly adjusted using the edge of the specimen nearby and the objective pole piece controls, see section 1.4.1.

(3) Form a CDF image on the opposite spot using the beam tilt controls as described in section 1.5.2, see figure 1.12(b).

(4) Return to BF and tilt the specimen until the diffraction spot 1, actually used to make the CDF image is bright, with the bright Kikuchi line lying just outside it, see figure 1.12(c).

(5) Record the diffraction pattern.

(6) Form a BF focussed image and record it.

(7) Switch to CDF and record the WB image without refocussing.

(8) Record the diffraction pattern. It will be found, figure 1.12(d), that the bright Kikuchi line will lie just outside the third-order reflection.* With this technique the position of this bright Kikuchi line in relation to the third-order reflection will always be the same as that at point 3 above relative to the first-order reflection, that is outside, through, or inside it.

In effect we have produced a strong reflection and used it to form CDF rather than the opposite reflection as outlined in section 1.5.2. Typical images with diffraction patterns are shown in figure 3.52.

If the very highest resolution is not required, a smaller value of s may be tolerated and the image may be viewed directly on the screen. A conventional CDF image may be formed, section 1.5.2, then the specimen tilted to increase s until the desired resolution is obtained. However, in general it will be necessary to refocus the image after tilting. Consequently, this technique is not satisfactory for very faint images.

Note that, for all of the above procedures, the

resolution obtainable from magnetic specimens will be inferior to that from non-magnetic specimens. The magnetic field of the specimen will introduce astigmatism in the WB image, because the magnetic correction described in section 1.7.1 is performed in BF before either the final beam, or specimen, tilt. The WB image is too faint to permit this correction.

The major experimental problem in obtaining WB images is the long exposure time which necessitates very low specimen drift rate. In general exposure times should be minimised, typical values being $\lesssim 15$ s. This may be achieved by the following.

(1) Maximising both beam intensity and film speed (consistent with retaining a reasonably fine grain size in the film). The beam intensity may be improved by increasing the gun bias and working with a well, but not fully, focussed condenser 2. Do not overfocus condenser 1 from normal operating conditions because this will increase the specimen temperature and therefore specimen drift.

(2) Use of reasonably large condenser 1 and 2 apertures (because the improvement in resolution obtained by reducing them is offset by the reduction in resolution arising from increased exposure times).

In general pointed filaments should be avoided because their instability largely offsets any likely improvement in resolution. Thin film objective apertures should be used because their low contamination rate does not significantly reduce resolution. Finally, image magnifications in the range $30\,000\times$ – $60\,000\times$ should be used followed by enlargement during printing.

1.5.4 Multiple Dark Field Images

It can sometimes be useful to form low magnification images of each diffracted spot in a diffraction pattern so that the crystal defects giving rise to each spot may be rapidly identified. Unfortunately these images cannot both be in focus and come from exactly the same region of the specimen.

It was pointed out in section 1.5.1 that the action of forming the diffraction pattern involves focussing either the diffraction or intermediate lens on the back focal plane of the objective lens. Referring to figure 1.10(a), it will be clear that, if the diffraction lens is focussed at a level between the back focal and image planes of the objective lens, each diffracted spot becomes broadened and is a low magnification image of the selected area of the specimen. However, the images are out of focus

* For a $\{220\}$ reflection in copper this corresponds to $s \gtrsim 3 \times 10^{-2} \text{ \AA}^{-1}$. However, for a spot closer to the transmitted, s will be less than this value. In such a case, it will be necessary to tilt the specimen initially until the second-order reflection 2, is bright to obtain the bright Kikuchi line outside the fourth-, fifth- or sixth-order reflection and a satisfactory value for s .

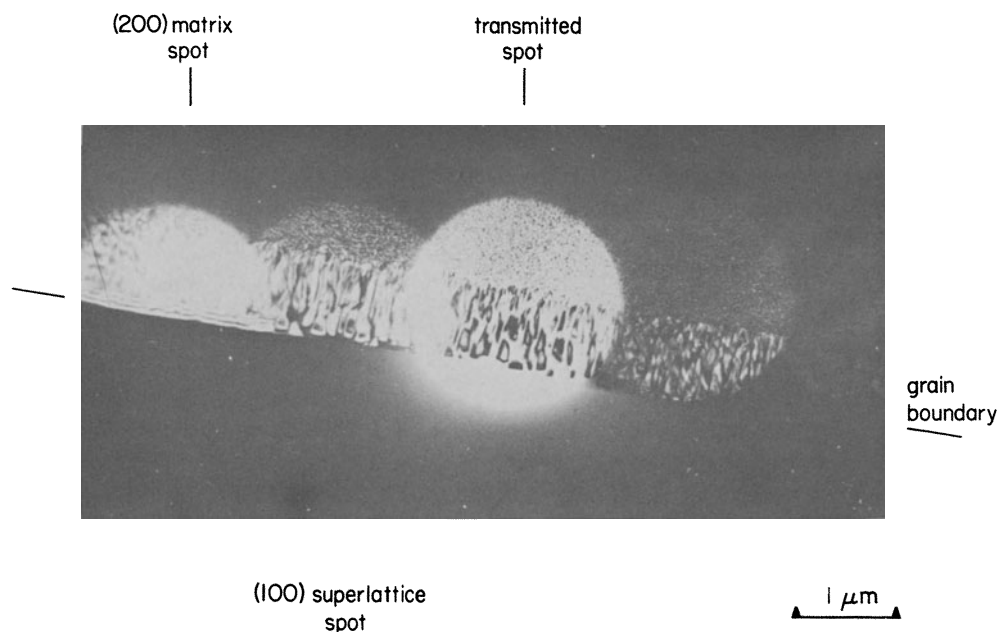


Figure 1.13 An in-focus multiple dark field image of a lamellar precipitate formed by a discontinuous reaction at a grain boundary in an aged Al–10.7 wt% Li alloy. Fine precipitates are visible in grain [Courtesy of D. B. Williams]

because the diffraction lens is not focussed on the objective image plane. An in-focus image may only be produced if the objective lens is refocussed to move its image plane up to the level of focus of the diffraction lens. Then the selected area condition is no longer preserved because the objective image plane and the selected area aperture are not coincident. Thus, as pointed out in section 1.5.1, each diffracted beam arises from a different part of the specimen and shows a different area.

It is a major advantage of the in-focus multiple dark field image that the contribution of any particular feature of the specimen to each spot may be readily determined. An example is shown in figure 1.13 for a discontinuous precipitate at a grain boundary. Clearly the (100) spot arises from the precipitate itself, that is only the precipitate is bright. In contrast the (200) spot contains contributions from both precipitate and matrix (both bright) because the ordered precipitate has lattice parameter close to that of the matrix. A further advantage of this approach is that lens rotations need not be allowed for when transferring crystallographic directions from the diffraction pattern to the image* because both image and diffraction pattern are here formed at the same lens currents.

The magnification of multiple dark field images is limited by their overlap when there is continuity of the actual image. As pointed out in section 1.5.1, the area selected is displaced parallel to the line joining the transmitted and diffracted spots. This effect is clearly visible in figure 1.13. By using a sufficiently small selector aperture it is possible to

work at screen magnifications of up to $20\,000\times$, which is adequate for most studies because the resolution of the dark field images is no better than that for displaced aperture dark field, described in section 1.5.2.

1.5.5 Phase Contrast Images

Phase contrast effects are important in direct lattice plane resolution (section 3.19), multi-beam lattice images (section 3.20), Moiré patterns (section 3.21), some high-resolution dark field images (section 3.22) and point-to-point resolution tests using carbon replicas (section 1.12). As pointed out in section 3.18 the electrons leaving the bottom surface of a thin electron microscope specimen have small phase differences arising from scattering in the specimen. However, to convert these phase differences into intensity differences in the final image, it is necessary to introduce additional phase shifts using the spherical aberration and defocussing effects of the objective lens. The following simple physical picture demonstrates why these additional phase shifts occur.

Consider a specimen that scatters electrons which pass through the objective aperture and are recombined with the transmitted (undeviated) electrons to form the image. Assume that objective astigmatism has been corrected, section 1.4.2, and that chromatic aberration is negligible, that is a thin specimen with no inelastic scattering. For the in-focus situation both image and viewing plane coincide, see figure 1.14. A ray leaves the object at an angle β such that it just enters the objective

* See section 1.11.3.

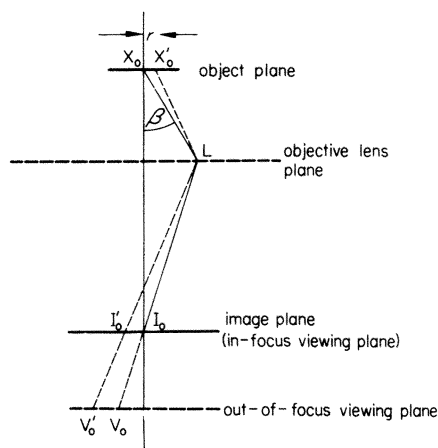


Figure 1.14 A ray diagram illustrating the phase difference produced by spherical aberration and defocussing the objective

aperture, that is β is the aperture semi-angle, figure 1.4. If the lens is perfect this ray will be focussed back on the optic axis at I_0 in the image plane. However, allowing for spherical aberration, the ray will be focussed back to the optic axis short of I_0 in figure 1.14, and will follow the path X_0LI_0 to intersect the image plane at I'_0 . Thus it will appear to have originated at X'_0 in the object. The path difference introduced relative to the perfect lens case is $X_0LI_0 - X'_0LI'_0 \sim \beta r$. However, $r = C_s \beta^3$ so that the path difference is $C_s \beta^4$.

If the viewing plane is actually below the image plane, that is the objective lens is defocussed by Δf , a further path difference is introduced $V_0I_0 - V'_0I'_0$, figure 1.14, which Heidenreich (1964) has shown to be $\frac{1}{2}\Delta f \beta^2$.

The total phase shift in the defocussed case, including both effects is $2\pi/\lambda$ (path difference), that is

$$-\frac{2\pi}{\lambda} (C_s \beta^4 - \frac{1}{2}\Delta f \beta^2) \quad (1.12)$$

for small aperture angles.

Two important conclusions may be drawn from this treatment.

(1) The defocus term rapidly becomes dominant because of the small angles ($\sim 10^{-3}$ rad) involved. Thus when Δf is a few hundred angströms the spherical aberration term may be neglected.

(2) For a given amount of defocus, Δf , only certain spacings within the object will be transferred (that is be visible) in the image. This arises because maximum phase contrast occurs for phase shifts $(2n - 1)\pi/2$ where n is $\pm 1, \pm 2$, etc. These may be

substituted in equation (1.12) leading to a series of specific values for the angle β which are equivalent to particular distances Λ in the specimen. Thus for diffraction the spacing Λ in the image is $\lambda \sin \beta = \lambda \beta$. These particular spacings Λ are transferred into the image but those between are not.[†] The minimum spacing transferred for a given defocus will depend on objective aperture size up to the resolution limit of the lens, as defined in section 1.3.

The last conclusion is most important because it shows that only a limited amount of phase contrast information is transferred to the image for a given aperture and a particular value of defocus. This may be demonstrated experimentally using images of thin carbon films and an optical diffractometer. Carbon films contain a complete range of particle spacings Λ which produce a complete series of phase contrast information at the exit surface of the specimen. Consequently the transfer of phase information from object to specimen may be readily checked.

Figure 1.15 gives an example of the evaluation of a phase contrast image. The upper series of images were taken from a carbon film passing through focus from left to right. The lower series of pictures are their light optical diffraction patterns obtained from a laser-source optical diffractometer. These diffraction patterns consist of clearly defined rings that correspond to the resolution of particular distances in the image.

In figure 1.15(b), near focus gives very poor imaging and only one small range of image spacings (one ring) are transferred to the image, $\equiv n = 1$. With increasing defocus more spacing information is transferred to the image (more rings present) but specific object spacings are not transferred to the image as discussed above. Optical diffraction patterns of this type provide a better resolution check of the electron microscope than direct measurement of spacings in the image.

In using the defocussed objective to form phase contrast images it is conventional to obtain a through-focus series of images, close to exact focus. Scherzer (1949) has defined an optimum defocus as

$$\Delta f = (C_s \lambda)^{1/4}$$

which provides an indication of the approximate amount of objective defocus to use. However, there is no general concept of optimum defocus because the effect of defocus on the image depends on the specimen. Thus, for point-to-point resolution in a carbon film the defocus chosen will depend

* A more accurate relationship of almost the same form but with a smaller spherical aberration term has been given by Scherzer (1949).

† More rigorous treatments (Thon, 1971; Lenz, 1971) lead to the same general conclusions. The transfer of object spacings from object to image is expressed graphically in terms of 'phase contrast transfer functions' that enable the imaging properties of a lens to be defined in terms of its design parameters.

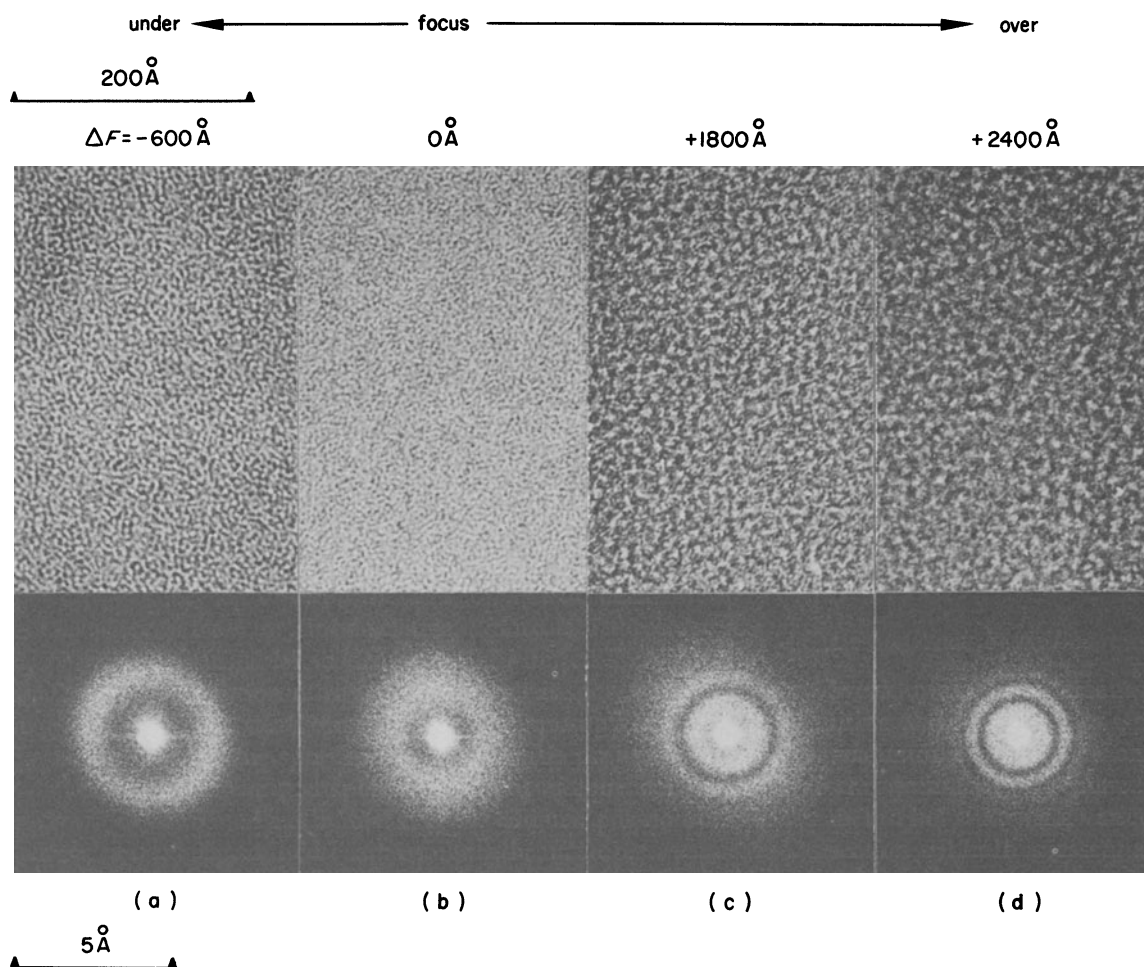


Figure 1.15 A focussing series on a carbon film (top sequence) and the corresponding light optical diffraction patterns (bottom sequence). The rings in the optical diffraction pattern give the spacings present in the image. The top scale gives the magnification of the image, while the bottom scale describes distances in the diffraction pattern 100 kV, C_2 aperture 200 μm , no objective aperture, Δf is increment of defocus [Courtesy of Philips, Eindhoven]

upon the resolution of the instrument through the contrast transfer function. However, for direct lattice images, the defocus required will depend upon the correspondence between image and specimen detail, see section 3.20.

Two alternative types of imaging technique may be employed in forming phase contrast images. These involve axial or tilted illumination. For a given microscope the latter produces the higher resolution.

Axial illumination for two-beam* and three-beam† diffracting conditions is shown schematically in figure 1.16(a), (b). In both cases the objective aperture remains on the optic axis and admits the axial transmitted, together with one or two non-axial diffracted beams. These all interfere to form the final image as described in section 3.19. This situation may be generalised to include a large number of diffracted beams, see section 3.20, for reflections with large interplanar spacings, that is close-in diffracted spots, when resolution is not significantly degraded by spherical aberration.

* Two-beam conditions are defined in section 3.2.1.

† In this case, positive (hkl) and negative ($\bar{h}\bar{k}\bar{l}$) reflections are used, as defined in section 2.7.2.1.

Axial illumination is used for point-to-point resolution tests with carbon replicas but without an objective aperture. Tilted illumination is commonly used for lattice resolution using metal specimens. In this case the incident illumination is tilted as described in section 1.5.2 for CDF images but only until the optic axis lies at the centre of the array of diffraction spots to be included within the objective aperture. This is shown schematically in figure 1.16(c), (d).

High-resolution phase contrast images require the microscope to be aligned for maximum resolution and require careful and precise operation technique. Particular attention should be paid to alignment of the objective lens axis on the optic axis, and to the correction of objective astigmatism. The latter may be minimised by using a self-cleaning objective aperture which should be inserted before astigmatism correction using the image background structure or a nearby hole to give $\Delta f \lesssim 500 \text{ \AA}$ (see section 1.4.2). In general the microscope is operated at 100 kV to minimise energy losses in

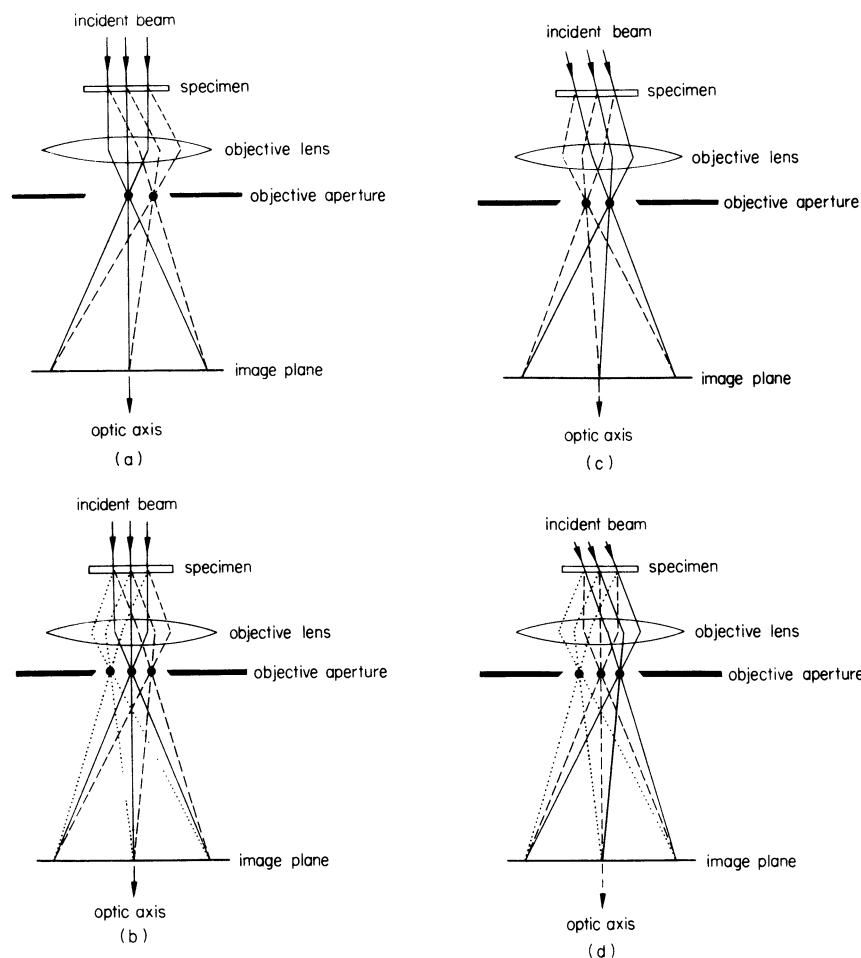


Figure 1.16 Ray diagrams illustrating axial objective illumination for (a) two-beam and (b) three-beam conditions, and tilted illumination for (c) two-beam and (d) three-beam conditions

the specimen which degrade resolution through chromatic aberration. Suitable values for specimen drift, and contamination, rates are $<0.1 \text{ \AA s}^{-1}$. A coherent incident beam must be used. Depending upon the microscope this may be achieved by strengthening condenser 1, varying the diameter of Wehnelt cap, see section 1.1, or using a pointed filament. Condenser lens aperture diameters must be minimised (to reduce condenser lens aberration effects on coherence) consistent with retaining enough intensity for comfortable visual observation of the image. Finally suitable periods must be allowed for stabilisation of HT, lenses and specimen illumination: and mechanical vibration must be minimised.

In general the area selected should be close to a grid-bar to minimise thermal drift or charging of the specimen. It is better to photograph quickly with a little residual astigmatism than to spend a long time correcting astigmatism, and thereby contaminate the area. A suitable value of under-focus for fringe images is $500\text{--}750 \text{ \AA}$ but for point-to-point resolution on carbon replicas, a through focus series is required because of the limited contrast transfer, section 1. For stable specimens

the beam intensity may be increased to give 2–5 second exposure times at plate magnifications $<250\,000\times$ but lower intensity may be required if specimens are unstable in the electron beam.

In most cases only a part of the micrograph will show lattice, or maximum point-to-point, resolution. It is important to emphasise that high-resolution images are not obtained easily and depend upon achieving a high standard of maintenance of both the vacuum system and power supplies. Those pictures included here represent the best of a number of series of images.

The condition of the specimen is important; a poor specimen can produce misleading high-resolution micrographs. Freshly prepared specimens are recommended; and these should be as clean and as uniformly thin as possible, with minimum bending in the regions of interest. Metal specimens should be as free of oxide as possible. Suitable carbon replicas for point-to-point resolution tests may be prepared using the technique outlined in appendix 12. A typical useful thickness is $\sim 30 \text{ \AA}$ which is consistent with the minimum thickness to avoid significant film breakage on a 200 mesh support grid. For direct lattice resolution,

optimum thickness is 0.25 or 0.75 ξ_g^* at which maximum contrast occurs. Specimens with large areas of suitable thickness may be prepared by extremely careful electro-thinning (Phillips, 1973) or evaporation onto heated rocksalt or mica (Menter, 1958). Satisfactory partially graphitised carbon black specimens of similar thickness may be prepared using the approach outlined by Heidenreich *et al.* (1968). Typical pictures of lattice resolution in gold are shown in figure 1.21(a) while pictures of graphitised carbon black and point-to-point resolution carbon replicas are shown in figures 1.21(c) and 1.15 respectively.

1.6 Stereo Microscopy

Stereo microscopy is an important technique in both replica and transmission studies. Its primary use is to provide a three-dimensional picture of the field of view and to enable 'depth' measurements to be made.

The stereo technique is simply an extension of human binocular vision. Thus an object is observed from two slightly different directions simultaneously and the two images are combined in a stereo viewer to give a three-dimensional picture. In the electron microscope, the technique is to photograph the field of view at two different settings of the specimen tilt, that is from two directions, and to observe the negatives or prints in a stereo viewer.

For carbon replicas it is possible to use the tilt/rotation holder to tilt about a particular direction in the micrograph. However, for thin foils where the same diffracting conditions must be maintained during tilting, see section 2.10.12, the double-tilt holder is more convenient.

Stereo microscopy enables a further and most important measurement to be made, namely the depth of defects in a thin foil. It has been shown by Diepers (1967) that the height difference Δh between two points in a thin foil is

$$\Delta h = \frac{\Delta p}{2M} \sin \frac{\phi}{2} \quad (1.13)$$

where M is the magnification, ϕ is the angle of tilt and Δp is the parallax between the two points viewed in stereo. The latter parameter can be determined using a Carl Zeiss mirror stereoscope N2 and for $\phi/2 = 5^\circ \pm 0.14^\circ$, and with M accurately known to within 5%, Δh can be measured to $\pm 10 \text{ \AA}$ as the maximum error. The direction of the tilt axis is not important and the experiment can best be performed with the double-tilt stage to maintain constant reflecting conditions as described in section 3.10.1.2. The absolute depth of a defect can also be measured if the specimen surface is

decorated with gold (Diepers, 1967), see sections 3.5.5, 3.5.6. Commonly angles of tilt of 20–30° are used, which can be measured accurately from diffraction patterns using the techniques outlined in sections 2.7.2, 2.7.3, 2.10.1. These techniques are very important in determining the sense of strain field images from small defects, see sections 3.5.6, 3.11. It should be noted that photographs at exactly the same magnification can be obtained if the specimen height adjustment is set before tilting, because this almost removes any vertical movements of the field of view during tilting. Thus only minor adjustment of the objective lens is necessary to maintain focus. Where the tilt axis cannot be set coincident with the field of view, significant vertical movement of the specimen often occurs during tilting, and the change in magnification arising from refocussing the objective lens must be taken into account.

In studies of thin foils, stereo techniques are extremely useful, for example to determine if precipitates lie in thin foils or on the surface. A much improved appreciation of the true three-dimensional nature of defect configurations in thin foils is obtained by this method. Although it is common to perform stereo microscopy under two-beam dynamical conditions the technique can also be applied to the high-resolution weak beam images described in section 3.17.

1.7 Magnetic Specimens

1.7.1 Magnetic Correction

Ferromagnetic specimens can seriously reduce resolution in conventional BF and CDF images because their own magnetic field distorts that of the objective lens. The problem is particularly severe for microscopes using a side entry specimen holder because the objective is then an immersion lens. In general the field distortion is asymmetric because the electron-transparent areas are generally at the edge of the foil and the bulk of the specimen is situated to one side of the area of interest. As a result the transmitted beam deviates from the optic axis of the objective lens with consequent loss in quality of the image. The incident beam must therefore be tilted to counteract this effect but the amount of tilt is much less than that necessary for high-resolution dark field described in section 1.5.2. The essential requirement of the technique is to tilt the illumination until the centre of rotation of the objective lens returns to the screen centre, as described in section 1.4.1.

The procedure for most microscopes is as follows.

- (1) Centre a recognisable point at the edge of specimen and focus the second condenser lens.

* ξ_g is the extinction distance defined in section 3.2.3.1.

Focus the image and remove the objective aperture. Centre the beam. This situation is the standard condition to which you should return after alignment.

(2) *Overfocus* the second condenser lens by one coarse click, *defocus* the objective to as near minimum as possible on the coarsest control.

(3) The point will move away from screen centre and should be returned by the beam or gun-tilt controls depending upon the microscope.

(4) Refocus the beam with second condenser lens, refocus image, and recentre the beam.

(5) Repeat (2)–(4) and continue until the point remains central.

In effect this is the procedure for aligning the rotation centre of the objective lens, as described in section 1.4.1. The details may vary slightly from instrument to instrument, depending upon its design features. Note that, if the same set of beam tilt controls are used to obtain CDF images as described in section 1.5.2, the magnetic correction will be lost for the BF image. Consequently, in microscopes where two methods of tilting the beam are available, one should be reserved for CDF. If not, it is a relatively simple matter to install an additional set of potentiometers for this purpose.

Because of the variation of local magnetic field produced by specimen translation or tilting, the magnetic correction must be repeated frequently. For ease of correction small foil window specimens are preferable because of their small magnetic field. Disc specimens should be avoided because of their large magnetic field. In all cases the specimen should be securely clamped in position or it may move on tilting because of its interaction with the magnetic field of the objective lens.

1.7.2 Lorentz Microscopy

Transmission electron microscopy may also be used to observe magnetic domain boundaries directly, a technique known as Lorentz microscopy, see section 3.23. There are two approaches, both of which aim at reducing the magnetic saturation of the specimen.

(1) The objective lens is either switched off or operated at a very low current, that is usually below the normal range provided by the manufacturer (Fuller and Hale, 1960; Blackburn *et al.*, 1969). Under these conditions the intermediate and/or the projector lenses are used to produce a low magnification image.

(2) The objective lens is excited at a low current but the specimen is raised above its field by using a special (Lorentz) holder.

The microscope operation conditions for a three-lens magnification system are described in

the papers by Fuller and Hale (1960) and Blackburn *et al.* (1969). Under these conditions the method of determining image magnification and defocussing distance described by Wohlenberg and Henriksen (1971) may be used.

Out-of-focus images of domain walls may be produced in the EM300/301 with the specimen in its normal position. The objective lens is switched off and both the intermediate and projector lenses, see figure 1.1, are used to change magnification while the image is focussed with the diffraction lens. In-focus images may be formed in the same way but the SAD aperture is used to limit the part of the diffraction spot contributing to the image. The resolution of the diffraction pattern may be controlled by the condenser 1 setting.

The procedure is as follows.

(1) Focus the diffraction lens on the plane of the C_2 aperture.

(2) Focus condenser 2 in this plane.

(3) Leaving condenser 2 fixed, focus the diffraction lens on the specimen.

(4) Use the intermediate and projector lenses to alter the image magnification.

Under these conditions the area of the specimen illuminated is defined by the condenser 2 aperture which may need to be increased in size.

1.8 Beam-sensitive Materials

Organic materials such as polymers, proteins, etc., together with inorganic salts such as chlorides and bromides degrade during observation in the electron microscope because of damage from the electron beam. These are discussed separately below.

Nevertheless, it is possible to make direct studies of the deformation modes of polymer single crystals (Allan *et al.*, 1973) and commercial polymers (Beahan *et al.*, 1972). In addition, spherulite size may be measured directly and nodular size and distribution may be determined (Carr *et al.*, 1968).

The process of polymer breakdown has been studied by a number of workers; for a review see Grubb and Keller (1972). In practice, in order to prolong the life of a polyethylene single crystal for only 3 min, it is necessary to use the lowest beam current possible, that is a very faint image viewed on the screen at $2000\times$ magnification. Under these conditions the spots in the diffraction pattern are lost in 2–4 min and diffraction contrast is lost, see figure 1.17. At present image intensifiers do not enable the life of the specimen to be prolonged significantly but improvements may occur as their efficiency improves.

It is important to realise that, although image detail is present for all polymer specimens after

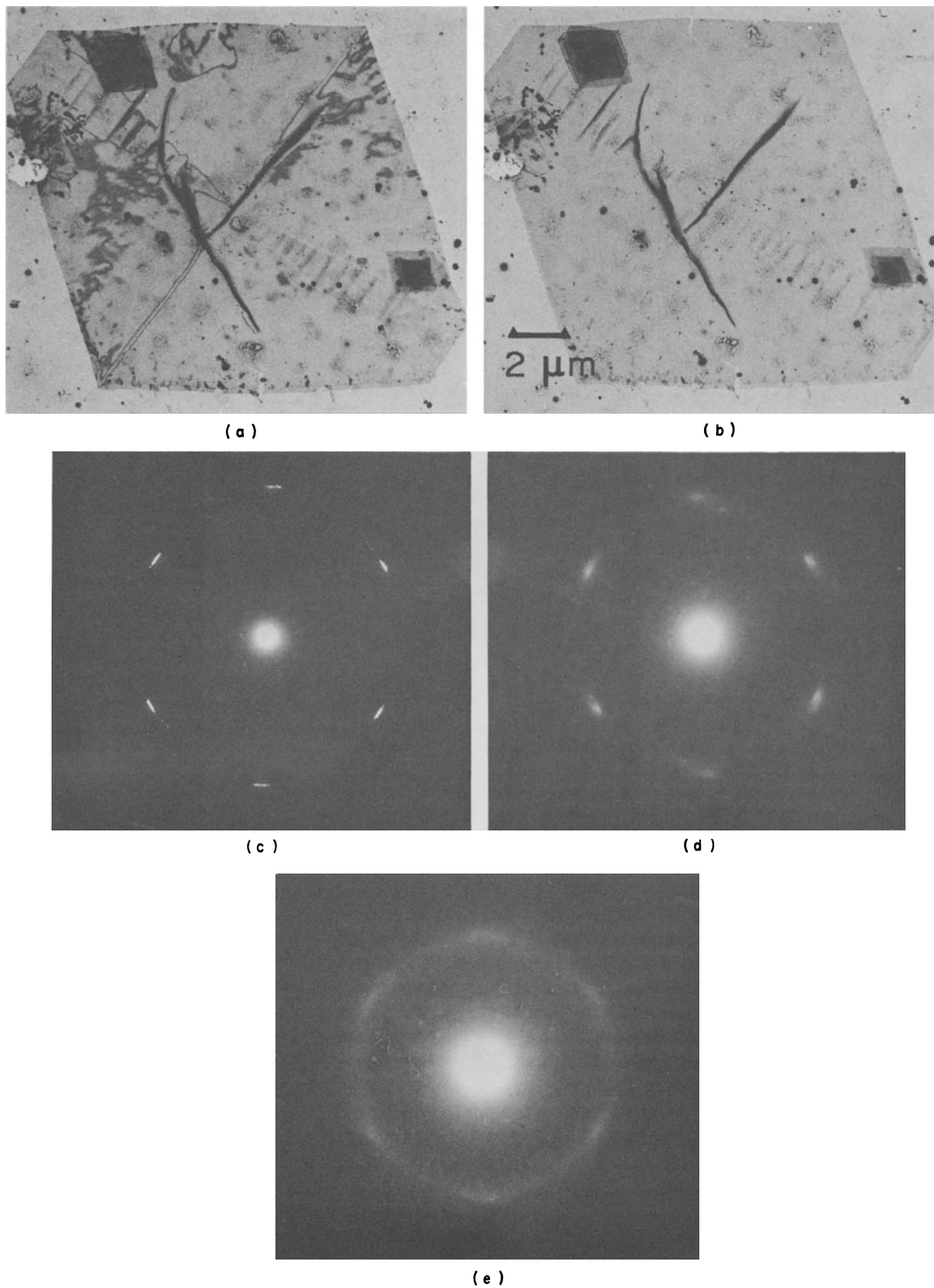


Figure 1.17 Transmission electron micrographs of single-crystal polyethylene (a) crystalline, 5 second exposure after 10 seconds irradiation, (b) after observation for 2½ min at 100 kV when crystallinity is largely destroyed by the electron beam dose 75 cm^{-2} , (c)–(e) a sequence of selected area diffraction patterns from a polyethylene single crystal showing loss of crystallinity during observation in the electron microscope (c) 20 seconds (d) 3½ min (e) 6½ min (dose 90 cm^{-2}), exposure [Courtesy of D. T. Grubb]

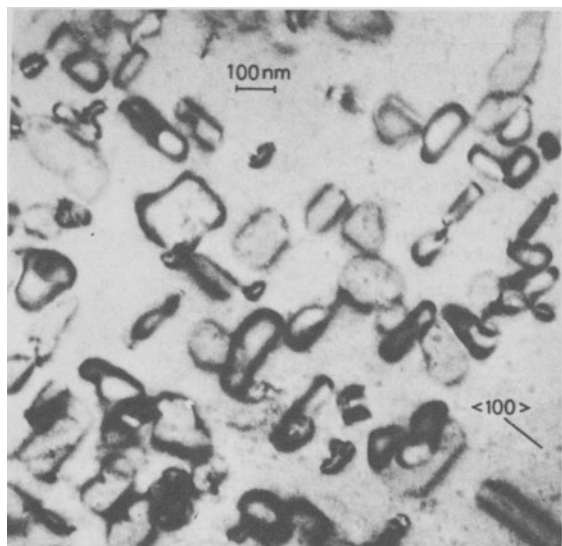


Figure 1.18 Dislocation loops produced in potassium iodide by observation in the electron microscope at room temperature [Courtesy of L. W. Hobbs]

crystallinity is destroyed, the specimen frequently becomes distorted during degradation. Consequently, image detail may not be simply related to the original structure.

In the case of alkali halides, thin foils suffer extensive irradiation damage in the microscope. Thus dislocation loops are formed by interstitial condensation in potassium iodide observed at room temperature, see figure 1.18. The observable damage may be reduced by lowering the specimen temperature to $\sim 10^\circ\text{K}$ which is below the anion interstitial mobility temperature of $\sim 30^\circ\text{K}$. In this way ~ 30 s observation time is possible at a current density at the specimen of $10^{-3} \text{ A cm}^{-2}$ before damage masks existing features. Alternatively, dopants which lead to damage reduction (Pooley, 1966) may be added or the specimen temperature may be maintained in a range for which the halide exhibits reduced ionisation cross section, for example KI at low temperatures, NaBr, NaI and LI at room temperature. Alternatively, the beam current may be reduced and an image intensifier system used.

1.9 Low Angle Diffraction

In studies of materials with large repeat distances ($20\text{--}20\,000 \text{ \AA}$) such as inorganic molecules and polymers, very large camera lengths (up to 150 metres) are essential. Specialised operation procedures have been reported by Bassett and Keller (1964), Drahos and Delong (1966), Wade (1967), Wade and Silcox (1967) and Curtis *et al.* (1967). In these cases the area of the specimen contributing to the diffraction pattern is much greater than for conventional SAD, section 1.5.1. The procedure due to Wade for the EM300 is given below.

The first condenser lens is used at or near maximum excitation to produce a strongly demagnified spot. The second condenser is used to image this spot in a plane well above the specimen plane. Normal condenser apertures are used but clearly this method of use of the double condenser provides a beam of extremely high coherence.

The objective lens is switched off, the specimen remains in its normal position and the area giving rise to the diffraction pattern is selected by the objective aperture. Very small area selection is thus not possible.

The pattern produced by the specimen is focussed by the diffraction lens and magnified by the intermediate and projector lenses. Variation of the intermediate lens produces variation of the camera length and thus of the dispersion. The projector is not varied from its optimum excitation.

The area from which the pattern arises is made visible simply by refocussing the diffraction lens to focus the image rather than the pattern. The image so obtained is similar to the images obtained using the microscope in its very low magnification conditions.

The maximum camera length available with this type of technique is about 150 metres: spacings of $\sim 2 \mu\text{m}$ are resolvable in the diffraction pattern.

1.10 Analysis of Plates

All manipulations of plates, rotation of diffraction patterns relative to images, determinations of the sense of tilt, etc., are performed with the plates *emulsion side up*. All prints and enlargements are obtained with the *negative emulsion side up*, which is contrary to normal photographic practice. This approach preserves continuity between specimen manipulations and events on the viewing screen/negative/print. If this procedure is not followed 180° inversions occur and errors in many of the analyses described in sections 2.10.3, 2.11.2, 2.14.1, 2.14.2, 3.4.3, 3.5.2, 3.5.4, 3.5.6, 3.6.2, 3.6.4, 3.7.2, 3.8.2, 3.9.2, 3.11.2, 3.12.2, 3.13.2 will occur.

1.11 Calibration of the Microscope

Accuracy in calibration of the instrument for magnification of image and diffraction pattern, and the relative rotation between the two, is critically important for quantitative electron microscopy.

There are two types of calibration.

(1) *Permanent* calibration of the parameter against standard instrument settings. This calibration is always obtained for a new microscope then checked periodically during its life, or if the instrument is modified, for example specimen stage changed. This calibration is normally adequate for most purposes.

Table 1.3 Suitable specimens for magnification calibration or resolution checks

Fringe spacing (Å)	Material	Reference	Comments
1.44 and 2.04	single-crystal gold <i>B</i> [100]	Komoda (1966)	stable in electron beam
3.4	partially graphitised carbon black*	Heidenreich <i>et al.</i> (1968)	stable in electron beam
4.5	mica	Yada and Hibi (1968)	reasonably stable in electron beam
4.4	pyrophyllit*	Dowell (1962)	reasonably stable in electron beam
6.94	potassium and chloroplatinate	Komoda and Sakaba (1959)	reasonably stable in electron beam
4.55 and 9.03	crocidilite Asbestos*	Agar (1973)	reasonably stable in electron beam
12.6	copper pthalocyanine	Menter (1958)	unstable in electron beam
	platinum pthalocyanine	Menter (1958)	unstable in electron beam
67.5 and 87.5	catalase*	Wrigley (1968), Murray and Ferrier (1968)	reasonably stable in electron beam

(2) *In situ* calibration against a standard specimen. This is usually performed when greater accuracy than the permanent calibration is required. However, considerable attention to sample preparation and analysis is necessary.

Both approaches are discussed in the following sections 1.11.1–1.11.3. Generally one should note that, if the microscope has axis-centred tilt facilities, section 1.4.4, provided that these are set, then it is operated at a standard objective lens current and the calibrations are relatively simple. If not, the specimen will change height by up to ± 1 mm during tilting, necessitating considerable changes in objective lens current to retain focus. In such cases, all calibrations should be performed as a function of objective lens current monitored on a meter. A suitable range of currents may be obtained by working with a region near the edge of the grid.

1.11.1 Image Magnification

Permanent calibration of magnification is generally only accurate to $\pm 5\%$ and to improve to $\lesssim \pm 2\%$ using *in situ* calibration requires considerable attention to detail. A wide range of methods has been reported, including diffraction grating replicas (Oster and Skillman, 1962; Bahr and Zeitler, 1965), direct lattice images (Dowell, 1964; Murray, 1968; Cox and Horne, 1968), latex spheres (Backus and Williams, 1948, 1949; Gerould, 1950), metal mesh (Skinner, 1969) and Moiré patterns (Dowell, 1964; Dowell *et al.*, 1956, 1958). The most commonly used are diffraction grating replicas for low and intermediate magnifications and direct lattice images for high magnifications. These are described below.

1.11.1.1 Diffraction grating replicas

Standard specimens in either carbon or silicon monoxide are available from most accessory

suppliers, the latter being recommended because of their greater stability in the electron beam. The number of lines per millimetre available are 617, 1134 and 2160, suitable for the magnification range $100\times$ – $200\,000\times$. At low magnifications the distances between the lines may be measured, whereas at higher magnifications distances between two recognisable points may be used.

For *in situ* calibration the grating replica may be mounted with the specimen, or alternatively the less accurate latex balls may be used.

1.11.1.2 Direct lattice images

These images may be produced by allowing the transmitted and one or more diffracted beams to interfere and form an image, see section 3.19. The separation of the lines in the image corresponds to the reflecting plane spacing within the limitations discussed in section 3.19. Table 1.3 lists some of the materials that may be used for this purpose. The only materials recommended are those which are stable in the electron beam. Those classified as reasonably stable should only be used with a low beam current otherwise they may degrade, leading to changes in interplanar spacing and, finally, loss of fringe image.

The major problems to overcome are variations in interplanar spacing arising naturally from the method of preparation, or from the effect of electron bombardment (Kamiya *et al.*, 1959). Both Dowell (1964) and Murray (1968) have described methods of dealing with such a situation. This latter method involved determination of the periodicity of a catalase crystal by recording its low angle diffraction pattern (section 1.9) combined with that of polycrystalline aluminium to act as a standard. This was followed by direct observation and measurement of the fringe image of catalase.

Since several of the materials (denoted by an

asterisk) in Table 1.3 are available in aqueous solutions or powders they may be used for in-situ calibration.

1.11.2 Diffraction Pattern Magnification

Knowledge of the camera constant λL (equation (1.11)) greatly simplifies indexing of diffraction patterns (section 2.7) and is essential for the identification of second phases (sections 2.13). The accuracy of the calibration depends upon the experiment, but an error of $\pm 1\%$ should be achieved reasonably easily and further improvements are possible, down to $\pm 0.1\%$, with considerable care both in operation of the microscope and in measurement and corrections on the plate. Thallous chloride is a satisfactory material because its lattice parameter is independent of evaporation conditions and exposure to air (Hambling, 1953). However, high beam intensity should be avoided because local evaporation may occur (Reidmüller and Schwaighofer, 1970). An alternative is evaporated gold which is equally acceptable and is commonly used.

Both thallous chloride and gold may be evaporated directly onto the specimen to provide an *in situ* calibration. The specimen may be shadowed to create calibrant-free areas. Alternatively a thin film carried on one of the specimen support grids may be used.

Finally, in some microscopes, for example EM301, a known standard beam tilt may be applied which moves the centre spot on the

diffraction pattern. This may be used to calibrate distance in the pattern directly against Bragg angle to determine λL .

1.11.3 Image-diffraction Pattern Rotation

For many of the analyses described in sections 2, 3 and 4, it is essential to be able to transfer crystallographic information from the diffraction pattern to the image. However, there is a rotation between object and image produced by all magnetic lenses. Since this rotation depends on lens excitation, the image rotates on the viewing screen as magnification is changed. This rotation must be calibrated relative to the diffraction pattern, the latter always being produced with the same lens excitations.

Although several methods have been suggested, that involving $\alpha\text{-MoO}_3$ single crystals is almost always used. This material is prepared by heating ammonium molybdate to red heat in a crucible. Small crystals form on the crucible lid and may be washed into an aqueous suspension. A drop of this is allowed to dry on a grid covered with a carbon support film. Molybdenum trioxide is pseudo-orthorhombic with lattice parameters $a = 3.966 \text{ \AA}$, $b = 13.848 \text{ \AA}$, $c = 3.696 \text{ \AA}$, and is a ribbon with long edges parallel to $[001]$ and surface normal $[010]$ (Hägg and Magneli, 1945).

In practice a series of double exposed negatives are produced, each with a diffraction pattern and an image of the crystal superimposed, see figure 1.19,

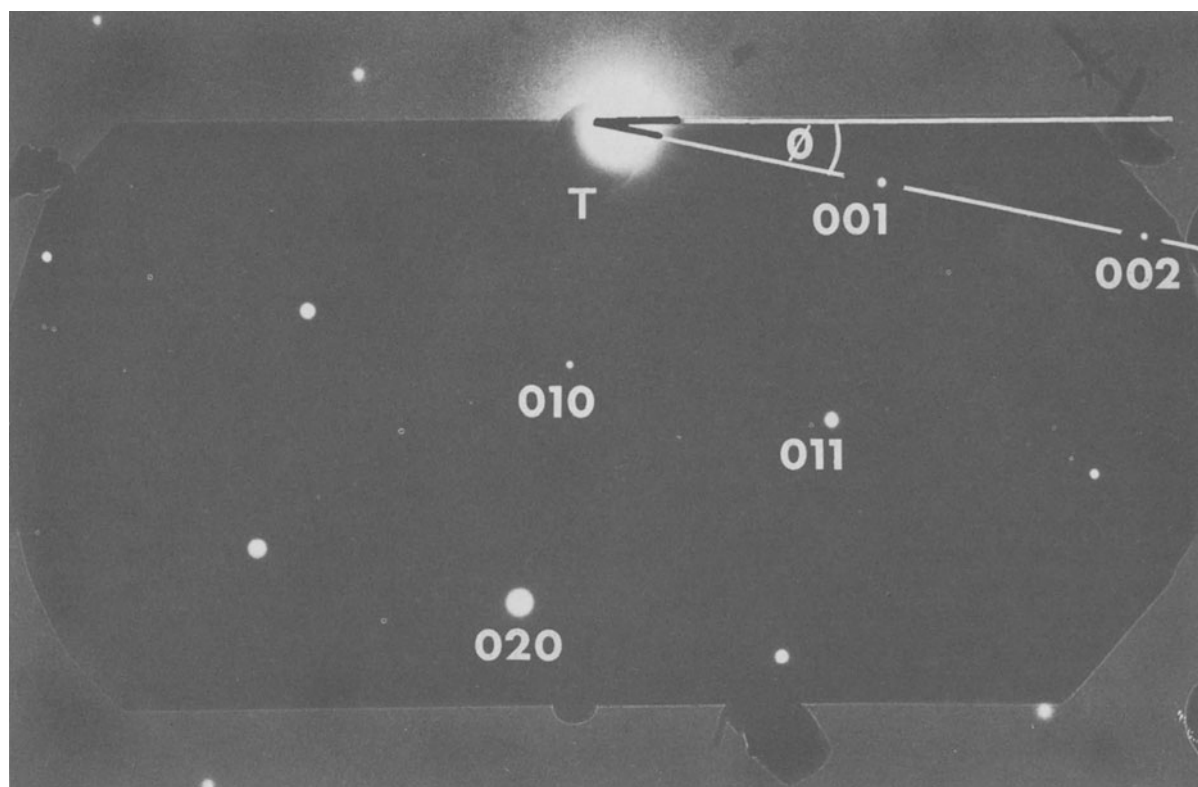


Figure 1.19 A micrograph and superimposed selected area diffraction pattern of a molybdenum trioxide crystal showing ϕ , the angle of rotation, between them [Courtesy of D. B. Williams]

Table 1.4 Image to diffraction pattern 180° inversions for various operational settings for EM300 for standard diffraction conditions

SA or M range	D + I coarse potentiometer on range 4
M1 → 10	180° inversion
SA1 → 10	no inversion
M11 → 25	} 180° inversion
SA11 → 25	

for each magnification. The (010) spot in the diffraction pattern may be indexed on the basis of its interplanar spacing using the camera constant approach outlined in section 2.7.2.1. Then the relative rotation may be measured for each magnification and a graph of rotation versus magnification constructed.

In addition to the above rotation it is necessary to allow for any 180° inversions which may occur between image and diffraction pattern.

As explained in section 1.5.1 the objective lens inverts the image but not the diffraction pattern relative to the object. Thus there is a 180° inversion between image and diffraction pattern which is preserved on the viewing screen of a microscope with a three-lens imaging system. This is because the intermediate and projector lens each inverts both image and diffraction pattern. Consequently in such a microscope the 180° rotation must always be included. However, in a microscope with a four-lens imaging system, such as the EM300/301, the 180° inversion is not always present because some lenses are switched off over parts of the magnification range. Table 1.4 shows these ranges for the EM300 for the standard SADP magnifications used for studies of most metals, that is D + I setting with the coarse intermediate lens potentiometer set at 4 while the fine potentiometer is on maximum ($\lambda L \approx 27 \text{ mm } \text{\AA}$, 100 kV electrons). Conventionally, the permanent image-diffraction pattern rotation calibration is performed with diffraction patterns obtained at this setting; figure 1.20 shows the rotation calibration for the EM301 for these conditions, versus image magnification setting. As with all other calibrations the rotations are measured with plates emulsion side up to preserve geometrical truth relative to the specimen, see section 1.10. The rotation is specified in terms of rotation of the image and must be preceded by 180° rotation of the diffraction pattern within the ranges specified in Table 1.4.

1.11.4 Errors in Use of Permanent Calibration

Permanent calibrations are normally performed at standard lens currents which must be subsequently reproduced exactly during normal operation of the microscope, if the calibration is to apply. If the

microscope has an axis-centred specimen tilt facility, section 1.4.4, then it may be operated at an almost constant objective lens current if the specimen height is correctly adjusted. This minimises errors to $\lesssim 2\%$. However, if axis-centred tilt is not available, different objective lens currents may be required to maintain image focus during $\pm 60^\circ$ specimen tilting because the area of interest may change height by up to 1 mm. Consequently for such a microscope all calibrations in sections 1.11.1–1.11.3 will be affected and they must be performed as a function of measured objective lens current using a permanently incorporated ammeter. The objective lens current must be monitored during subsequent work. If this is not done errors in λL of up to 25% may occur (Armitage and MacConnail, 1964) and magnification changes of the same order are possible.

Lens hysteresis is a further source of error and it arises because the magnetic field of the soft iron components of the lens depends on its recent history through the magnetic hysteresis loop. Thus the focal length of the lens may not be constant for a given lens current.

There are several methods of obtaining reproducible magnetisation of the lens.

- (1) The lens current may be slowly cycled 3 or 4 times to a maximum then to the required focus condition.
- (2) The polarity of the lens may be reversed 3 or 4 times.
- (3) The lens may be switched off and on 3 or 4 times.

For a specific lens current, each of the above procedures causes the lens to adopt a different equilibrium magnetisation. Consequently, the most convenient of the above procedures should be adopted as standard.

In addition there are a number of geometric factors that introduce errors into determination of the magnification of diffraction patterns. These effects may be conveniently discussed in terms of the ring patterns described in section 2.7.1 and figure 2.18, although the results are also valid for spot patterns. The width of the diffracted ring depends upon the grain size of the specimen, its thickness, any elastic strain present and the grain size of the photographic plate. These effects will limit the accuracy of measurement of ring diameters. In some microscopes, lens aberrations may lead to eccentricities in the diffraction patterns and errors of $\pm 0.1\%$ may arise. However, this latter effect can be minimised if an *in situ* standard pattern is used, section 1.11.2.

In addition, the camera constant increases with increasing ring diameter, because the Ewald sphere is only approximately a plane and the error

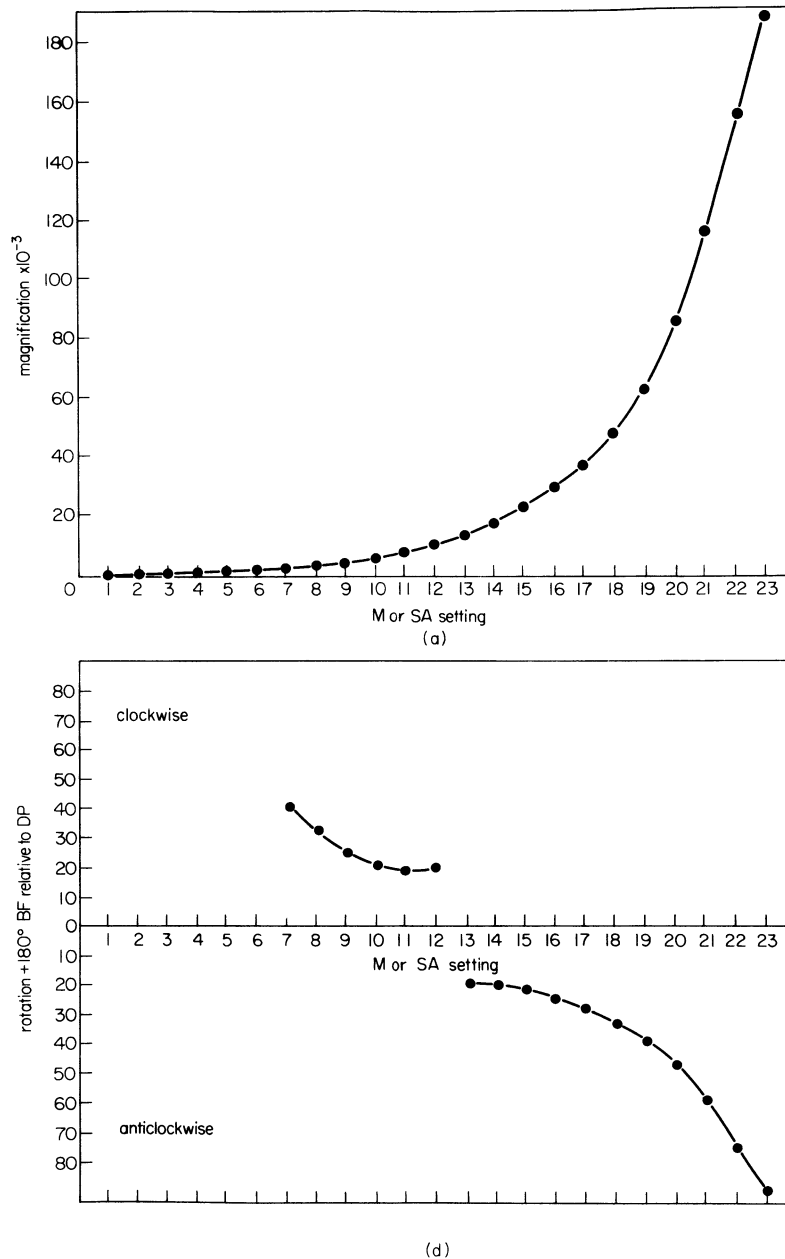


Figure 1.20 Calibration of (a) image magnification, (b) image-diffraction pattern rotation versus magnification (M) or selected area (SA) setting on the EM301 with goniometer stage [Courtesy of D. B. Williams]

in assuming it is a plane increases with increasing Bragg angle. Andrews *et al.* (1971) expressed the correction as

$$Rd = \lambda L \left(1 + \frac{3R^2}{8L^2} \right) \quad (1.14)$$

More recently Lodder and van der Burg (1972) showed that this equation did not represent the correction required in practice and suggested the following empirical relation:

$$Rd = \lambda L + KR^2 \quad (1.15)$$

The experimental correction factor K appeared to be 10 to 20 times greater than the geometrical correction factor $3\lambda/8L$ in equation (1.14). The origin of the discrepancy is unknown.

An additional factor in the case of spot patterns is that the specimen tilt can influence spot spacing, see section 2.7.5.

Finally errors peculiar to the image-diffraction pattern rotation calibration must be considered. These arise primarily from difficulties in aligning plates. If the edges of the plates are used, errors of up to 3° are common because of misalignments in the camera. However, if the edge of the automatic plate numbering mask is used or the SADP and image are superimposed or recorded on continuous roll film, the error may be reduced to <1°.

1.11.5 Additional Measurements

There are a number of calibrations that will be found to be useful. These are discussed below.

1.11.5.1 Accelerating voltage

The accelerating voltage may differ by up to $\pm 10\%$ from the nominal value, and it should be measured as part of the permanent calibration procedure. There are several methods of calibrating accelerating voltage in terms of electron wavelength λ (Uyeda *et al.*, 1965; Høier, 1969; Thomas, 1970).

The method of Thomas (1970) is particularly simple being based on the indexing of two Kikuchi intersections A, B, figure 2.27(a), as described in section 2.7.3.2. Referring to figure 2.27(b) the angle ϕ between A and B may be calculated using the formula from appendix 1 for the particular crystal structure. Then $L = AB/\phi$ and

$$\lambda = \frac{x \, d\phi}{y} \quad (1.16)$$

1.11.5.2 Specimen drift rate

Every electron microscope specimen drifts laterally while under observation owing to differential thermal expansion between specimen and support grid arising from local heating by the electron beam. Backlash in the specimen traverse or tilt mechanisms is a further common contributory factor.

Specimen drift rate may be measured by superimposing on one plate consecutive images of the same area, separated by 1 or 2 min, during which the specimen manipulators remain untouched. A holey carbon film is generally used to monitor the performance of the specimen holder and stage, paying particular attention to careful seating of the specimen and holder. The grid should be $\lesssim 200 \mu\text{m}$ mesh and standard illumination conditions, frequently specified by the manufacturer should be used. It is important to wait 1–2 min after specimen movement or tilt to allow the specimen to reach equilibrium before measurement. Note that, for magnetic or contaminated specimens, and those containing particles that are poor electrical conductors, etc., the drift rate is frequently determined by the specimen itself. Consequently, it may be much greater than that specified by the manufacturer. Furthermore, if intense illumination conditions are used or if the anticontaminator is inoperative, excessive specimen drift may occur.

1.11.5.3 Specimen contamination rate

During observation in the electron microscope the area of the specimen illuminated by the electron beam becomes contaminated with carbon. The resolution is seriously reduced and stresses are generated in the specimen that can cause dislocation movement. Carbon contamination arises from the breakdown of hydrocarbons in the vicinity of the specimen. These hydrocarbons may be produced from the vacuum pumping system or

outgassing of vacuum seals or metal surfaces. Contamination may be reduced to $< 1 \text{ \AA min}^{-1}$ in modern electron microscopes by using a liquid nitrogen cooled anticontaminator shroud around the specimen holder, such that hydrocarbons are condensed out before reaching the specimen.

Conventionally, the contamination rate is measured as the rate of reduction in diameter of a hole in a carbon film. The rate of contamination increases with increased beam intensity or reduced spot size. Consequently, it is necessary to specify the illumination conditions, suitable values being a spot diameter of $2 \mu\text{m}$ and a current density of 1 A cm^{-2} on the specimen.

1.11.5.4 The position of the specimen tilt axis and sense of tilt

Calibration of the position of the tilt axis and sense of tilt in the image may be obtained using a specially shaped test specimen (Feltner and Sefton, 1964). Alternatively, in side entry specimen stages such as the EM300/301, one tilt axis is parallel to a specimen traverse direction. In the EM300/301, turning the left-hand traverse clockwise moves the specimen from left to right, viewed from the front of the microscope. This specifies the left to right specimen direction in the image. It is then a simple matter to determine the sense of tilt from the known geometry of the specimen tilt relative to this direction.

In the diffraction pattern mode, the simplest approach in the EM300/301 is to form an out-of-focus multiple dark field image, section 1.5.4, then note the direction of movement in the image for clockwise movement as above. Alternatively, the position of the tilt axis and the sense of tilt may be determined from the direction of Kikuchi line movement (see section 2.6.3). The direction of motion is perpendicular to the tilt axis and towards the edge of the specimen tilted up.

1.11.5.5 Focal increments of the objective lens

Heidenreich *et al.* (1968) have shown that this parameter may be measured from small regular crystals of magnesium oxide smoke on a carbon support film. An in-focus and an out-of-focus image is superimposed on one plate. The spacing between the images is Δx_g and

$$\Delta f = \Delta x_g / 2Mm\theta \quad (1.17)$$

where Δf is the change in focal length, m is the number of focal steps between images, θ is the Bragg angle for the (200) reflection used for formation of the image and M is the magnification.

1.11.5.6 Beam current

The most convenient method of measuring electron currents of the magnitude found in electron

microscopes is to use a Faraday cage (Grubb, 1971). This is an electrode of cup shape so that most secondary and backscattered electrons are retained, and the total beam current can be measured directly. The cage is normally surrounded by a similar electrode to prevent stray electrons, which could strike the outside of the cup, from being detected.

If measurements of the beam current incident on the specimen are required, it is important to site the Faraday cage as close to the specimen as possible, that is immediately above the objective lens. To measure the current falling on photographic emulsion, a cage would normally be placed just above the microscope camera. Some commercial instruments use the current to the fluores-

cent screen corrected for electron backscattering, for the same purpose.

1.12 Resolution Tests

There is no general agreement on a test specimen for evaluating instrument resolution. Four types have been suggested.

- (1) Fresnel fringe width.
- (2) Crossed lattice fringes from single-crystal gold films.
- (3) Lattice fringes from partially graphitised carbon black.
- (4) Fine structure in a shadowed carbon replica.

The Fresnel fringe approach relies on the fact that the fringe thickness decreases on approaching

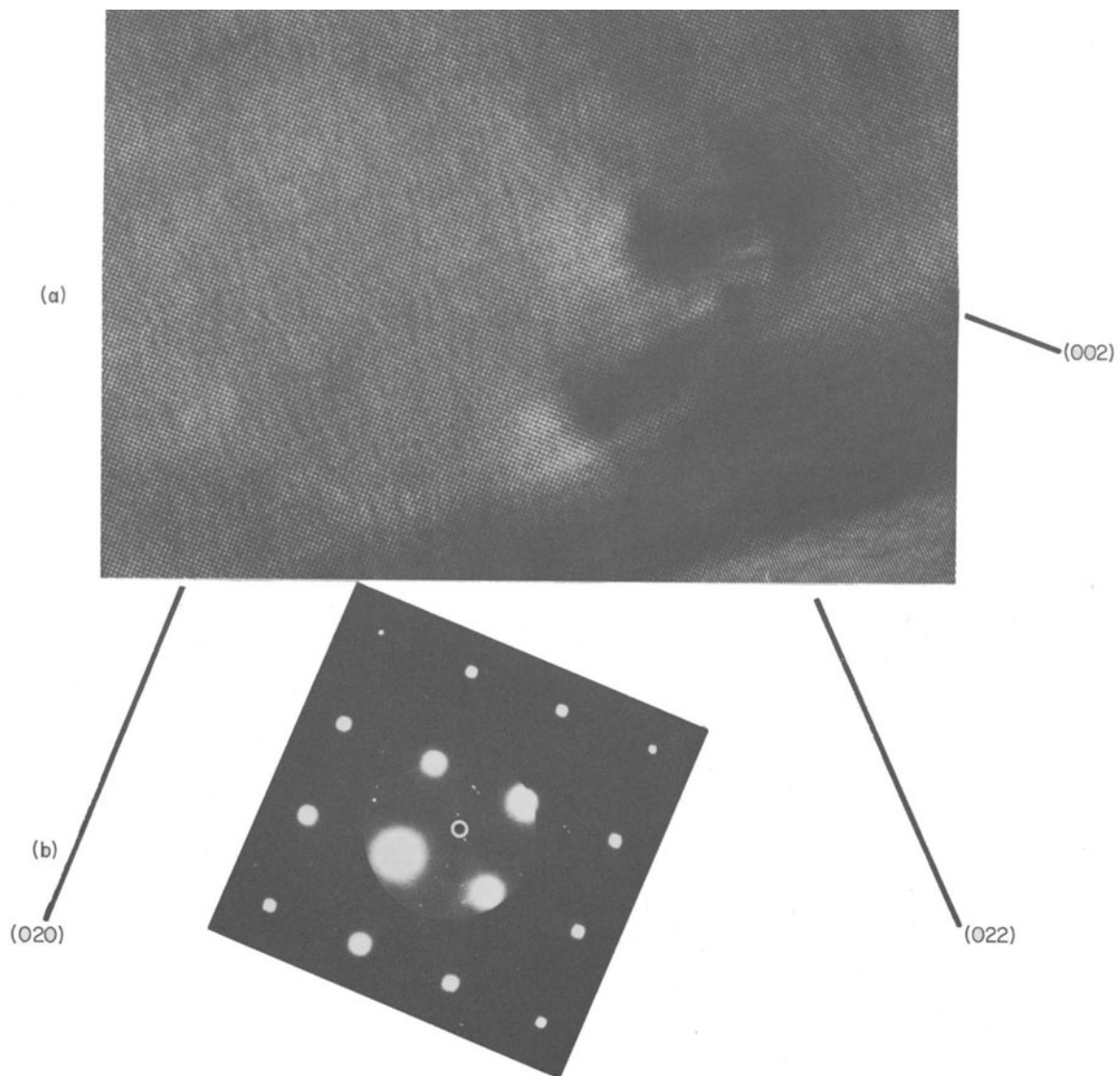
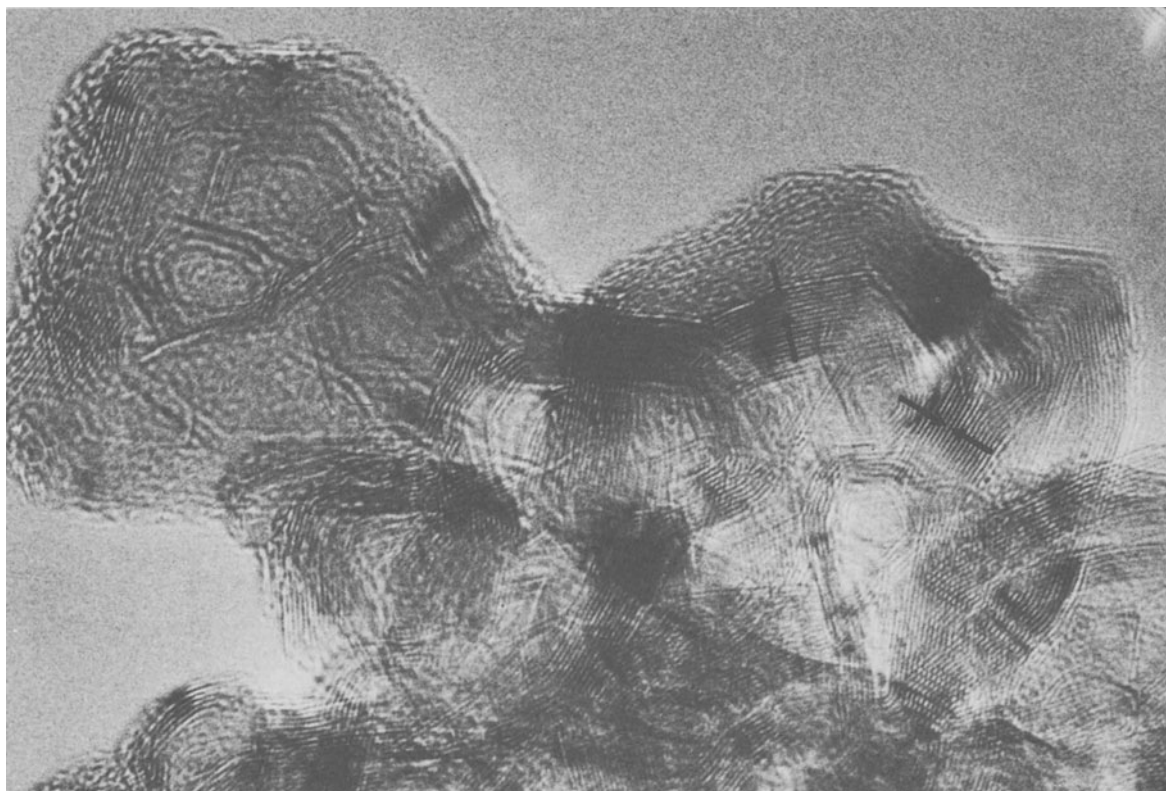
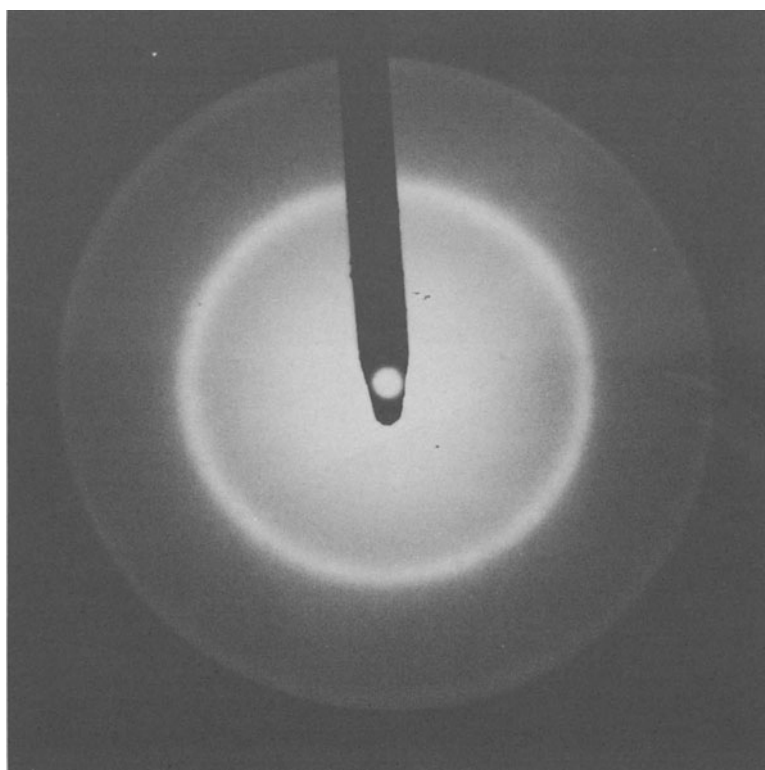


Figure 1.21 (a) An electron micrograph of crossed fringes showing (200)(020) and (220) in a [001] gold film $d(020)(002) = 2.039 \text{ \AA}$, $d(020) = 1.442 \text{ \AA}$. (b) the selected area diffraction photograph showing the position of the optic axis and objective aperture, (c) fringe image in partially graphitised carbon black (arrows indicate interplanar spacing $d(002) = 3.4 \text{ \AA}$), (d) selected area diffraction photograph showing the position of the objective aperture, axial illumination [Courtesy of F. Sheldon]



(c)



(d)

Figure 1.21 (continued)

focus, see section 1.4.2. Thus a series of micrographs taken sequentially approaching focus may be used to determine the narrowest fringe observable. Unfortunately, the fringe becomes difficult to interpret at a thickness of ~ 5 Å because of the granularity of the carbon film. Nevertheless, this approach is useful in the intermediate resolution range, for example ≥ 8 Å, because it can simultaneously provide an estimate of objective astigmatism and enables resolution to be measured in all directions in the image. Correction for astigmatism may be achieved using either of the methods outlined in section 1.4.2. The major disadvantage of this technique is that it does not include specimen effects and, while it estimates the performance of the instrument, this bears little relationship to the real resolution expected in an actual specimen.

Nevertheless lattice spacing images can be related to a real specimen with a known structural separation. Crossed lattice fringes from gold single crystals, figure 1.21(a) and appendix 12, or direct resolution of curved fringes in carbon black, figure 1.2(b) and appendix 12, require the objective astigmatism to be adjusted in the plane of the lens. However, for a single array of straight fringes astigmatism need only be correctly adjusted perpendicular to them and this approach is not recommended as a true indication of instrument performance. If the resolution of the microscope is insufficient to use either of the above specimens, any material from table 1.3 may be used, preferably avoiding the pthalocyanines because their life in a fully focussed electron beam can be < 1 min.

The direct resolution of the fine structure in a carbon replica lightly shadowed with gold or tungsten, see appendix 12, involves obtaining a series of images approaching focus. The spacing of points in the image may be measured directly in the image, or preferably optical diffractometry may be used to determine resolution, see section 1.5.5 and figure 1.15. However, this approach involves assuming that the two 'points' in the image are in the same plane which may not be true because the specimen has a finite thickness.

In the opinion of the author the crossed lattice fringe or carbon black resolution test provides the best resolution test for a microscope because it is related to a well-defined measurement in the specimen and includes the effect of the specimen itself. Consequently it is a good indicator of the performance of the instrument. However, it is important to realise that chromatic aberration effects are still much less than those in the average thin foil, when this resolution cannot be achieved.

In practice, axial rather than tilted illumination, section 1.5.5, provides a better estimate of instrument resolution. Tilted illumination reduces the effect of chromatic aberration, see section 3.19.1.

Thus, as a rule of thumb, if it is possible to resolve 5–6 Å in a carbon replica with axial illumination, the instrument should be capable of 3–4 Å carbon black resolution with tilted illumination. Furthermore, 3–4 Å fringe resolution with axial illumination is equivalent to < 5 Å point to point under the same illumination conditions.

In general it is preferable to use an objective aperture because it improves image contrast. However, it will be necessary to correct objective astigmatism with the aperture inserted, which is best performed using the background structure of the support replica where possible, see section 1.4.2. In the case of single-crystal gold films, an image of a dislocation may be used to correct astigmatism, adjustment of the astigmators being made to place the four images of the dislocation as close together as possible. The image should be viewed at screen magnifications $> 250\,000\times$, carbon black being particularly useful because of its high contrast images. Finally, the objective aperture size, usually 50–70 μm diameter, should be chosen just to include those portions of the diffraction pattern necessary to produce interference to form the lattice fringe image. In the case of the crossed lattice gold and carbon black, the situation is shown in figure 1.21(c), (d). For the crossed lattice image all four spots in the diffraction pattern should have approximately equal intensity. Consequently, CDF images, section 1.5.2, on all spots 1, 2, 3, figure 1.21(c), may be used to locate a region which contributes strongly to all spots.

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APPENDIX 12.

PREPARATION OF HIGH-RESOLUTION TEST SPECIMENS

Conventionally, three types of specimen are used as high-resolution test specimens.

- (1) Single-crystal gold films with (001) foil plane.
- (2) Partially graphitised carbon black.
- (3) Shadowed replicas for point-to-point resolution.

The preparation techniques are described below.

A12.1 Single-crystal (100) Gold Films

Single-crystal gold films have been shown to produce crossed fringe images (Komoda, 1966) with spacing 2.039, and 1.442 Å, figure 1.21a. In general these test specimens must be very thin 30–40 Å with a low defect density. To achieve such a thin film it is essential to vacuum evaporate the material. The sequence of events involves evaporation of an intermediate layer of silver, ~1000 Å thick, onto a freshly cleaved {100} rocksalt substrate held at 450 °C (Bruck, 1936; Shirai, 1943; Basset, 1965). This is followed by evaporation of a thin layer 30–40 Å of gold onto the silver at the same temperature. The combined silver–gold film may be floated off in distilled water, transferred to 50 % nitric acid to dissolve the silver, washed in distilled water and mounted on a 200 mesh grid. These films are not in general completely continuous and a few polycrystalline regions may occur.

Direct evaporation of gold onto rocksalt at 450 °C is not recommended because the films tend to be much thicker to maintain continuity.

A12.2 Partially Graphitised Carbon Black

This test specimen was first suggested by Heidenreich *et al.* (1968) who also describe its preparation. A picture is shown in figure A12.1. Standard carbon black is used (U.S. designation I.S.A.F., ASTM-N-220) which consists of small particles ~250 Å diameter primarily used to impart high abrasion resistance to tyre treads. This powder may be partially graphitised by heating at 2600–3000 °C for 30 min under argon in a graphite crucible.

The carbon black may then be dusted directly onto a holey carbon support film. Alternatively it may be dispersed ultrasonically in chloroform and then transferred to the support film by dipping.

A12.3 Replicas for Point-to-point Resolution Tests

These test specimens consist of a thin carbon film shadowed with platinum. The preparation procedure described here is that due to Nauta and Van Wijngaarden (1964). The form of the specimen on its support grid is shown in figure A12.2. Several methods have been suggested to produce the support film, and the most successful is as follows.

Prepare 6 ml of 0.25 % Formvar in chloroform and 0.05 ml of 25 % Teepol in distilled water. Mix to form a milky stock solution. Add 0.35 ml of this suspension to 10 ml of the 0.25 % Formvar in chloroform then dip a clean glass slide into the solution. When the film is dry it is floated off on distilled water and mounted on grids. For this stage lay a number of support grids on a coarse mesh gauze and use the gauze to scoop up the Formvar film. Dry on a filter paper and pick off the grids with tweezers.

Holes in the Formvar film may now be produced by placing the support grids in a dish filled with 20–25 % amyl acetate in benzene. After 15–30 min the grids are removed and dried on a filter paper. As with other films, the speed with which the glass is withdrawn from the solution influences the nature of the films. Fast withdrawal results in a thick film, slow withdrawal in a thin one. With a thick film, larger holes result and such a film is subsequently suitable as a basis for supporting a thinner film or delicate specimens (the so-called microgrid). In a thin film, smaller holes result and this is suitable for astigmatism correction.

The size of the holes is, however, also influenced by the time of immersion in the amyl acetate/benzene bath, the longer times producing, in general, larger holes.

The holey film is then covered with evaporated carbon and then gold, to build up the support grid for the final high-resolution film. The thin

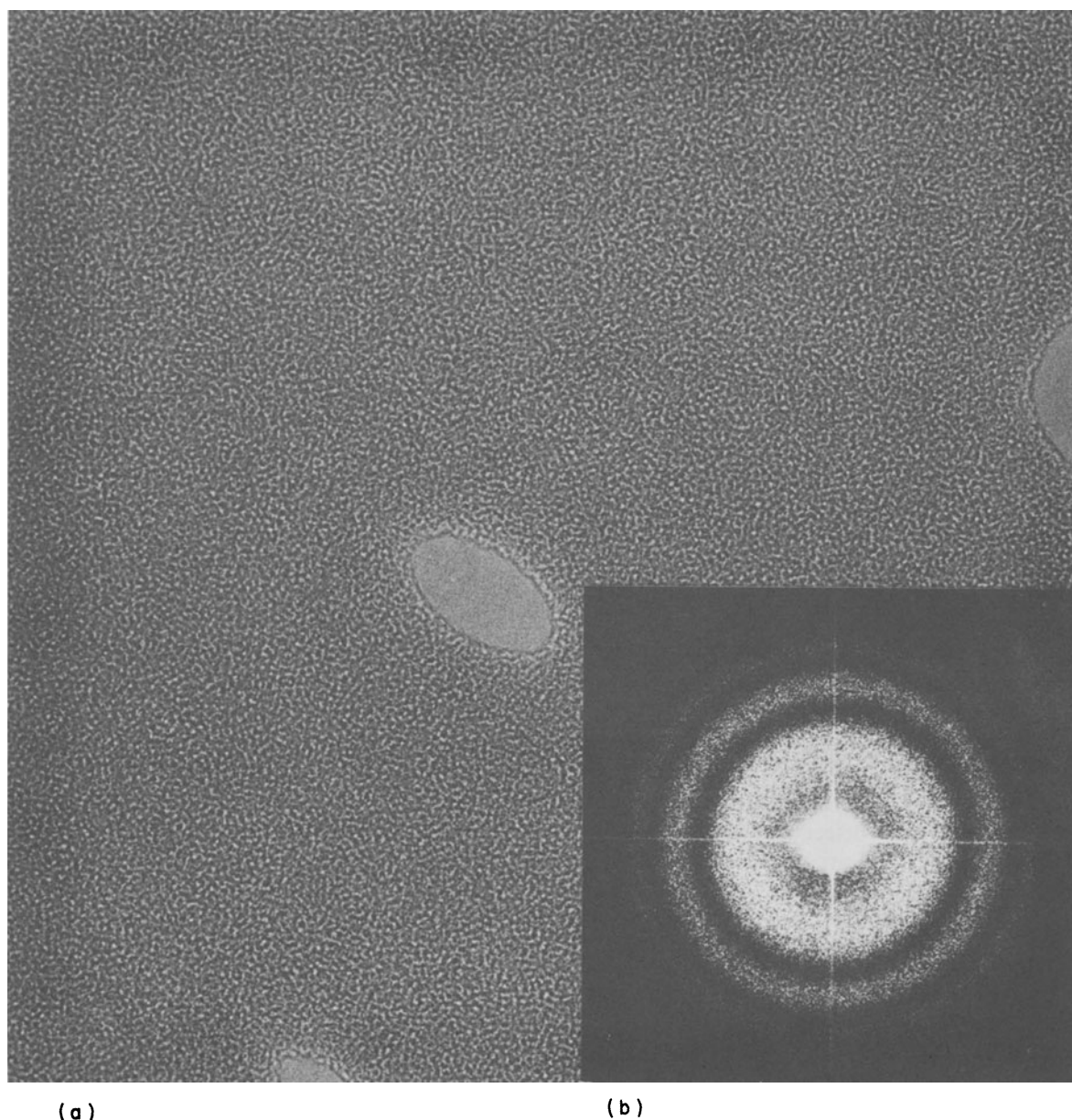


Figure A12.1 (a) A typical image of a high-resolution carbon replica showing minimum point-to-point resolution of 5 Å. (b) The corresponding light optical diffraction pattern. The inner ring is the 5 Å spacing [Courtesy of P. N. T. Unwin]

carbon film is evaporated onto a piece of freshly cleaved mica, the thinner the film the better, then stripped onto water and collected on the prepared grids as for the holey Formvar film above. Allow to dry.

The final step is to evaporate a little platinum or

other heavy metal onto the film. The platinum islands themselves may be used to check resolution above 10 Å but the structure of the support film itself is more suitable below this value.

Figure A12.1(a) shows a typical example of an image from a high-resolution replica. The resolution

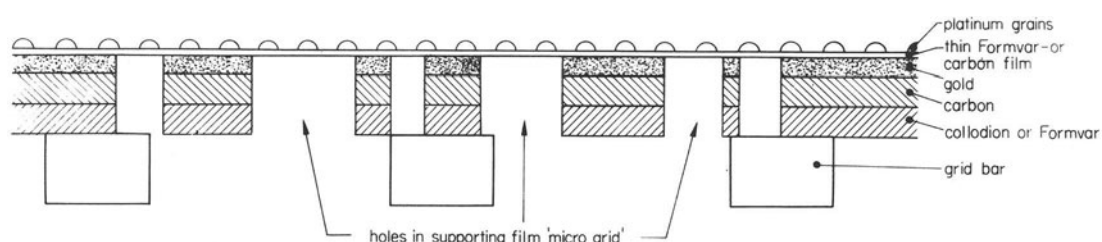


Figure A12.2 A schematic diagram showing the layer structure of a high-resolution carbon replica for point-to-point resolution tests [Courtesy of R. Nauta and J. D. van Wijngaarden]

is best checked by optical diffractometry (Thon, 1971), see figure A12.1(b).

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APPENDIX 13.

HOLEY CARBON REPLICAS

These replicas are commonly used for astigmatism correction by observation of the Fresnel fringes at a small hole. Several preparation techniques have been suggested (Harris, 1962; Fukami and Adachi, 1965; Johnston and Reid, 1971). The technique due to Harris (1962) is easy to use and is described below. An emulsion of glycerol in 0.25 % Formvar solution is used. A cold (0 °C) glass microscope slide is dipped in the solution, allowed to dry and the Formvar replica floated off onto distilled water. This film contains holes arising from the droplets of glycerol. It is scooped up on a coarse grid with several support grids (200 mesh) on

it. After drying an $\sim 100 \text{ \AA}$ thick carbon film is evaporated onto the film *in vacuo*. The Formvar is dissolved away in chloroform and the support grids then contain a holey carbon film suitable for observation in the microscope.

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