MICROSCOPY HANDBOOKS 47

Electron Diffraction in the Transmission Electron Microscope

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Electron Diffraction in the Transmission Electron Microscope

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Taylor & Francis
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LONDON AND NEW YORK

© Taylor & Francis 2001

First published by Taylor & Francis
2 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN
52 Vanderbilt Avenue, New York, NY 10017

Transferred to Digital Printing 2008

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A CIP catalogue record for this book is available from the British Library.

ISBN 1 85996 147 9

Production Editor: Paul Barlass.

Typeset by Marksbury Multimedia Ltd, Midsomer Norton, Bath, UK.

Front cover: [001] Tanaka CBED pattern of the 24R polysome of the Al, Sn oxide mineral nigerite (see *Figure 6.1*) and [111] schematic Kikuchi map for diamond cubic crystals (see *Figure 4.7*).

Publisher's Note

The publisher has gone to great lengths to ensure the quality of this reprint but points out that some imperfections in the original may be apparent.

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Abbreviations

APB	antiphase domain boundary
CBED	convergent-beam electron diffraction
CCD	charge-coupled detector
FEG	field-emission gun
FOLZ	first-order Laue zone
FWHM	full width at half maximum
FWTM	full width at 10th maximum
HOLZ	higher-order Laue zone
K-M	Kossel–Möllenstedt
relp	reciprocal lattice point
relrod	reciprocal lattice rod
SAD	selected-area diffraction
SOLZ	second-order Laue zone
STEM	scanning transmission electron microscope
TEM	transmission electron microscope
ZAP	zone-axis pattern
ZOLZ	zero-order Laue zone

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List of symbols

a, b, c	lattice parameters
a^*, b^*, c^*	reciprocal lattice parameters
A	amplitude
C_s	spherical aberration coefficient
d	spacing of periodic object
d_{hkl}	spacing of lattice planes (hkl)
D	diameter of diffraction aperture
E	energy
f	atomic scattering factor
f	focal length of lens
F_{hkl}	structure factor
$\mathbf{g}_{hkl}$	reciprocal lattice vector
G	radius of HOLZ rings
hkl	indices of reflection from (hkl) planes
(hkl)	Miller indices of a plane
$\{hkl\}$	form or set of planes related by symmetry
H^*	spacing of reciprocal layers
I	intensity
$\mathbf{k}_0$	vector of the incident beam
$\mathbf{k}$	vector of the diffracted wave
L	camera length
M	magnification
m	pitch of screw axis
n	integer
N	$h^2+k^2+l^2$
N	total number of atoms in unit cell
N	order of screw axis
nm	nanometre (10^{-9} metres)
pm	picometre (10^{-12} metres)
R	distance in the diffraction pattern as measured on the film or screen
$\mathbf{s}$	deviation parameter
s'	effective deviation parameter
t	thickness of specimen
t	lattice repeat
u	fourth crystallographic axis used in hexagonal and trigonal systems

$[UVW]$	zone direction
$\langle UVW \rangle$	family of zones related by symmetry
v	velocity
V	voltage
V_c	volume of unit cell
x	distance
y	displacement
x, y, z	direction of crystallographic axes
x_n, y_n, z_n	fractional coordinates of n th atom in unit cell
x^*, y^*, z^*	direction of reciprocal crystallographic axes

Greek symbols

α	primary tilt angle
α	scattering angle
2α	convergence angle
α, β, γ	angles between crystallographic axes
$\alpha^*, \beta^*, \gamma^*$	angles between reciprocal crystallographic axes
β	secondary tilt angle
Δ	path difference
Δf	defocus
ΔR	displacement of Kikuchi line from diffraction spot on film or screen
ϕ	angle between $\mathbf{g}$ vectors/normal to planes
ϕ	phase difference
ϕ_{hkl}	phase angle for reflection hkl
ξ	extinction distance
λ	wavelength
Ψ_{hkl}	amplitude of diffracted beam
Ψ_0	amplitude of undeviated beam
θ	Bragg angle

Preface

This text is the result of many years' experience of teaching the elements of electron diffraction to postgraduate students in the University of Manchester and UMIST, and to students attending the annual Royal Microscopical Society School in Electron Microscopy. I have tried to minimize the use of mathematics, but where I considered mathematics unavoidable, I have made the derivations as full as possible. I have also tried to illustrate physical concepts with suitable diagrams, wherever appropriate.

My examples have been taken from a range of metals, minerals and ceramics, and I have tried to make the coverage as broad as possible in terms of crystal symmetry. My experience has been that students who are only familiar with the cubic and hexagonal systems come unstuck when confronted with a system of lower symmetry! I also hope that biologists will find the book accessible.

The appendices cover material that is not dealt with in the main text. They include a summary of essential crystallography and an introduction to space groups. Other appendices and parts of the text provide tables of data and crystallographic formulae that I hope will provide the reader with a useful source of reference.

P.E. Champness

Acknowledgements

I am extremely grateful to the following people who have made helpful comments on various parts of the text: Alwyn Eades, Chris Hammond, Peter Kenway, Gordon Lorimer and Jian-Guo Zheng. I am also grateful to Sue Maher, Robin Hadley and Margaret Banton, who printed the photographs, and to Richard Hartley who produced the line drawings from my, often rough, originals. Feridoon Azough, Chris Hammond, Peter Kenway, Gordon Lorimer and Phil Prangnell kindly supplied photographs.

A number of copyright holders have permitted me to reproduce figures, the acknowledgement of which is made in the figure captions.

Safety

Attention to safety aspects is an integral part of all laboratory procedures and both the Health and Safety at Work Act and the COSHH regulations impose legal requirements on those persons planning or carrying out such procedures.

In this and other Handbooks every effort has been made to ensure that the recipes, formulae and practical procedures are accurate and safe. However, it remains the responsibility of the reader to ensure that the procedures which are followed are carried out in a safe manner and that all necessary COSHH requirements have been looked-up and implemented. Any specific safety instructions relating to items of laboratory equipment must also be followed.

1 Diffraction and the electron microscope

When we look at a distant street light through a finely woven net curtain or an umbrella we see a pattern of bright spots. This pattern is not an image, it is a diffraction pattern. We see a regular array of spots because the fabric of the curtain or the umbrella also has a regular, periodic weave. The pattern that we see is telling us about the periodicity of the weave and its orientation; if you rotate the fabric about a direction perpendicular to its surface, the pattern of spots will also rotate. We will look at this phenomenon in more detail at the end of the next section.

1.1 How a lens forms a diffraction pattern

We are most familiar with the use of lenses to form a (usually) magnified image of an object; for instance, with a magnifying glass or an optical microscope. However, any converging lens used in this way is also forming a diffraction pattern. What happens can be understood by reference to *Figure 1.1*. Here we have a periodic object (it could be a piece of net curtaining) placed in front of a converging lens which is illuminated by a parallel beam of monochromatic (single wavelength) light. Some of the light is transmitted undeviated through the object and some is 'scattered' (some will also be absorbed). The undeviated light travels parallel to the optic axis of the lens and is focused by the lens so that it passes through the **principal focal point** F_0 at a distance from the lens equal to the focal length, f . A plane drawn through F_0 perpendicular to the axis is the **back focal-plane** of the lens (note that there is a **conjugate**, i.e. optically equivalent, **front focal-plane** at a distance f from the lens on the object side of the lens). Light scattered at angles $\pm \alpha$ by the object will form parallel beams which are focused to points F_1 and F_1' in the back focal-plane, at a distance from the axis proportional to the angle α . This pattern of spots formed when the transmitted and scattered beams are focused into the back focal-plane is the diffraction pattern. In fact, for an infinite, periodic object such as our net curtain the spots are only formed for rays that are scattered in specific directions (we will learn

Figure 1.1. Formation of an image and diffraction pattern by a lens from an infinite periodic object. For parallel, monochromatic illumination, the transmitted and diffracted beams are focused into spots, F_0 , F_1 , F_1' etc., which form the diffraction pattern in the back focal-plane of the lens at a distance f , the focal length, from the lens. Only three diffraction spots are shown for clarity. The diffracted beams recombine to form a magnified image in the image plane. Note that the image is inverted with respect to the object, but the diffraction pattern is not. In effect, the screen of the electron microscope is in the plane I_1 – I_3 in the imaging mode and in the plane F_1 – F_1' in the diffraction mode.

the rule that governs these directions later). The rays that form the diffraction spots in the back focal-plane go on to form an inverted image in the image plane. If you follow the three rays shown in *Figure 1.1* that are emerging from any of the three apertures O_1 , O_2 or O_3 of the object, you will find that they end up at the same point in the image (rays from O_1 end up at I_1 for example, as you would expect), each having passed through a *different* diffraction spot. In other words, information about the object is contained in *each* diffraction spot. A direct consequence of this effect is that, in order for the lens to form an image of the object (i.e. in order to resolve the weave of the curtain material), at least two diffracted beams should enter the objective lens and be allowed to recombine in the image plane. This principle was first described by Ernst Abbe in the 19th century and is known as the **Abbe criterion**.

If we explore a little further, we find that there is a definite relationship between the periodicity and orientation of the object and the spacing and orientation of the spots in a diffraction pattern. You can easily see what this relationship is if you hold a set of meshes, net-curtain materials or diffraction gratings of varying periodicity in front of a laser pointer (do NOT look directly into the laser beam) or other small torch and view the diffraction pattern produced on the wall on the opposite side of the room. You will find that the spacing of the diffraction spots is inversely proportional to the object's periodicity (for instance, if you halve the spacing of the grating or mesh, the spacing of the diffraction spots doubles) and that the separation of the spots produced by the grating or by the weave of the net is perpendicular to the lines of the grating (*Figure 1.2*). (If you do not have a set of such objects, you can see the size effect by rotating a mesh about the direction of one of the threads, thus effectively shortening the mesh periodicity perpendicular to the direction of rotation.

Figure 1.2. Optical diffraction patterns taken with a laser and recorded under the same conditions. (a) Pattern from a diffraction grating with spacing 0.126 mm. The lines of the grating were parallel to the length of the page; notice that the row of diffraction spots is perpendicular to this direction. (b) Pattern from a 100-mesh electron-microscope grid, grid spacing 0.25 mm. (c) Pattern from a 200-mesh electron-microscope grid, grid spacing 0.125 mm. (d) Pattern from a 400-mesh electron-microscope grid, grid spacing 0.0625 mm. (e) Diffraction pattern from the rectangular two-dimensional 'crystal' shown in (f). Note the reciprocal relationship between the spacings in the objects and the spacing of the corresponding row of diffraction spots. Note also that the row of spots is perpendicular to the grating from which it is diffracted.

The spacing of the corresponding diffraction spots will increase). What we have been looking at in the diffraction pattern is also known as the **reciprocal lattice** because of the inverse (reciprocal) relationship it bears to the **direct lattice** – the object.

1.2 Introduction to diffraction in the TEM

A transmission electron microscope (TEM) consists of a source of electrons (the electron gun) and a series of electromagnetic lenses, as shown in *Figure 1.3* (see also *Table 1.1*). The most critical components of a magnetic lens are the soft-iron pole-pieces which produce an axially symmetric magnetic field for focusing the electrons. The rest of the lens is a magnetic yoke containing the windings for energizing the lens with a d.c. current. By varying this current, the magnetic field, and hence the

Figure 1.3. Schematic diagram of the lenses and apertures in a modern TEM. The nomenclature shown for the imaging lenses follows that adopted for FEI/Philips instruments. The nomenclature used by other manufacturers is given in *Table 1.1*. Note: the scan coils will only be present if the instrument is designed for scanning transmission electron microscopy.

Table 1.1. Nomenclature used by different manufacturers for the imaging lenses in a TEM

FEI/Philips	JEOL	Hitachi
Objective	Objective	Objective
Diffraction	First intermediate	First intermediate
Intermediate	Second intermediate	Second intermediate
Projector 1	Third intermediate	First projector
Projector 2	Projector	Second projector

focal length of the lens, is changed. This ability to change the focal length is one of the most important ways in which magnetic lenses differ from glass ones. Another difference is that, unlike the glass lenses in a light microscope, magnetic lenses in an electron microscope, because of their construction, size and weight, cannot be moved physically with respect to one another in order to focus the image, although the specimen can be moved up or down in the holder to achieve a certain amount of focusing. We will see some other differences between the two types of microscope later.

The specimen is placed in a holder in the front focal-plane of the objective lens (*Figure 1.3*), i.e. at a distance f in front of the lens. *Figure 1.4* shows schematically the ray paths for the imaging system of a TEM that employs three stages of magnification. The magnified image produced by the objective in its image plane serves as an object for the diffraction lens, which produces a second intermediate image, which is further magnified by the projector lens to produce the final image on the fluorescent viewing screen (or on the photographic film which is just

Figure 1.4. Schematic diagram showing the imaging system of a TEM. A three-stage magnification system is shown. (a) The ray paths for the imaging mode in which the image formed by the objective lens forms the object for the diffraction lens. Note: in this mode there is an objective aperture in the back focal-plane of the objective lens which restricts the number of diffracted beams that recombine in the image plane. This arrangement improves contrast in the image. (b) The ray paths for the diffraction mode. Here the objective aperture is withdrawn and a diffraction aperture is inserted in the image plane of the objective lens (the first intermediate image). The diffraction lens is weakened (its focal length is changed from f_1 to f_D) so that its object plane is the back focal-plane of the objective lens. Hence, the diffraction pattern is magnified by the projector lens and the diffraction pattern appears on the screen of the instrument. Note that the top part of the diagrams (from the specimen to the first image plane) is identical to *Figure 2.1*.

below the screen). This is the imaging mode (*Figure 1.4a*); the action of the objective lens and the diffraction/projector lenses parallels that of the objective and eyepiece, respectively, in the optical microscope. However, the diffraction lens can be reduced in strength (its focal length is increased from f_I to f_D) by decreasing the current in the windings so that an image of the diffraction pattern in the back focal-plane of the objective lens is focused on the final screen (*Figure 1.4b*); in other words the 'object' for the diffraction lens in the diffraction mode is the diffraction pattern produced by the objective lens. Referring back to the diagram of a simple lens in *Figure 1.1*, the screen of the electron microscope is effectively in the plane I_1 – I_3 in the imaging mode and in the plane F_1 – F_1' in the diffraction mode. The weakening of the current in the diffraction lens is simply achieved by selecting the diffraction mode on the microscope's console. If an aperture of diameter D (called the selected-area or diffraction aperture) is placed in the first image plane (*Figure 1.4b*), and if the objective lens behaves as a 'perfect' lens (i.e. one without aberrations; see Section 1.5), only those electrons passing through an area of diameter D/M in the specimen will reach the final screen, where M is the magnification of the objective lens. This can be seen in *Figure 1.5* which shows the **virtual aperture** produced in the object plane. In practice, D may be about $50\ \mu\text{m}$ and since M is about 50, the diameter of the area selected is about $1\ \mu\text{m}$. The diffraction pattern from this area only is therefore observed. This technique is known as **selected-area**

Figure 1.5. Ray diagram showing the formation of a selected-area (SAD) pattern. The insertion of a (diffraction) aperture in the image plane of the objective lens results in the creation of a 'virtual' aperture in the object plane. Only electrons falling inside the virtual aperture will pass through into the imaging system; all other electrons (for instance the dashed rays) will hit the diffraction aperture and will not contribute to the image or the diffraction pattern seen on the screen. If the diameter of the diffraction aperture is D and the magnification of the lens is M , then the area selected in the object has diameter D/M . Diagram courtesy of P.B. Kenway.

diffraction or **SAD**. It allows observation of diffraction patterns from small areas of the specimen so that, among other things, a correlation between features seen in the image and the crystallography of the specimen can be made. Unfortunately, the technique is subject to certain errors, both systematic and random, which are described in Section 1.5. These errors, which are in part due to aberrations in the lens, limit the minimum size of the area that can be selected.

Modern TEMs usually have at least two condenser lenses to focus the image of the electron source onto the specimen and five imaging lenses (*Figure 1.3*) which are given varying names by different manufacturers; I will adopt the nomenclature used for FEI/Philips instruments. This larger number of imaging lenses allows a greater range of magnification, both of the image and of the diffraction pattern, than is possible with the three-lens arrangement in *Figure 1.4*.

1.3 SAD in the TEM

Once you have chosen the area from which you wish to obtain a diffraction pattern, you only need to: insert a diffraction aperture, select the diffraction mode, select the required **camera length** (the magnification of the diffraction pattern), remove the objective aperture and focus the pattern. An example of a diffraction pattern from a single crystal taken at two different camera lengths is shown in *Figure 1.6*. If the area selected is to be accurate, it is important that the image of the specimen is accurately focused in the plane of the diffraction aperture (see Section

Figure 1.6. Diffraction patterns of the mineral titanite ($\sim \text{CaTiSiO}_5$), which is monoclinic ([100] zone axis), taken at camera lengths of (a) 707 nm and (b) 1.43 m, allowing for print size. Note the rotation of about 15° clockwise between the patterns in (a) and (b).

1.6). The diffraction and the projector lenses are programmed within a given range of magnification (the SA, or selected-area, range) so that the plane of the diffraction aperture (the first image plane) is always focused on the viewing screen. At the same time, the objective lens operates at a more-or-less fixed excitation so that it focuses the image in that plane.

Further experimental details of how to obtain a SAD pattern are given in Section 1.9.

1.4 Low- (or small-) angle diffraction

Some biological and other organic crystals have atomic repeats much greater than 10 nm, and the range of camera lengths available by normal SAD may only give a small diffraction pattern from them (remember the reciprocal relationship that we noticed in Section 1.1 between the object and its diffraction pattern; more detail is given in Section 2.3). For instance, a spacing of 100 nm in the object requires a camera length of 54 metres for 100 kV electrons, if the spacing of the spots in the pattern is to be 2 mm (for how this value is arrived at, see Section 2.3), a minimum value for easy measurement on the negative. This camera length is about eight times larger than the maximum normally available in the SAD mode.

Larger camera lengths can be obtained using a different procedure. Here, the objective lens is switched off or reduced to a minimum and the condenser system is focused on the object plane of the diffraction lens. A small **spot size** (the nominal diameter of the beam at the object), obtained by a strongly excited C1 lens, and a small C2 aperture are essential. The subsequent lenses further magnify the diffraction pattern, and camera lengths of the order of several hundreds of metres can be obtained. Details of the procedure differ from one microscope to another, but the technique can be used with any microscope for which there is a flexibility in control over all the lenses.

1.5 Limitations to the accuracy of SAD

The area of the specimen defined by the diffraction aperture in the image plane of the objective lens will not generally correspond exactly to the area from which the associated diffraction pattern comes. There are three sources of error. Firstly, if the image is incorrectly focused, the plane of the diffraction aperture will not coincide precisely with the first image plane and diffracted rays originating from outside the area selected by the aperture will contribute to the pattern. The error involved is proportional to Δf , where Δf is the focusing error. A similar error will

occur if the specimen is not in the **eucentric plane** (when the specimen is in this plane and the image is in focus, the objective lens current is an optimum value). This is because the focal length of the objective lens, even when accurately focused on the object, will differ by some value Δf from its correct value if the specimen is at an incorrect height. Adjustment of the specimen height is possible on all modern microscopes.

The third error in the area selected is caused by **spherical aberration** in the objective lens; a phenomenon that can only currently be corrected for with a customized (and expensive) lens (Haider *et al.*, 1998; Krivenek *et al.*, 1999). Spherical aberration is a lens defect that causes electrons scattered furthest from the optic axis to be brought to focus closer to the lens than rays that are near-axial (*Figure 1.7*); in other words, the focal length f is shorter for rays that are scattered at a high angle. Thus, even if you manage to focus the image accurately, diffracted beams coming from areas outside the selected one will contribute to the diffraction pattern. The effect is shown in *Figure 1.8*, which shows one diffracted beam and the undeviated beam. The undeviated rays from the area x - y in the specimen (the dot-dashed lines) are focused through the principal axial focal-point at O in the back focal-plane at a distance equal to the focal length, f_0 , from the lens. If the lens were perfect, the rays diffracted by the specimen at an angle α to the optic axis would follow the path shown by the dotted lines, after passing through the lens, and the diffraction spot would appear at B in the back focal-plane. Both the undeviated and diffracted rays would be focused in the normal image plane at YX (note the inversion of the image with respect to the object, as is also apparent in *Figure 1.1*). A diffraction aperture placed at YX in the image plane would then select the area x - y in the specimen.

For a lens with spherical aberration, however, the diffracted ray is more strongly focused along the full line to position C, which is slightly closer to the lens than B and lies in a different (back focal-) plane at a distance f_α from the lens. The result is that the image of the object x - y formed by the rays entering the objective lens at angle α is at Y' - X' ; i.e. the end points of the image are displaced distances XX' and YY' (which are unequal) in the plane of the diffraction aperture relative to the image

Figure 1.7. Spherical aberration in a lens. Marginal rays are brought to focus nearer to the lens than near-axial rays. The former therefore have a shorter focal length, f_α , than the latter, f_0 .

Figure 1.8. The effect of spherical aberration of the objective lens on the accuracy of the area selected by the diffraction aperture. The diagram shows one diffracted beam at an angle α to the optic axis and the undeviated beam. B is the position of the diffraction spot for a perfect lens and C is the position of the spot for a lens with spherical aberration. C is slightly closer to the objective lens than O and B because the focal length, f_α , is slightly shorter for marginal rays than the focal length for axial rays, f_0 . The image of the object x-y formed by the diffracted rays is at Y'-X', whereas the image formed by the undeviated beam is at Y-X. The displacement between the two images is proportional to $MC_s\alpha^3$ where M is the magnification of the objective lens and C_s is the spherical aberration constant for the lens. Diagram courtesy of P.B. Kenway.

from the undeviated beam. These distances are proportional to $MC_s\alpha^3$ where M is the magnification of the lens and C_s is the spherical aberration constant of the lens. So, for a lens with spherical aberration, the area selected in the object using the diffraction aperture corresponds to XY *only* for the direct beam; it is displaced by a distance proportional to $C_s\alpha^3$ for each diffracted beam; in other words each diffracted beam 'selects' a different area of the specimen and the error increases very rapidly with scattering angle because of its dependence on the cube of α . Thus there is little point in trying to isolate an area smaller than about $0.5\ \mu\text{m}$ in the specimen. If the magnification of the objective lens is 40 times, this figure translates to an aperture size of $20\ \mu\text{m}$ in the first image plane. Even with a high-resolution TEM (very low C_s) operating at 300–400 kV, the area that can be isolated for SAD is no better than $0.1\ \mu\text{m}$.

The accuracy of SAD can be improved if the operating voltage is increased. This is a consequence of the fact that the scattering angle α is proportional to λ , the wavelength of the radiation, and λ decreases with voltage (see Appendix D). However, the improvement is not spectacular

as λ decreases from 3.7×10^{-3} nm for 100 kV electrons to 1.64×10^{-3} nm for 400 kV electrons, a change of 56%. A more effective (and cheaper!) alternative is described in the next section.

1.6 Diffraction using small probes

We can achieve a dramatic decrease in the size of the area from which the diffraction pattern comes by focusing the electron beam down onto the specimen and dispensing with the diffraction aperture in the first image plane. The beam is no longer parallel and the area selected is, to a first approximation, equal to the size of the beam, known as the **probe**. Convergent-beam electron diffraction (CBED), as the technique is generally known, was first discovered in 1939 by Kossel and Möllenstedt, but has only been developed since the 1970s with the advent of instruments designed for high-resolution microanalysis. The only major problem with using this technique arises in beam-sensitive specimens (see Section 1.8.2) because of the high current densities in the focused electron beam. For such specimens CBED may be impossible, even with the use of a cooling stage and high voltages.

As we have seen, in conventional SAD an approximately parallel electron beam is incident on the specimen, and the resulting diffraction pattern from a periodic sample consists of an array of sharp spots in the back focal-plane of the objective lens (*Figures 1.1, 1.4, 1.9a*). If, instead, the incident beam is convergent (*Figure 1.9b*), the spots become discs and their diameters depend upon the convergence angle 2α , which, in turn, is controlled by the size of the C2 aperture and the lens configuration (see below). *Figure 1.10* shows diffraction patterns in which four different convergence angles were used. As you can see, if the convergence angle is large, the discs overlap and it can be difficult to measure the periodicities in the pattern. In fact, these wider-angle patterns can give us a lot of useful crystallographic information (a topic that will be covered in Chapter 6), but for the moment we will confine ourselves to the situation in which the discs do not overlap and will refer to such patterns as **microdiffraction patterns**. Sometimes you will come across the term **Kossel-Möllenstedt** (or K-M) patterns for such patterns and the term **Kossel patterns** for the situation when the discs overlap.

In modern TEMs, a convergent probe may be formed in most operating modes; i.e. the **TEM mode** (**microprobe** and **nanoprobe**) and the **STEM** (scanning transmission electron microscope) mode. You can form a CBED pattern in the TEM mode in any TEM that can produce a small probe ($\ll 1 \mu\text{m}$ in size) with a convergence angle of > 20 mrad. This may not be possible on TEMs made before the late 1970s. Most modern TEMs use a condenser-objective lens, as illustrated in *Figure 1.11*. The specimen is placed in the centre of the very strong magnetic field

Figure 1.9. The formation of (a) a conventional (selected-area) electron diffraction pattern and (b) a convergent-beam electron diffraction pattern. In (a) the area AA' is selected by means of an aperture in the first intermediate image plane (see *Figure 1.4b*), whereas in (b) the area BB' selected is determined by the probe size. The diameter of the discs in the CBED pattern depends on the convergence angle 2α .

between the pole pieces of the objective lens, the upper part of which, the **prefield lens**, acts as a very strong condenser lens immediately in front of the specimen to focus the electron beam onto the specimen. The second half of the lens is the objective lens that forms the image and diffraction pattern of the specimen. The **auxiliary lens** (or **mini-lens**) above the prefield lens in *Figure 1.11* has the effect of partially nullifying the converging effect of the prefield lens, allowing a larger area to be illuminated in the imaging mode. When a CBED pattern is formed in the TEM mode all the lenses in the illumination system are used (*Figure 1.11a*) and the electron beam is focused on the specimen using the C2 lens.

In the STEM mode of modern TEMs the auxiliary lens is switched off, the first condenser lens is strong (small spot size) and the second condenser lens is either very weak or switched off. In the STEM imaging mode a small probe is scanned over the area of interest using two pairs of scan coils situated below the second condenser lens as shown in *Figure 1.3* (see Keyse *et al.* 1998 for an introduction to STEM techniques). Clearly your TEM must be fitted with a STEM system for you to be able to do this! In this mode the electron beam is focused on the specimen using the objective lens. The convergence angle at the specimen in the STEM mode is several times larger than that obtained in the TEM mode for the same C2 aperture (*Figure 1.11b*).

Figure 1.10. Convergent-beam electron diffraction patterns of synthetic Mg_2GeO_4 spinel (F cubic, $a = 0.825$ nm) taken using convergence angles of: (a) 1.4×10^{-3} , (b) 2.3, (c) 4.9 and (d) 8.1 milli-radians. (a) and (b) are referred to as microdiffraction or Kossel-Möllenstedt (K-M) patterns as their diffracted discs do not overlap; they were taken in the 'microprobe mode' of a Philips 400T TEM using 30 and 50 μm C2 apertures, respectively. (c) and (d) were taken in the 'nanoprobe mode' with 30 and 50 μm C2 apertures, respectively. The zone axis was near $[110]$.

In the Philips 400 series of microscopes a so-called high-resolution STEM mode, in which both the C2 and auxiliary lenses are switched off, is also available for CBED. Note that in older machines STEM attachments were available, but the electron optical design varied from machine to machine.

In the FEI/Philips range of microscopes (the 400, CM and Tecnai series), a **nanoprobe** mode is available which uses the same lens configuration as in STEM operation. Here, however, C2 is used to focus the beam onto the specimen, as in the TEM mode. The convergence angles produced in the nanoprobe mode are intermediate in size between those in the TEM and STEM modes for the same size of C2 aperture. The convergence angles produced in the STEM and nanoprobe modes will be too large to produce a microdiffraction pattern in many samples (the patterns shown in *Figure 1.10c* and *d* were both taken in the nanoprobe mode).

Details of how to obtain CBED patterns are given in Section 1.9.

Figure 1.11. Schematic ray diagrams for probe formation in a TEM with a condenser-objective lens. The objective lens consists of two parts: the prefield and the imaging objective lenses; the specimen sits between the two. The auxiliary lens has the effect of partially nullifying the converging effect of the prefield lens, allowing a larger area to be illuminated in the imaging mode. (a) TEM mode, (b) mode in which the auxiliary lens is switched off. In STEM operation, the beam is focused using the objective lens. This lens configuration is also used in the so-called nanoprobe mode on FEI/Philips instruments. In the nanoprobe mode the C₂ lens is used to focus the beam. Note that, although the focal lengths of the auxiliary and prefield lenses are shown as roughly equal, in reality the former is several times larger than the latter. Adapted from Champness PE, *Mineral. Mag.*, 1987, with the permission of the Mineralogical Society, London.

1.7 Spatial resolution

What spatial resolution can be achieved using CBED methods? I have said earlier that the resolution is, to the first approximation, equal to the diameter of the electron probe; but what is that? You will find, in manufacturers' brochures and manuals, tables showing nominal probe sizes for different spot sizes and operating conditions (TEM or STEM), but what exactly do these values mean? They are normally the calculated values at 'full width at half maximum', FWHM; in other words, the width of the probe at half its maximum intensity, and assume that the intensity profile of the beam is Gaussian (in a Gaussian probe 50% of the total current is contained within a disc of diameter equal to the FWHM and 90% is contained within the full width at 10th maximum, FWTM). This

assumption may be far from the truth. The images of focused electron probes under various conditions are shown in *Figure 1.12*, together with their profiles. The probe on the far left is approximately Gaussian in shape, but the others are not. The 'tails' in the intensity distributions of the other probes have been produced by spherical aberration because the probes are highly converging (remember that spherical aberration causes electrons travelling furthest from the optic axis to be brought to focus closer to the lens than rays that are near-axial). For the probe in *Figure 1.12d* the tail contains an estimated 95% of the total probe current and extends to 1000 nm, despite having a FWHM of only 10 nm. If the probe size is defined as the diameter that contains 90% of the beam current, the spatial resolution for CBED when such a STEM probe is used can be more than 40 times the FWHM, compared with 1.82 times the FWHM for a Gaussian probe.

One way of decreasing the convergence angle is to use a smaller C2 aperture (*Figure 1.11*), at the expense, of course, of beam intensity. But it

Figure 1.12. Micrographs and schematic drawings of the electron density distributions in electron probes formed under various operating conditions in a Philips EM 400T. A 70- μm C2 aperture was used in each case, and the same excitation of C1 was used in (a), (b) and (c). In (d) a lower excitation of C1 was used. (a) In the 'conventional' TEM, or microprobe, mode the convergence angle is low and the probe is essentially Gaussian. (b) In the TEM nanoprobe mode, when the auxiliary lens is switched off, the convergence angle is greater and there is significant spherical aberration. This results in a non-Gaussian probe. (c) The STEM mode shows similar characteristics to the nanoprobe mode. (d) A STEM probe with an even higher convergence angle gives rise to an even larger 'tail' in the electron distribution. In (b), (c) and (d) the tails would be reduced if a smaller condenser aperture had been used. Reproduced from Cliff G and Kenway PB, *Microbeam Analysis*, 1982, with the permission of the San Francisco Press Inc.

will not be possible to use the ideal size of aperture for each operating condition (unless your microscope has a facility for continually varying α , see Section 1.10.1) as there are usually only three or four apertures to choose from. To minimize the effects of spherical aberration, in addition to using the most appropriate aperture, the beam must be well aligned, the condenser astigmatism corrected and all the apertures in the illumination system accurately centred. If you are concerned with knowing the characteristics of your probe, the best solution is to photograph it and obtain its profile using a microdensitometer on the photographic image, as shown in *Figure 1.12*.

Another factor that affects the size of the area from which a microdiffraction pattern comes is the thickness of the specimen. This is because, as the beam penetrates the specimen, it spreads out. The beam spreading is proportional to the thickness of the specimen to the power $^{3/2}$ and inversely proportional to the accelerating voltage (see for instance Williams and Carter, 1996). Thus, it is sensible to use as high a voltage and as thin a sample as possible to attain the highest resolution, though of course very thin samples and very small probes will not produce very intense diffraction patterns and will require long exposures.

Although I have sounded a note of caution about nominal 'probe sizes' and beam broadening, it is still possible to obtain microdiffraction patterns from areas as small as 2 nm with current instrumentation and a field-emission gun (FEG). With a LaB₆ gun the equivalent value is about 10 nm.

In modern TEMs, diffraction patterns with sharp spots can be obtained from areas a few tens of nanometres in diameter by first creating a small probe in TEM or nanoprobe mode, by using a small C2 (30 μ m or less) aperture, and then defocusing the probe to give parallel illumination. The **Riecke** diffraction pattern, sometimes called **micro-micro diffraction**, is then seen when the diffraction mode is selected. There are also a number of scanning methods for obtaining diffraction patterns from small areas which are outside the scope of this Handbook, but are covered by Williams and Carter (1996) and Keyse *et al.* (1998).

1.8 Problems with specimens

1.8.1 Specimen preparation

The standard methods for preparing biological specimens for the TEM are covered by Misell and Brown (1987), and the methods for non-biological specimens are covered by Goodhew (1984) and Williams and Carter (1996). Artefacts may arise in the preparation process that introduce spurious detail into the electron diffraction pattern.

Negative staining of biological samples may result in modification of the structure of the sample and the stain itself may recrystallize under the electron beam. One solution is to dispense with the stain and use a supporting medium such as glucose; an even better method is to observe the specimen in the frozen-hydrated state or to use an environmental cell in which the specimen may be observed in the wet state (Misell and Brown, 1987).

Electropolishing of metals sometimes produces a crystalline and/or amorphous oxide film on the surface of the specimen, for example on Mg and Al, and ion thinning may also re-deposit material on the surface of non-metallic specimens. Careful ion polishing will normally remove these surface films.

1.8.2 Beam damage

Organic materials such as polymers, proteins etc., together with inorganic salts, such as chlorides and bromides, and some silicate minerals, such as quartz, feldspar and clays, degrade during observation in the electron microscope because of damage due to ionization and/or heating from the electron beam. Often the material becomes amorphous (because the electron beam causes atomic bonds to be broken) and the diffraction pattern is lost (*Figure 1.13*); other materials transform to different compounds that can be identified by electron diffraction and X-ray analysis. Often the damage is so rapid that it is difficult to record a diffraction pattern under normal operating conditions.

There are several methods that you can use to minimize this type of beam damage.

- Operate at the highest possible kV. (The so-called **capture cross-section** for damage is *lower* the *higher* the kV.)
- Cool the specimen to the temperature of liquid nitrogen in a specially designed cold-stage. (The use of liquid helium appears only to be a marginal improvement over liquid nitrogen and is a lot more expensive!)
- Make preliminary adjustments such as setting the illumination conditions and focusing the pattern in a different area of the specimen from that you intend to use for recording. This can be done automatically on some instruments.
- Use a low beam-current and increase the exposure time accordingly. Or use an image intensifier.
- Use a short camera length, thus decreasing the exposure required. The pattern can be enlarged later.
- Encapsulate the specimen in a thin film of, for instance, carbon or calcium stearate. The protective effect is thought to be the result of the reduction in secondary damage. The diffusion of atomic and molecular species through the specimen is reduced and their recombination with damaged species is increased (see Misell and Brown, 1987).

Figure 1.13. Effect of radiation damage on the alkali feldspar albite, $\text{NaAlSi}_3\text{O}_8$, at 200 kV on the image (left) and diffraction pattern (right). (a) After 1 min, (b) 5 min, (c) 12 min. The nominal beam flux was $3.2 \times 10^{-2} \text{ cm}^2$. Note that the extinction contours visible in the image in (a) and (b) are no longer visible in the image in (c). The diffraction pattern present initially has disappeared after 12 min and is replaced by a faint, diffuse ring. Reproduced from Lorimer GW and Champness PE, *High Voltage Electron Microscopy*, 1974, with the permission of Academic Press.

The damage suffered by metals under the impact of the electron beam is of a different nature to that described above. This is called **knock-on** or **displacement damage** and occurs when electrons knock atoms out of their sites, so is directly related to the beam energy. Light metals such as Al and Mg suffer knock-on damage at 200 kV and, at 400 kV, metals with an atomic weight below that of Ti are damaged (see Williams and Carter, 1996). However, this damage is very slow and does not normally cause a problem for recording electron diffraction patterns.

1.9 Obtaining and recording a SAD pattern in the TEM

The complete procedure for obtaining a pattern is as follows.

- Select the required field of view; this must be within the SA range of magnification for FEI/Philips microscopes. Centre the beam and spread it to fill the field of view.
- Focus the image by using the **Objective focus** knob. Note that the image has minimum contrast when the objective aperture is removed and does not wobble when the **Wobbler** is switched on.
- Adjust the specimen height so that the specimen is in the eucentric plane (to ensure that the specimen is always at the same height for diffraction; i.e. the height for which the camera constant has been calibrated and the objective lens is working at its optimum setting).
 - a. Use the beam stop to mark a prominent feature of the specimen and tilt the specimen by about 20° about the major tilt axis; the image will move across the screen.
 - b. Adjust the specimen height using the z control (the thumbwheel on older Philips models) until the image returns to the centre of the screen.
 - c. Re-zero the tilt, re-centre the image with the specimen translators and re-focus the image.
 - d. Repeat steps a–c until there is minimum movement of the image. Note that on the CM and later series of FEI/Philips TEMs there is an **Autofocus** switch which sets the eucentric height; you then only have to refocus the image. Alternatively there is an ' α -**Wobbler**' control that, when depressed, rocks the major tilt by $\pm 15^\circ$ about zero; the z control is then used to adjust the height until the image no longer appears to move about the centre of the screen.
- Remove the objective aperture.
- Focus the second condenser lens (the **Intensity** control on FEI/Philips machines and the **Condenser** control on JEOL machines) and centre the beam using the **Deflection** (FEI/Philips) or **Trans** (JEOL) control.
- Defocus the second condenser lens and refocus the image.
- Insert a diffraction aperture (you will have a choice of three or four sizes) and centre it over the region whose diffraction pattern you wish to see. If the aperture does not appear in the image, you will need to remove the aperture, decrease the magnification, spread the illumination and re-insert the largest aperture, centre it, and gradually increase the magnification again while keeping the aperture in view with the alignment controls.
- On JEOL microscopes depress the **Sam/Rock** switch and focus the aperture with the **Diffraction focus** knob.
- Select the diffraction mode.

- Choose the required camera length using the selector switch. (The camera length is changed by variation of the excitation of the intermediate and projector lenses.) *Figure 1.6* shows an example of a pattern recorded at two different camera lengths.
- Underfocus the second condenser lens (usually an anti-clockwise rotation of the knob) by several coarse steps to obtain a more parallel electron beam. The more C2 is defocused, the sharper the diffraction spots will be. Aperture sizes of 200 μm in the first condenser lens, C1, and 100 μm in C2 are typical values for imaging purposes, but if high beam coherence is required in diffraction, for instance for diffraction from materials with large atomic repeats such as biological crystals or polymers, smaller C1 and C2 apertures should be used. Reduction of the **Spot size** using the C1 control (increasing the excitation of C1) also increases the **coherence** of the electron beam (coherence is a difficult concept in physics, but is a way of defining how well the electrons in the beam are 'in step' or 'in phase' with each other; a parallel beam is more coherent than a convergent beam). It should be noted that all these measures for increasing the coherence of the beam will also reduce the intensity of the diffraction pattern and result in the need for longer exposures. Cooling the specimen to the temperature of liquid nitrogen also improves the sharpness of the spots because it reduces thermal-diffuse scattering in the specimen, the scattering produced by the thermal motion of the atoms.
- Focus the diffraction pattern using the **Diffraction focus** (FEI/Philips) or **SA/HD diffraction camera length** control (JEOL). This focuses the diffraction lens accurately on the back focal-plane of the objective lens. (If the objective aperture is replaced, you should also see a sharp image of it).
- If necessary, the pattern can be centred on the screen using the **Diffraction point alignment** control (FEI/Philips) or the shift knob of the **Deflector-Projector** control (JEOL). In either case this procedure is achieved by deflection coils. It may be necessary to centre the pattern for each camera length.
- Correct any astigmatism of the diffraction lens (Section 1.11).
- If you want to take a photograph of the pattern, the exposure time needed will depend on the amount of defocus of C2, the type of specimen, etc. The exposure meter *cannot* be used for this purpose. The only way of finding the correct exposure is by experience or making a series of photographs at different exposure times. If the central beam is very intense compared with the other spots, the beam stop can be inserted and a double exposure taken. The first exposure includes the beam stop and the second is recorded without it for a length of time of about one 10th of the first exposure. An example of the use of the beam stop is shown in *Figure 1.6*. If you have a CCD (charge-coupled detector) imaging system or an image-plate system you will need to refer to the manufacturer's manual. Note that the CCD camera could be damaged when an intense diffraction pattern is focused on it.

1.10 Obtaining a CBED pattern

1.10.1 TEM mode

To form a microdiffraction pattern in the TEM mode the procedure is as follows.

- Make sure the specimen is in the eucentric plane and that the image is focused.
- Select a small C2 aperture (10–50 μm in diameter) and centre it.
- Decrease the spot size two or three clicks with the **Spot size** control (the excitation of C1 determines the spot size, which, in turn, determines the size of the area from which the diffraction pattern will be obtained). Remember that decreasing the spot size also decreases the electron-beam current at the specimen (it is proportional to α^2). You will probably have to correct the condenser astigmatism as you change the spot size.
- Focus the beam down onto the area of interest using the C2 lens control.
- Switch to the diffraction mode and remove the objective aperture. The pattern should now be visible. If the discs overlap, you will have to repeat the procedure from the second point using a smaller C2 aperture. Note: on the FX series and later JEOL machines the α -**Control** provides a continuous control of the convergence angle by changing the excitation of the auxiliary or mini-lens. A similar device will soon be available on the FEI/Philips Technai range.
- Use **Diffraction focus** to focus the image of the C2 aperture at the edge of one of the discs.
- You should check that the beam is accurately focused on the specimen. The procedure is to view the central beam through the binoculars and to overfocus (strengthen) either the C2 or the objective (**Focus**) lens so that an (out-of-focus) image is visible in the disc (*Figure 1.14a*). Then weaken the lens and the image will expand to higher magnifications until an inversion point is reached at exact focus (*Figure 1.14b*). At underfocus, an image will again be seen that is inverted with respect to the image at overfocus (*Figure 1.14c*).

1.10.2 STEM mode

The procedure is as follows:

- Obtain a focused image in STEM on the cathode-ray tube (see Keyse *et al.*, 1998 or Williams and Carter, 1996 for a description of the operation in STEM).
- Stop the beam scanning by selecting **Spot** on the STEM console.
- You may have to remove the STEM detector if it is above the TEM screen, or lower the TEM screen if the detector is below. Position the

Figure 1.14. Procedure for focusing a CBED pattern by adjusting the strength of the objective or C2 lens. Under overfocus (a) or underfocus (c) conditions you see an image of the specimen in the central disc, but at exact focus (b) the disc contains non-spatial contrast. Reproduced from Williams DB and Carter ECB, *Transmission Electron Microscopy*, 1996, with the permission of Plenum Press, New York.

spot on the region of interest. A CBED pattern should be visible on the fluorescent screen of the TEM because the microscope is operated in the diffraction mode during STEM.

- You can change the camera length and focus the image of the C2 aperture as in the TEM modes. However, in TEMs in which the C2 lens is switched off in STEM operation, the diffraction pattern can only be focused using the objective lens.

1.11 Alignments that are important for diffraction

The routine alignments of the illumination and imaging systems that need to be carried out before operation of the TEM are described by Chescoe and Goodhew (1990). One of these alignments that directly affects the diffraction pattern is correction of the **astigmatism** in the condenser lens. Astigmatism arises when the lens field is not perfectly symmetrical about the optic axis and results in a 'smearing out' of the image when it is not precisely in focus, and an unsharp image when apparently at focus. In the diffraction pattern, condenser astigmatism results in the diffraction spots being elliptical. Correction of astigmatism in the condenser lens is achieved by adjustment of the condenser stigmators, as described by Chescoe and Goodhew (1990).

Astigmatism in the diffraction lens also affects the diffraction pattern; in effect, it makes the magnification of the pattern on the screen or photograph unequal in different directions. 'Ring' patterns (see Section 2.5) show a series of concentric ellipses rather than circles. Any astigmatism is corrected as follows.

- Focus the image of a specimen within the SA range of magnifications and at the eucentric height.
- Remove the specimen.
- Select a small spot size with the C1 control and centre on the optic axis.
- Select the diffraction mode.
- Defocus C2 by several coarse clicks to obtain defocused illumination.
- Select the required camera length and centre the beam on the screen if necessary.
- Adjust the diffraction focus control until the image crossover is visible on the screen (*Figure 1.15a*)
- Adjust the diffraction stigmator controls until the crossover is a symmetrical three-pointed pattern as shown in *Figure 1.15b*.

Figure 1.15. Correction of astigmatism in the diffraction lens. (a) Uncorrected; (b) corrected.

Note that not all microscopes have the requisite stigmators to allow this correction.