CRYSTALS AND DIFFRACTION

Part
I

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Introduction

1

Much of our present knowledge of the architecture of molecules has been obtained from studies of the diffraction of X rays or neutrons by crystals. X rays* are scattered by the electrons of atoms and ions, and the interference between the X rays scattered by the different atoms or ions in a crystal can result in a diffraction pattern. Similarly, neutrons are scattered by the nuclei of atoms. Measurements on a crystal diffraction pattern can lead to information on the arrangement of atoms or ions within the crystal. This is the experimental technique to be described in this book.

* "X ray" for a noun, "X-ray" for an adjective.

X-ray diffraction was first used to establish the three-dimensional arrangement of atoms in a crystal by William Lawrence Bragg in 1913 (Bragg, 1913), shortly after Wilhelm Conrad Röntgen had discovered X rays and Max von Laue had shown in 1912 that these X rays could be diffracted by crystals (Röntgen, 1895; Friedrich et al., 1912). Later, in 1927 and 1936 respectively, it was also shown that electrons and neutrons could be diffracted by crystals (Davisson and Germer, 1927; von Halban and Preiswerk, 1936; Mitchell and Powers, 1936). Bragg found from X-ray diffraction studies that, in crystals of sodium chloride, each sodium is surrounded by six equidistant chlorines and each chlorine by six equidistant sodiums. No discrete molecules of NaCl were found and therefore Bragg surmised that the crystal consisted of sodium ions and chloride ions rather than individual (noncharged) atoms (Bragg, 1913); this had been predicted earlier by William Barlow and William Jackson Pope (Barlow and Pope, 1907), but had not, prior to the research of the Braggs, been demonstrated experimentally. A decade and a half later, in 1928, Kathleen Lonsdale used X-ray diffraction methods to show that the benzene ring is a flat regular hexagon in which all carbon–carbon bonds are equal in length, rather than a ring structure that contains alternating single and double bonds (Lonsdale, 1928). Her experimental result, later confirmed by spectroscopic studies (Stoicheff, 1954), was of great significance in chemistry. Since then X-ray and neutron diffraction have served to establish detailed features of the molecular structure of every kind of crystalline chemical species, from the simplest to those containing many thousands of atoms.

We address ourselves here to those concerned with or interested in structural aspects of chemistry and biology who wish to know how

crystal diffraction methods can be made to reveal the underlying three-dimensional structure within a crystal and how the results of such structure determinations may be critically assessed. In order to explain why molecular structure can be determined by single-crystal diffraction of X rays or neutrons, we shall try to answer several questions: Why use crystals and not liquids or gases? Why use X rays or neutrons and not other types of radiation? What experimental measurements are needed? What are the stages of a typical structure determination? How are the structures of macromolecules such as proteins and viruses determined? Why is the process of structure analysis sometimes lengthy and complex? Why is it necessary to "refine" the approximate structure that is first obtained? How can one assess the reliability of a crystal structure analysis?

This book should be regarded not as an account of "how to do it" or of practical procedural details, but rather as an effort to explain "why it is possible to do it." We aim to give an account of the underlying physical principles and of the kinds of experiments and methods of handling the experimental data that make this approach to molecular structure determination such a powerful and fruitful one. Practitioners are urged to look elsewhere for details.

The primary aim of a crystal structure analysis by X-ray or neutron diffraction is to obtain a detailed three-dimensional picture of the contents of the crystal at the atomic level, as if one had viewed it through an extremely powerful microscope. Once this information is available, and the positions of the individual atoms are therefore known precisely, one can calculate interatomic distances, bond angles, and other features of the molecular geometry that are of interest, such as the planarity of a particular group of atoms, the angles between planes, and conformation angles around bonds. Frequently the resulting three-dimensional representation of the atomic contents of the crystal establishes a structural formula and geometrical details hitherto completely unknown. Such information is of great interest to chemists, biochemists, and molecular biologists who are interested in the relation of structural features to chemical and biological effects. Furthermore, precise molecular dimensions (and information about molecular packing, molecular motion in the crystal, and molecular charge distribution) may be obtained by this method. These results expand our understanding of electronic structure, molecular strain, and the interactions between molecules.

Atoms and molecules are very small and therefore an extensive magnification is required to visualize them. The usual way to view a very small object is to use a lens, or, if even higher magnification is required, an optical or electron microscope. Light scattered by the object that we are viewing is recombined by the lens system of the microscope to give an image of the scattering matter, appropriately magnified, as shown in Figure 1.1a. This will be discussed and illustrated later, in Chapter 3. What is important is how the various scattered light waves interact with each other, that is, the overall relationship between the relative phases** of the various scattered waves (defined

** Relative phases (discussed in Chapter 3) describe the relationships between the various locations of peaks and troughs of a series of sinusoidal wave motions. They are described as "relative" phases because they are measured with respect to a fixed point in space, such as but not necessarily the selected origin of the unit cell.

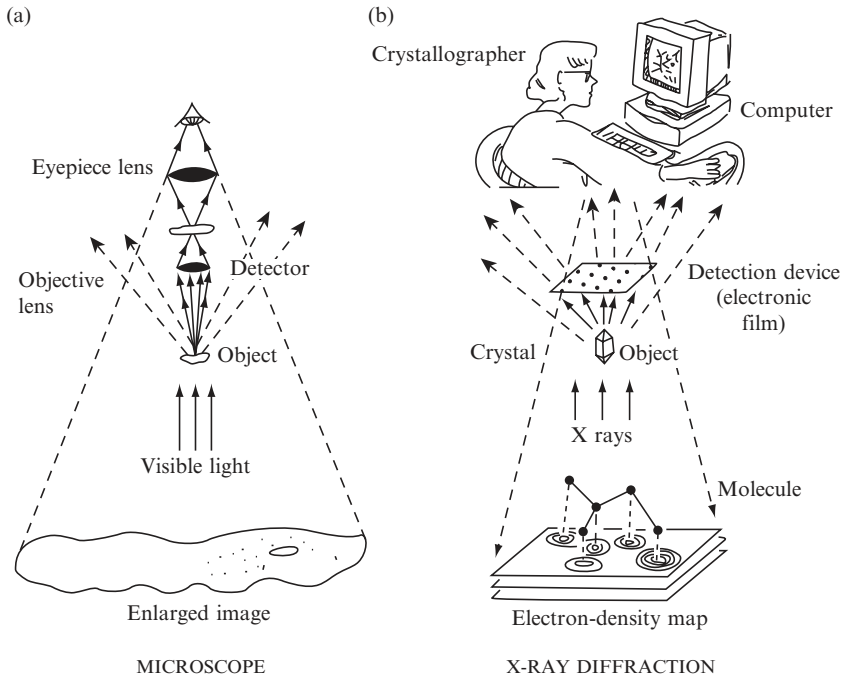

Fig. 1.1 Analogies between light microscopy and X-ray diffraction.Analogies between the two methods of using scattered radiation for determining structure are shown here—optical microscopy on the left, X-ray diffraction on the right. The sample that is under study in both instruments scatters some of the incident radiation and this gives a diffraction pattern.

(a) In the ordinary optical microscope there are two lenses. The lower objective lens gathers light that has been scattered by the object under study and focuses and magnifies it. The eyepiece or ocular lens, which is the one we look through, increases this magnification. There is no need to record a diffraction pattern because the light that is scattered by the object under examination is focused by these lenses and gives a magnified image of that object. The closer the objective lens is to the object, the wider the angle through which scattered radiation is caught by this lens and focused to form a high-resolution image. The rest of the radiation is lost to the surroundings.

(b) With X rays the diffraction pattern has to be recorded electronically or photographically, because X rays cannot (at this time) be focused by any known lens system. Therefore the recombination of the diffracted beams (which is done by an objective lens in the optical microscope) must, when X rays are used, be done mathematically by a crystallographer with the aid of a computer. As stressed later (Chapter 5), this recombination cannot be done directly, because the phase relations among the different diffracted beams cannot usually be measured directly. However, once these phases have been derived, deduced, guessed, or measured indirectly, an image can be constructed of the scattering matter that caused diffraction—the electron density in the crystal.

in Figure 1.2); this is because, when two scattered waves proceed in the same direction, the intensity of the combined wave will depend on the difference in the phases of the two scattered beams. If they are "in phase" they will enhance each other and give an intense beam, but if they are "out of phase" they will destroy each other and there will be no apparent diffracted beam. Generally it is found that such enhancement or destruction is only partial, so that the diffracted beams have some intensity and the diffraction pattern that is obtained contains diffracted beams that have differing intensities—some are weak and some are intense.

In an optical microscope, that is, a microscope that uses light that is visible to the human eye, the radiation scattered by the object is

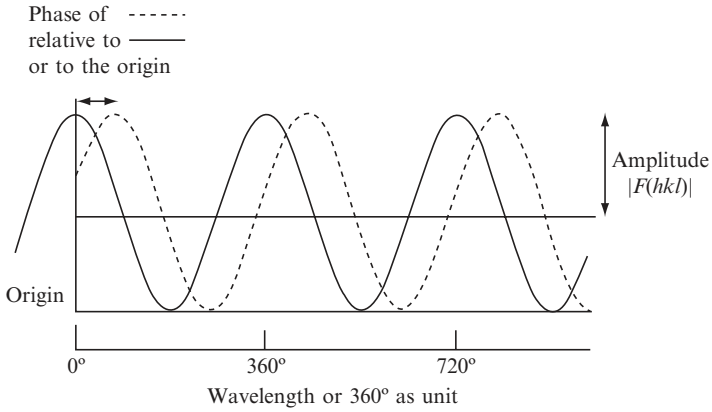

Fig. 1.2 A sinusoidal wave.

A sinusoidal wave, showing its amplitude, phase relative to the origin, and wavelength. Sine and cosine functions are sinusoidal waves with different phases [$\cos x = \sin(x + \pi/2)$ when the distance traveled is measured in radians]. Shown is a cosine wave (black line), which has a peak at the wave origin. This wave origin coincides with the origin in space that has been selected by the crystallographer. A second wave (dashed line) has its peak in a different location. The distance between these two peaks defines their "relative phase."

recombined by the lens system (the objective lens) so that a magnified image of the object under study is obtained (Figure 1.1a). Light flows through and beyond the lens system of the microscope in such a way that the relationships between the phases of the scattered waves are maintained, even after these waves have been recombined by the second lens (the eyepiece lens). In a similar way, X rays are scattered by the electrons in atoms and ions (Figure 1.1b), but, in contrast to the situation with visible light, these scattered X rays cannot be focused by any presently known experimental techniques. This is because no electric or magnetic field or material has yet been found that can refract X rays sufficiently to give a practicable X-ray lens. Therefore an X-ray microscope cannot yet be used to view atoms (which have dimensions too small to permit them to be visible with an ordinary light microscope). Much research on a possible X-ray lens is currently in progress (see Shapiro et al., 2005; Sayre, 2008). The information obtained from an X-ray diffraction experiment, however, is three-dimensional, and therefore the great usefulness of this method will doubtless continue after an X-ray lens can be made.

Since a lens system cannot be used to recombine scattered X rays to obtain images at atomic resolution, some other technique must be used if one wishes to view molecules. In practice, the diffracted (scattered) X rays or neutrons are intercepted and measured by a detecting system, but this means that the relationships between the phases of the scattered waves are lost; only the intensities (not the relative phases) of the diffracted waves can be measured. If the values of the phases of the diffracted beams were known, it would be possible to combine them with the experimental measurements of the diffraction pattern

and *simulate the recombination of the scattered radiation*—just as if a lens had done it—by an appropriate, though complicated, calculation (done by a crystallographer and a computer in Figure 1.1b). Then we would have an electron-density map, that is, an image of the material that had scattered the X rays. This mathematical calculation, the *Fourier synthesis* of the pattern of scattered or "diffracted" radiation (Fourier, 1822; Porter, 1906; Bragg, 1915), is a method for summing sinusoidal waves in order to obtain a representation of the material that scattered the radiation. Such a Fourier synthesis is a fundamental step in crystal structure determination by diffraction methods and is a central subject of our discussion (described in detail in Chapters 5, 6, 8, and 9). The difficult part of correctly summing these sinusoidal waves is termed the "phase problem," that is, finding where the peaks of each sinusoidal wave should lie with respect to the others in the summation. Any of several methods (to be described) can be used to overcome this difficulty and determine the phases of the various diffracted beams with respect to each other. When the correct phases are known (that is, derived, deduced, guessed, or measured indirectly), the three-dimensional structure of the atomic contents of the crystal (and hence of the molecules or ions that it contains) will be revealed as a result of a Fourier synthesis.

Why make the effort to carry out a crystal structure analysis? The reason is that when the method is successful, it is unique in providing an unambiguous and complete *three-dimensional representation of the atoms in the crystal.* This three-dimensionality is incredibly useful because chemical and biological reactions occur in three dimensions, not two; surface and internal structures of molecules, plus information on their interactions with other molecules, are revealed by this powerful technology. Other experimental methods can also provide structural information. For example, large molecules, such as those of viruses, can also be visualized by use of an electron microscope, but individual atoms deep inside each virus molecule cannot currently be distinguished. Newer technologies such as field ion microscopy and scanning tunneling microscopy (or atomic force microscopy) are now providing views of molecules on the surfaces of materials, but they also do not provide the detailed and precise information about the internal structure of larger molecules that X-ray and neutron diffraction studies do. Infrared and microwave spectroscopic techniques give quantitative structural information for simple molecules. High-field nuclear magnetic resonance (NMR), the main alternative method currently used for structure determination, can also provide distances between identified atoms and can be used to study fairly large molecules. No other method can, however, give the entire detailed three-dimensional picture that X-ray and neutron diffraction techniques can produce.

Crystal diffraction methods do, however, have their limitations, chiefly connected with obtaining samples with the highly regular long-range three-dimensional order characteristic of the ideal crystalline

state. The success of high-resolution diffraction analysis requires that the sample be prepared as an ordered array (e.g., a crystal). Molecular motion or static disorder within the regular array of molecules in a crystal may result in a time-averaged or space-averaged representation of the molecular structure. Freedom of molecular motion is, in general, much more restricted in solids than it is in liquids or gases. Even in solids, however, both overall and intramolecular motion can be appreciable, and precise diffraction data may reveal enlightening information about atomic and molecular motion.

When a crystal structure analysis by diffraction methods is completed, a wealth of information results. It reveals the shapes of molecules and the way they interact, and gives geometrical data for each. The method can be adapted to a wide range of temperatures, pressures, and environments and has been successfully used to establish the molecular architecture and packing of an enormous diversity of substances, from elementary hydrogen and simple salts to molecules such as buckminsterfullerene and to proteins and nucleic acids and their assemblages in viruses and other cellular structures. X-ray diffraction methods have also contributed significantly to our understanding of natural and synthetic partially crystalline materials such as polyethylene and fibers of DNA. Although structure determinations of organic and biochemically significant molecules have received the most attention in recent years, the contributions of the technique to inorganic chemistry have been equally profound, initially through the clarification of the chemistry of the silicates and of other chemical mysteries of minerals and inorganic solids, and then with applications to such diverse materials as the boron hydrides, alloys, hydrates, compounds of the rare gases, and metal-cluster compounds.

Throughout the book you may encounter symbols or terms that are unfamiliar, such as d_{010} or d_{110} in Figure 2.5. We have included a list of symbols at the start, and a glossary (to provide definitions of such symbols and words) and a list of references at the end of the book. We urge you to use all of these sections frequently as you work your way through the book.

Crystals

2

The elegance and beauty of crystals have always been a source of delight. What is a crystal? *A crystal is defined as a solid that contains a very high degree of long-range three-dimensional internal order of the component atoms, molecules, or ions*. This implies a repetitious internal organization, at least ideally.* By contrast, the internal organization of atoms and ions within a noncrystalline material is totally random, and the material is described as "amorphous." Studies of crystal morphology, that is, of the external features of crystals, have been made since early times, particularly by those interested in minerals (for practical as well as esthetic reasons) (Groth, 1906–1919; Burke, 1966; Schneer, 1977).

It was Max von Laue who realized in 1912 that this internal regularity of crystals gave them a grating-like quality so that they should be able to diffract electromagnetic radiation of an appropriate wavelength. From Avogadro's number (6.02×10^{23}, the number of molecules in the molecular weight in grams of a compound) and the volume that this one "gram molecule" of material fills, von Laue was able to reason that distances between atoms or ions in a crystal were of the order of 10^{-9} to 10^{-10} m (now described as 10 to 1 Å).** A big debate at that time was whether X rays were particles or waves. If X rays were found to be wavelike (rather than particle-like), von Laue estimated they would have wavelengths of this same order of magnitude, 10^{-9} to 10^{-10} m. Therefore, since diffraction was viewed as a property of waves rather than particles, von Laue urged Walther Friedrich and Paul Knipping to test if X rays could be diffracted by crystals. Their resulting diffraction experiment was dramatically successful. The crystal, because of its internal regularity, had indeed acted as a diffraction grating. This experiment was therefore considered to have demonstrated that X rays have wavelike properties (Friedrich et al., 1912). We now know that particles, such as neutrons or electrons, can also be diffracted. The X-ray diffraction experiment in 1912 was, in spite of this later finding, highly significant because it led to an extremely useful technique for the study of molecular structure. An analysis of the X-ray diffraction pattern of a crystal, by the methods to be described in this book, will give precise geometrical information on the molecules and ions that comprise the crystal.

The most obvious external characteristic of a crystal is that it has flat faces bounded by straight edges, but this property is not necessary

* Real crystals often exhibit a variety of imperfections—for example, short-range or long-range disorder, dislocations, irregular surfaces, twinning, and other kinds of defects—but, for our present purposes, it is a good approximation to consider that in a specimen of a single crystal the order is perfect and three-dimensional. We discuss very briefly in Chapter 13 the way in which our discussion must be modified when some disorder is present—for example, when the order is only one-dimensional, as in many fibers.

** Crystallographic interatomic distances are usually listed in Å. 1 Å = 10^{-8} cm = 10^{-10} m.

or sufficient to define a crystal. Glass and plastic, neither of which is crystalline, can be cut and polished so that they have faces that are flat with straight edges. However, they have not been made crystalline by the polishing, because their disordered internal structures have not been made regular (even though the word "crystal" is often used for some quality glassware). Therefore the presence of flat faces or straight edges in a material does not necessarily indicate that it is crystalline. It is the internal order, rather than external appearance, that defines a crystal. One way to check whether or not this internal order is present is to examine the diffraction pattern obtained when the material is targeted by a beam of X rays; the extent of the crystallinity (that is, the quality of its regular internal repetition) will be evident in any diffraction pattern obtained.

The fact that crystals have an internal structure that is periodic (regularly repeating) in three dimensions has long been known. It was surmised by Johannes Kepler, who wrote about the six-cornered snowflake, and by Robert Hooke, who published some of the earliest pictures of crystals viewed under a microscope (Kepler, 1611; Hooke, 1665; Bentley, 1931). They both speculated that crystals are built up from an ordered packing of roughly spheroidal particles. The Danish physician Nicolaus Steno (Niels Stensen) noted that although the faces of a crystalline substance often varied greatly in shape and size (depending on the conditions under which the crystals were formed), the angles between certain pairs of faces were always the same (Steno, 1669). From this observation Steno and Jean Baptiste Louis Romé de Lisle postulated the "Law of Constancy of Interfacial Angles" (Romé de Lisle, 1772). Such angles between specific faces of a crystal can be measured approximately with a protractor or more precisely with an optical goniometer (Greek: *gonia* = angle), and a great many highly precise measurements of the interfacial angles in crystals have been recorded over the past three centuries. This constancy of the interfacial angles for a given crystalline form of a substance is a result of its internal regularity (its molecular or ionic packing) and has been used with success as an aid in characterizing and identifying compounds in the old science of "pharmacognosy." Investigations of crystal form were carried out further by Torbern Olof Bergman in 1773 and René Just Haüy in 1782; they concluded independently, as a result of studies of crystals that had cleaved into small pieces when accidentally dropped, that crystals could be considered to be built up of building bricks of specific sizes and shapes for the particular crystal. These ideas led to the concept of the "unit cell," the basic building block of crystals (Bergman, 1773; Haüy, 1784; Burke, 1966; Lima-de-Faria, 1990).

Obtaining and growing crystals

The growth of crystals is a fascinating experimental exercise that the reader is urged to try (Holden and Singer, 1960; McPherson, 1982;

Ducruix and Giegé, 1999; Bergfors, 2009). Considerable perseverance and patience are necessary, but the better the quality of the crystal the more precise the resulting crystal structure. Generally crystals are grown from solution, but other methods that can be used involve cooling molten material or sublimation of material onto a surface.

In order to obtain crystals from solution it is necessary to dissolve the required substance (the solute) in a suitable solvent until it is near its saturation point, and then increase the concentration of the solute in the solution by slowly evaporating or otherwise removing solvent. This provides a saturated or supersaturated solution from which material will separate, and the aim is to make this separation occur in the form of crystals. During the growth process, solute molecules meet in solution and form small aggregates, a process referred to as "nucleation." Extraneous foreign particles (such as those from a person's beard or hair, or "seeds," or dust) may serve as initiators of such nucleation. More molecules are then laid down on the surface of this nucleus, and eventually a crystal may separate from the solution. Crystal growth will continue until the concentration of the material being crystallized falls below the saturation point:

Saturated solution → Supersaturation → Nucleation → Crystal growth

The crystallization process is essentially a controlled precipitation onto an appropriate nucleation site. If growth conditions are achieved too quickly, many nucleation sites may form and crystals may be smaller than those obtained under slower crystallization conditions. If too few nucleation sites form, crystals may not grow. Crystal habit (overall shape) may be modified by the addition of soluble foreign materials to the crystallization solution. These added molecules may bind to growing crystal faces and inhibit their growth. As a result, different sets of crystal faces may become more prominent.

When a molecule or ion approaches a growing crystal, it will form more interactions than otherwise if it can bind at a step in the formation of layers of molecules in the crystal. Various irregularities or defects (dislocations) in the internal order of stacking can facilitate the formation of steps and therefore aid in the crystallization process. Most real crystals are not perfect; that is, the regularity of packing of molecules may not be exact. In general, they tend to be composed of small blocks of precisely aligned unit cells (domains) that may each be slightly misaligned with respect to each other. The extent to which this occurs is referred to as the "mosaicity" of the crystal, and its measurement indicates the degree of long-range crystalline order (regularity) in the crystal under study. Most real crystals are described as "ideally imperfect" if they have a mosaic structure composed of slightly misoriented very small crystal domains.

Several of the methods that are now used to facilitate the growth of crystals involve changing the experimental conditions so that

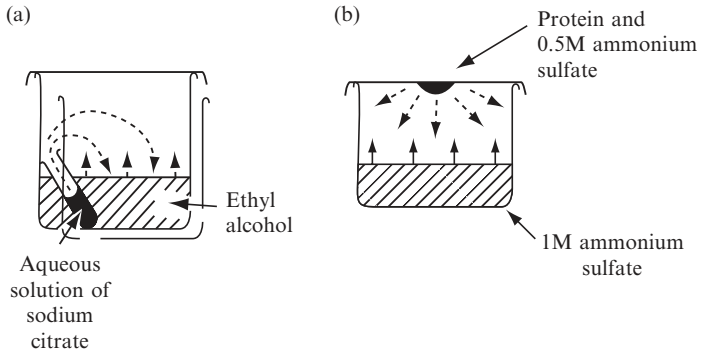

Fig. 2.1 Crystals being grown by the vapor diffusion method.

(a) The sample (sodium citrate) to be crystallized is soluble in water but is not very soluble in ethyl alcohol. A test tube containing sodium citrate dissolved in water is sealed in a beaker containing ethyl alcohol. An equilibrium between the two liquids is then approached. Vapor phase diffusion of the water molecules from the test tube into the larger volume in the reservoir and of alcohol into the smaller volume in the test tube takes place. The result is that crystals separate out in the test tube as the solution in it becomes more concentrated and the alcohol helps the citrate to separate out.

(b) Pure protein is usually available only in limited quantities and therefore the following scheme has been adopted to circumvent this problem. A drop of protein solution is placed on a cover slip, which is sealed with grease over a container (a beaker or one of the many small wells in a biological culture tray). In this sealed system, the protein drop contains precipitant at a concentration below the point at which protein precipitation would be expected; the sealed container (the well) contains a much larger volume of precipitant at or slightly above the concentration of the precipitation point of the protein. Water evaporates slowly from the protein-containing drop into the container until the concentration of precipitant in the hanging drop is the same as that in the well, and crystallization may occur. This method works best for a protein if it is highly purified.

saturation of the solution will be exceeded, generally by a slow precipitation method (see Figure 2.1). In one method, a precipitant (that is, a liquid or solution of a compound in which the substance is insoluble) is layered on a solution of the material to be crystallized. For example, alcohol, acting as a precipitant, when carefully layered on top of a saturated aqueous solution of sodium citrate and left for a day or so, will generally give good diffraction-quality crystals. Alternatively, some of the solvent may be slowly removed from the solution by equilibration through the vapor phase in a closed system, thereby increasing the concentration of the material being crystallized. This can be done, as shown in Figure 2.1a, with an aqueous solution of sodium citrate in a test tube, placed in a covered beaker containing ethyl alcohol alone; equilibration of the solvents in this sealed container will (hopefully) then cause the formation of crystals. This vapor diffusion method is also used for macromolecules. An aqueous solution of the protein, together with a precipitant (a salt such as ammonium sulfate, or an alcohol such as methylpentanediol) in the same solution but at a concentration below

that which will cause precipitation, is put in a dish, or suspended as a droplet on a microscope slide, in a sealed container. Then another, more concentrated, precipitant solution is placed at the bottom of the same sealed container (Figure 2.1b). Water will be transferred through the vapor phase from the solution that is less concentrated in the precipitant (but containing protein) to that which is more concentrated (but lacking protein). The result is a loss of water from the suspended droplet containing protein. As the precipitation point of the protein is reached in the course of this dehydration, factors such as pH, temperature, ionic strength, and choice of buffer will control whether the protein will separate from the solution as a crystal or as an amorphous precipitate.

In summary, the main factors affecting the growth of good crystals are an appropriate choice of solvent, suitable generation of nucleation sites, control of the rate of crystal growth, and a lack of any disturbance of the crystallization system (see Chayen, 2005). In practice the equipment for doing this is now increasingly sophisticated, and often, for macromolecules, a robot setup is used that provides a wide variety of conditions for crystallization (Snook et al., 2000). For example, it has been found that protein crystallization may be more successful on space shuttles, where gravity is reduced (DeLucas et al., 1999). The components do not then separate as quickly and fluid flow at the site of crystallization is reduced.

Crystals suitable for modern single-crystal diffraction need not be large. For X-ray work, specimens with dimensions of 0.2 to 0.4 mm or less on an edge are usually appropriate. Such a crystal normally weighs only a small fraction of a milligram and, unless there is radiation damage or crystal deterioration during X-ray exposure, can be reclaimed intact at the end of the experiment. Larger crystals are needed for neutron diffraction studies, although this requirement is becoming less strict as better sources of neutrons become available.

Sometimes a crystal is difficult to prepare or is unstable under ordinary conditions. It may react with oxygen or water vapor, or may effloresce (that is, lose solvent of crystallization and form a noncrystalline powder) or deliquesce (that is, take up water from the atmosphere and eventually form a solution). Many crystals of biologically interesting materials are unstable unless the relative humidity is extremely high; since such crystals contain a high proportion of water, they are fragile and crush easily. Special techniques, such as sealing the crystal in a capillary tube in a suitable atmosphere, cooling the crystal, or growing it at very low temperatures, can be used to surmount such experimental difficulties. Sometimes a twinned crystal may be formed as the result of an intergrowth of two separate crystals in a variety of specific configurations. This may complicate optical and diffraction studies, but methods have been devised for working with them because sometimes only twinned crystals, and no single crystals, can be obtained.

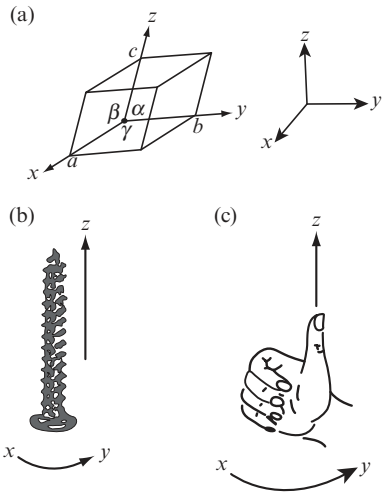

Fig. 2.2 Unit-cell axes.

(a) A unit cell showing the axial lengths a, b, and c and the interaxial angles (α between b and c, β between c and a, and γ between a and b). The directions of axes are given in a right-handed system, as shown by the screw in (b) and the human fist in (c). As x is moved to y, the screw in (b) or the thumb in (c) moves in the z-direction in a right-handed manner.

† A parallelepiped is a *three-dimensional* polyhedron with six faces, each a parallelogram that is parallel to a similarly shaped opposite face. It does not have any requirement that all or any angles at the corners of the six faces be 90°. Each face, a parallelogram, is a four-sided *two-dimensional* polygon with two pairs of parallel sides.

The unit cell of a crystal

Any crystal may be regarded as being built up by the continuing *three-dimensional translational repetition of some basic structural pattern*, which may consist of one or more atoms, a molecule or ion, or even a complex assembly of molecules and ions; the simplest component of this three-dimensional pattern is called the "unit cell." It is analogous to a building brick. The word "translational" in the above definition of a crystal implies that there is within it a repetition of an arrangement of atoms in a specific direction at regular intervals; this repeat distance defines a measure of the unit-cell dimension in that direction.

The basic building block of a crystal is an imaginary three-dimensional parallelepiped,† the "unit cell," that contains one unit of the translationally repeating pattern. It is defined by three noncoplanar vectors (the crystal axes) **a**, **b**, and **c**, with magnitudes a, b, and c (Figure 2.2a). These vectors are arranged, for convenience, in the sequence **a**, **b**, **c**, in a right-handed axial system (see Figures 2.2b and c). The angles between these axial vectors are α between **b** and **c**, β between **a** and **c**, and γ between **a** and **b** (see Figure 2.2). Thus, the size and shape of the unit cell are defined by the dimensions a, b, c, α, β, γ. As will be described later, atomic positions along each of the unit-cell directions are generally measured as fractions x, y, and z of the repeat lengths a, b, and c.

The unit cell is a complete representation of the contents of the repeating unit of the crystal. As a building block, it must pack in three-dimensional space without any gaps. The unit cells of most crystals are, of course, extremely small, because they contain comparatively few molecules or ions, and because normal interatomic distances are of the order of a few Å. For example, a diamond is built up of a three-dimensional network of tetrahedrally linked carbon atoms, 1.54 Å apart. This atomic arrangement lies in a cubic unit cell, 3.6 Å on an edge. A one-carat diamond, which has approximately the volume of

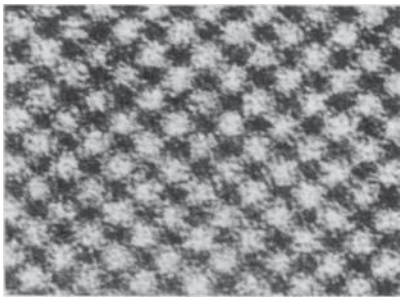

Fig. 2.3 An electron micrograph of a crystalline protein.

An electron micrograph of a crystalline protein, fumarase, molecular weight about 200,000. The individual molecules, in white, are visible as approximately spherical structures at low resolution. Note that several choices of unit cell are possible.
(Photograph courtesy of Dr. L. D. Simon)

a cube a little less than 4 mm on a side, thus contains 10^{21} unit cells of the diamond structure.‡ A typical crystal suitable for X-ray structure analysis, a few tenths of a millimeter in average dimension, contains 10^{12} to 10^{18} unit cells, each with identical contents that can diffract X rays in unison. Figure 2.3 shows an electron micrograph of a protein crystal and the regularity of its molecular packing. The existence of unit cells in this micrograph is evident.

‡ The unit cell of diamond is cubic. The unit cell edges are 3.6 Å. Given that the density is 3.5 g cm^{-3} we can calculate that there are 8 atoms of C in the unit cell. 1 carat weighs 0.2 g.

The faces of a crystal

There is a need to be able to describe a specific face of a crystal, and this is done with respect to the chosen unit cell. Finding three integers that characterize a given crystal face or plane is known as "indexing." As shown in Figure 2.4, a crystal face or crystal plane is indexed with three numbers, h, k, and l, with these indices relatively prime (not each divisible by the same factor), when the crystal face or plane makes intercepts a/h, b/k, c/l with the edges of the unit cell (lengths a, b, and c). This is derived from the "Law of Rational Indices," which states that each face of a crystal may be described by three whole (rational) numbers; these three numbers describing a crystal face are enclosed in parentheses as (*hkl*). This nomenclature was introduced by William Whewell and William Hallowes Miller (see Haüy, 1784, 1801; Miller, 1839). If a crystal face is parallel to one crystal axis, its intercept on that axis is at infinity, so that the corresponding "Miller index" is zero, as shown in Figure 2.4a. If a crystal face intersects the unit-cell edge at one-third its length, the value of the index is 3, as shown in Figure 2.4b. When the crystal faces have been indexed and the angles between them measured, it is possible to derive the ratio of the lengths of the unit

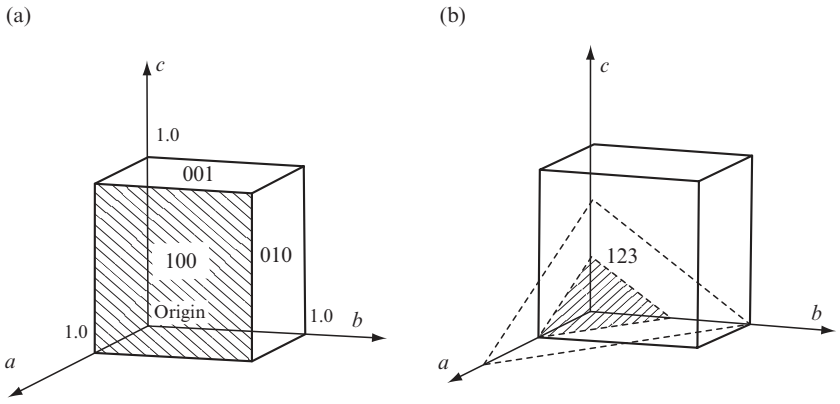

Fig. 2.4 Indexing faces of a crystal.

A crystal face or plane (*hkl*) makes intercepts a/h, b/k, c/l with the edges of the unit cell of lengths a, b, and c. (a) The (100), (010), and (001) faces are shown. (b) The (123) face makes intercepts $a/1$, $b/2$, and $c/3$ with the unit-cell axes. A parallel crystal plane (unshaded) is also indicated; it makes the same intercepts with the next unit cell nearer to the observer.

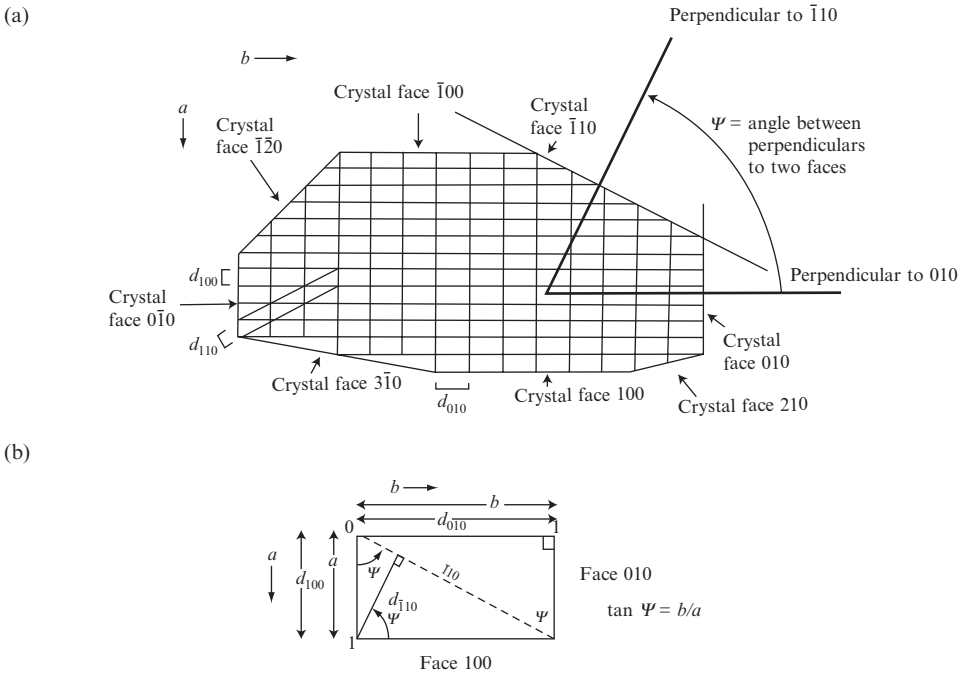

Fig. 2.5 The determination of the probable shape of the unit cell from interfacial angles in the crystal.

(a) A cross section of a crystal viewed down the *c*-axis. For each face in this figure, $l = 0$. If the faces can be indexed and the angles between these faces measured, it is possible to derive the ratio of the lengths of the unit cell edges (in this example b/a). This will then give the shape (but not the absolute dimensions) of the unit cell. (b) One unit cell, showing the indices of some faces and the interplanar spacings d_{hkl} (the spacing between crystal lattice planes (hkl) in the crystal).

cell edges and hence the shape (but not the absolute dimensions) of the unit cell.

The relative lengths of some interplanar spacings, d_{hkl} (the spacing between the crystal lattice planes (hkl) in the crystal), are indicated in both Figures 2.5a and b. An index (hkl) with a line above any of these entries means that the value is negative. For example, $(3\ \bar{1}\ 0)$ means $h = 3$, $k = -1$, $l = 0$; the intercepts with the axes are $a/3$ and $-b$, and the faces or planes lie parallel to c, since $l = 0$ (intercept infinity). Sets of planes that are equivalent by symmetry (such as (100), (010), (001), $(\bar{1}00)$, $(0\bar{1}0)$, and $(00\bar{1})$ constitute a crystal form, represented (with "squiggly" brackets) as $\{100\}$. Square brackets enclosing three integers indicate a crystal lattice row; for example, [010] denotes the direction of the **b** axis, that is, a line connecting the unit-cell origin to a point with coordinates $x = 0$, $y = 1$, $z = 0$. Before the discovery of X-ray diffraction in 1912, it was possible to deduce only the relative lengths of the unit-cell axes and the values of the interaxial angles from measurements of interfacial angles in crystalline specimens by means of a special instrument (an optical goniometer), as shown in Figure 2.5a. As we shall see shortly, however, X rays provide a tool for measuring the actual lengths of these axes, and therefore the size, as well as the shape, of the unit

cell of any crystal can be found. In addition, if the density of a crystal is measured, one can calculate the weight (and hence, in most cases, the atomic contents) of atoms in the unit cell. The method for doing this is described in Appendix 1.

The crystal lattice

The *crystal lattice* highlights the repetition of the unit-cell contents within the crystal. If, in a diagram of a crystal, *each complete repeating unit (unit cell) is replaced by a point*, the result is the crystal lattice. It is an infinite three-dimensional network of points that may be generated from a single starting point (at a chosen position in the unit cell) by an extended repetition of a set of translations that are, in most cases, the conventional unit-cell vectors just described. This highlights the regularly repeating internal structure of the crystal, as shown in Figure 2.6.

The term "crystal lattice" is sometimes, misleadingly, used to refer to the crystal structure itself. It is important to remember that a *crystal structure is an ordered array of objects (atoms, molecules, ions) that make up a crystal, while a crystal lattice is merely an ordered array of imaginary points*. Although crystal lattice points are conventionally placed at the corners of the unit cell, there is no reason why this need be done. The crystal lattice may be imagined to be free to move in a straight line (although not to rotate) in any direction relative to the structure. A crystal lattice point may be positioned anywhere in the unit cell, but exactly the same position in the next unit cell is chosen for the next crystal lattice point. As a result every crystal lattice point in the unit cell will have the same environment as every other crystal lattice point in the crystal. The most general kind of crystal lattice, composed of unit cells with three unequal edges and three unequal angles, is called a triclinic crystal lattice. Once the crystal lattice is known, the entire crystal structure may be described as a combination (convolution[§]) of the crystal lattice with the contents of one unit cell, as shown in Figure 2.6.

The two-dimensional example of the regular translational repetition of apples, illustrated in Figure 2.6, might serve as a pattern for wallpaper (which generally has two-dimensional translational repetitions). Several possible choices of unit cell, however, can be made from the two-dimensional arrangement of apples in it. How, then, can we speak of *the unit cell* for a given crystal? In general, we can't. The conventional choice of unit cell is made by examining the crystal lattice of the crystal and choosing a unit cell whose shape displays the full symmetry of the crystal lattice—rotational as well as translational—and that is convenient. For example, the axial lengths may be the shortest possible ones that can be chosen and the interaxial angles may be as near as possible to 90°. There may be several possibilities that fit these conditions. It is usual to derive the Niggli reduced cell (Niggli, 1928; de

[§] A convolution (with axes u, v, w) is a way of combining two functions $A(x, y, z)$ and $B(x, y, z)$ (with axes x, y, z). It is an integral that expresses the extent to which one function overlaps another function as it is shifted over it. The convolution of these two functions A and B at a point (u_0, v_0, w_0) is found by multiplying together the values $A(x, y, z)$ and $B(x + u_0, y + v_0, z + w_0)$ for all possible values of x, y, and z and summing all these products. This process must then be repeated for each value of u, v, and w of the convolution. A crystal structure, for example, can be viewed as the convolution of a crystal lattice (function A) with the contents of a single unit cell (function B) (see Figure 2.6). This is a simple example because the crystal lattice exists only at discrete points and the rest of this function A has zero values. This convolution converts a specific unit of pattern into a series of identical copies arranged on the crystal lattice. All that is needed is information on the geometry of the crystal lattice and on the unit of pattern; the convolution of these two functions gives the crystal structure.

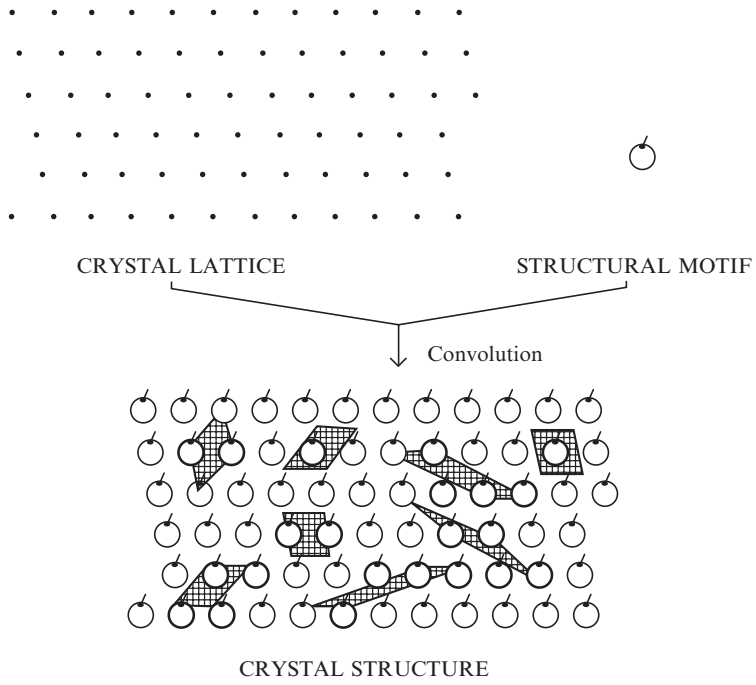

Fig. 2.6 The crystal lattice and choices of unit cells.

The generation of a two-dimensional "crystal structure" from a crystal lattice and a structural motif (an apple in this example). The crystal lattice is obtained from the crystal structure by replacing each complete repeating unit by a point. The replacement of each point in the crystal lattice by an apple would lead to a two-dimensional crystal structure. This *crystal structure* may be described alternatively as the *convolution of an apple and the crystal lattice*. There are many ways in which unit cells may be chosen in the repeating pattern of apples. Some possible alternative choices are shaded, each having the same area despite varying shape. This can be verified by noting that the total content of any chosen unit cell in this figure is one apple. Infinite repetition in two dimensions of any one of these choices for unit cell will reproduce the entire pattern.

Wolff and Gruber, 1991), that is, to select the three shortest noncoplanar vectors in the crystal lattice. This may help in establishing whether two crystals with different unit-cell dimensions are really the same or not.

It is a common misconception, perhaps arising from the abundance of illustrations of the simplest elementary and ionic structures in textbooks, that an atom must lie at the corner (origin) of each unit cell. It is possible to choose the origin arbitrarily and place it at the site of an atom, but in most structures the choice of origin is dictated by convenience, because of its relation to symmetry elements that may be present (i.e., the appropriate space group), and in the great majority of known structures no atom is present at the origin. Another misconception is that what a chemist finds convenient to regard as a single molecule or formula unit must lie entirely within one unit cell. Portions of a single bonded aggregate may lie in two or more adjacent unit

cells. If this does happen, however, any single unit cell will necessarily still contain all of the independent atoms in the molecule—the atoms simply comprise portions of different molecules. This is illustrated in Figure 2.6, which shows that a given unit cell may contain only one apple or portions of two or more apples.

Crystal symmetry

Unit cells and crystal lattices are *classified according to their rotational symmetry*. If an object is rotated 180° and then appears identical to the starting structure, the object is said to have a two-fold rotation axis (the axis about which the 180° rotation occurred). The presence of an n-fold rotation axis, where n is any integer, means that when the unit cell is rotated $(360/n)^\circ$ about this axis, the crystal lattice so obtained is indistinguishable from original before rotation. If you closed your eyes, rotated the crystal lattice, and opened your eyes again, nothing would appear to have changed. The symmetry of an isolated crystal can be found by examination, and it can give us some very useful information about the internal atomic arrangement. If the crystal is set down on a flat surface, it is possible to note if there is another face on top of the crystal that is parallel to the lower face lying on the flat surface. Then one can determine if there is a center of symmetry between these upper and lower faces of the crystal. Similarly, one can examine the crystal for two-, three-, four- and six-fold rotation axes. The result of such examinations is the determination of the point group of the crystal, that is, a group of symmetry operations, such as an n-fold rotation axis, that leaves at least one point unchanged within the crystal.

It is shown in Appendix 2 that there are seven ways in which different types of applicable rotational symmetry (such as two-, three-, four-, and six-fold rotation axes) lead to infinitely repeatable unit cells. These seven are called the *seven crystal systems*—triclinic, monoclinic, orthorhombic, tetragonal, trigonal/rhombohedral, hexagonal, and cubic. They are distinguished by their different rotational symmetries. For example, in a triclinic crystal lattice there is no rotational (only one-fold) symmetry; this defines the term "triclinic." As a result, usually (but not always) in a triclinic crystal lattice, all unit-cell lengths (a, b, and c) are unequal, as are all interaxial angles (α, β, and γ). A monoclinic crystal lattice ($\alpha = \gamma = 90^\circ$) has a two-fold rotation axis parallel to the b axis (where b is chosen, by convention for this crystal system, to be unique). This means that a rotation of the crystal lattice by 180° about the b axis gives a crystal lattice indistinguishable from the original. In an orthorhombic crystal lattice, with three mutually perpendicular rotation axes, all interaxial angles (α, β, and γ) are 90°. A cubic crystal is defined by three-fold axes along the cube diagonals, not by its four-fold axes. It must be stressed that it is the symmetry of the crystal lattice that is important in defining the crystal system, not the magnitude of the interaxial angles. Some monoclinic crystals have been found with

$\beta = 90^\circ$, and some triclinic crystals with all interaxial angles very close to 90°; this is why symmetry rather than unit cell dimensions are used to define which is the correct crystal system for the material under study. In the diagrams of these seven crystal systems in Appendix 2 all crystal lattice points (designated by small circles) are equivalent by translational symmetry. All crystal lattices except the triclinic crystal lattice display more than one-fold rotational symmetry (see Chapter 7 for details).

It is customary when choosing a unit cell to take advantage of the highest symmetry of the crystal lattice. If a unit cell includes only one crystal lattice point (obtained from the fractions at each corner), it is said to be primitive and the crystal lattice is designated P. Sometimes it is more convenient to choose a unit cell that contains more than one crystal lattice point (a "nonprimitive" unit cell). Nonprimitive unit cells are chosen because they display the full symmetry of the crystal lattice, or are more convenient for calculation; any given crystal lattice may always be described in terms of either primitive or nonprimitive unit cells. The latter type of crystal lattices have lattice points not only at the corners of the conventional unit cell, but also at the center of this unit cell (I for the German *innenzentrierte*), at the center of one pair of opposite faces (A, B, or C), or at the center of all three pairs of opposite faces (F) (see Appendix 2). More than one crystal lattice point is then associated with a unit cell so chosen, but the requirement that every crystal lattice point must have identical surroundings is still fulfilled. That there are 14, and only 14, distinct types of crystal lattices was deduced by Moritz Ludwig Frankenheim and Auguste Bravais in the nineteenth century, and these crystal lattices are named after Bravais (Bravais, 1850). The Bravais crystal lattices are obtained from a combination of the seven crystal systems (triclinic, monoclinic, orthorhombic, tetragonal, trigonal/rhombohedral, hexagonal, and cubic) with the four crystal lattice types (P, A or B or C, F, and I) after the elimination of any equivalencies. The unit cells of these 14 Bravais crystal lattices are shown in Appendix 2.

Space groups

Since the atomic contents in each unit cell are identical (or nearly so), the symmetry of the arrangement of atoms in each unit cell must be related by certain symmetry operations (in addition to translation) that ensure identity from unit cell to unit cell. This means that the atomic arrangement in one unit cell is related by defined symmetry operations to the arrangement in all other unit cells. The smallest part of a crystal structure from which the complete structure can be obtained by space-group symmetry operations (including translations) is called the asymmetric unit. The operation of the correct space-group symmetry elements (other than crystal lattice translations) on the asymmetric unit

will generate the entire contents of a primitive unit cell. When one considers the possible combinations of symmetry elements (centers of symmetry, mirror planes, glide planes, rotation axes, and screw axes) that are consistent with the 14 Bravais crystal lattices, and thus the possible symmetry elements of the structures that can be arranged on the crystal lattices, it is found that 230, and only 230, distinct combinations of the possible symmetry elements exist for three-dimensional crystals (and only 17 plane groups for two-dimensional wallpaper). Thus the many different ways of arranging atoms or ions in structures to give a regularly repeating three-dimensional arrangement in a crystal fall into 230, and only 230, different three-dimensional crystallographic space groups. They are listed in *International Tables for (X-ray) Crystallography* (referred to here as *International Tables*), and these Tables, listed at the end of this book in the "References and further reading" section, are constantly used by crystallographers. The important result is that if the location of one atom in a crystal of known space group has been found, then application of the space-group symmetry operations (listed for convenience in *International Tables*) will give the locations of all other such specific atoms in the unit cell. This can be repeated for each atom in the ions or molecules that make up the crystal. Symmetry and space groups are discussed further in Chapter 7.

Physical properties of crystals

Optical properties

The interaction of light with crystals is one of the reasons they are used for ornamentation (as jewelry). It may also reveal information about crystalline symmetry and, in certain cases, the internal structure of the crystal (Hartshorne and Stuart, 1950; Wood, 1977; Wahlstrom, 1979). Particularly useful information may be obtained from the refractive index of the crystal. This gives a measure of the change in the velocity of light when it enters the crystal. Refraction is evident when a straight stick or rod is partially inserted in water; the rod appears to be bent at the point of entry. The change in the velocity of light as it passes from air to water is revealed by the angle to which the rod appears to be bent; when this angle is measured it gives information on the ratio of the two velocities (that is, the refractive index of water). The refractive index of a crystal is generally measured by immersing it in liquids of known refractive index, and determining when the crystal becomes "invisible." The crystal and the liquid surrounding it now have the same refractive index.

Some crystals, such as cubic crystals, are optically isotropic: the refractive index is independent of the direction from which the crystal is viewed. Other crystals may be birefringent, with different refractive indices in different directions. When a test tube containing birefringent

crystals in their mother liquor is shaken, the crystals glisten (unlike the situation for isotropic cubic crystals). Birefringence, or double refraction, is the decomposition of light into two rays, each polarized. One ray, the "ordinary ray," travels through the crystal with the same velocity in every direction. The other ray, the "extraordinary ray," travels with a velocity that depends on the direction of passage through the crystal. The result of this can readily be seen for a calcite crystal (Iceland spar) in Figure 2.7, in which two images are formed when light passes through the crystal. If a birefringent crystal is colored, it may show different colors when viewed in different directions. If the crystal structure contains an approximately planar group, measurements of refractive indices may permit deduction of the orientation of this planar group within the chosen unit cell. This method, combined with unit-cell measurements, was used to study steroid dimensions and packing long before any complete structure determination was or could be initiated. It led to a correct chemical formula for atoms in the steroid ring structure (Bernal, 1932).

Fig. 2.7 The birefringence of calcite (Iceland spar).

View through an Iceland spar crystal (calcite) with the word "BIREFRINGENCE" written on a strip of paper behind it. Light is broken into two polarized beams as it passes through the crystal. The word is split into two images, hence the term "birefringence." As the crystal is rotated, the image made by the extraordinary ray moves around the image made by the ordinary ray. Iceland spar crystals are believed to have been used in the Arctic regions for ages in navigation to determine the direction of the sun on a cloudy day, and hence which direction to sail in.

There are many other interesting optical properties of crystals. Second-harmonic generation (SHG, also called frequency doubling) was first demonstrated when a ruby laser with a wavelength of 694 nm was focused into a quartz crystal (Dougherty and Kurz, 1976). Analysis with a spectrometer indicated that light was produced with a wavelength of 347 nm (half the wavelength and twice the frequency of the incident light). Only noncentrosymmetric crystal structures can double the frequency, and therefore SHG provides a useful method for testing the symmetry of a crystal. Green laser pointers combine a noncentrosymmetric (nonlinear) crystal with a red neodymium laser to produce green light.

Electrical properties

Certain crystals display piezoelectricity. This word is derived from a Greek word meaning "to squeeze" or "press." Piezoelectricity is the creation of an electrical potential by a crystal in response to an applied mechanical stress. This effect is reversible in that materials exhibiting the direct piezoelectric effect also exhibit the converse piezoelectric effect (the production of stress when an electric field is applied). The piezoelectric effect was first reported by Pierre and Jacques Curie in 1880, who detected a voltage across the faces of a compressed Rochelle salt crystal (Curie and Curie, 1880). The phenomenon has many industrial uses. For example, when the button of a cigarette lighter or gas burner is pressed, the high voltage produced by the compression of a crystal causes an electric current to flow across a small spark gap, so that the gas is ignited. Another example is in the airbag sensor of a car. The intensity of the shock of a car crash to a crystal causes an electrical signal that triggers expansion of the airbag. In the analogous phenomenon of pyroelectricity, a crystal can generate an electrical potential in response to a change in temperature.

The significance of the unit cell

In this chapter we have described crystals and their representation by a repeating component, the unit cell. Since a crystal is built up of an extremely large number of regularly stacked cells, each of which has identical contents, the problem of determining the structure of a crystal is reduced to that of determining the spatial arrangement of the atoms *within a single unit cell*, or *within the smaller asymmetric unit* if (as is usual) the unit cell has some internal symmetry. If there is some static disorder in the structure, the arrangements of atoms in different unit cells may not be precisely identical, varying in an apparently random fashion. There may also be dynamic disorder in a structure as various part of the molecules move. Since the frequencies of atomic vibrations are of the order of 10^{13} per second, and since sets of X-ray diffraction data are measured over periods ranging from seconds to hours, time-averaging of the atomic distribution is always involved. What one finds for the arrangement of atoms in a crystal is the space-averaged structure of all of its component unit cells.

Summary

A crystal is, by definition, a solid that has a regularly repeating internal structure (arrangement of atoms). This internal periodicity was surmised in the seventeenth century from the regularities of the shapes of crystals, and was proved in 1912 when it was shown that a crystal could act as a three-dimensional diffraction grating for X rays, since X rays have wavelengths comparable to the distances between atoms in crystals.

Crystals are generally grown by concentrating a solution of the material of interest until material separates (hopefully in a crystalline state). Experimental conditions should ensure a good choice of solvent, the generation of a suitable number of nucleation sites, control of the rate of growth, and a lack of disturbance of the setup.

The *unit cell* of a crystal is its basic building block and is described by three axial lengths a, b, c and three interaxial angles α, β, γ. When describing a crystal face or plane it is necessary to consider intercepts on the three axes of the unit cell. The *hkl* face or plane makes intercepts a/h, b/k, c/l with the three axes. The internal regularity of a crystal is expressed in the *crystal lattice*; this is a regular three-dimensional array of points (each with identical environments) upon which the contents of the unit cell (*the motif*) are arranged by infinite repetition to build up the crystal structure. There are seven ways in which rotational symmetry can lead to infinitely repeatable unit cells. These are the seven crystal systems—triclinic, monoclinic, orthorhombic, tetragonal, trigonal/rhombohedral, hexagonal, and cubic (see Appendix 2). These seven crystal lattices are combined with the four crystal lattice types (primitive P, single-face-centered A or B or C, face-centered F, and

body-centered *I*) to give 14 Bravais lattices. Symmetry elements (center of symmetry, mirror planes, glide planes, rotation axes, and screw axes) combined with these 14 Bravais lattices give the 230 different combinations of symmetry elements (the 230 space groups) that are possible for arranging objects in a regularly repeating manner in three dimensions, as in the crystalline state.

Diffraction

3

A common approach to crystal structure analysis by X-ray diffraction presented in texts that have been written for nonspecialists involves the Bragg equation, and a discussion in terms of "reflection" of X rays from crystal lattice planes (Bragg, 1913). While the Bragg equation, which implies this "reflection," has proved extremely useful, it does not really help in understanding the process of X-ray diffraction. Therefore we will proceed instead by way of an elementary consideration of *diffraction phenomena* generally, and then diffraction from periodic structures (such as crystals), making use of optical analogies (Jenkins and White, 1957; Taylor and Lipson, 1964; Harburn et al., 1975).

Visualizing small objects

The eyes of most animals, including humans, comprise efficient optical systems for forming images of objects by the recombination of visible radiation scattered by these objects. Many things are, of course, too small to be detected by the unaided human eye, but an enlarged image of some of them can be formed with a microscope—using visible light for objects with dimensions comparable to or larger than the wavelength of this light (about 6×10^{-7} m), or using electrons of high energy (and thus short wavelength) in an electron microscope. In order to "see" the fine details of molecular structure (with dimensions 10^{-8} to 10^{-10} m), it is necessary to use radiation of a wavelength comparable to, or smaller than, the dimensions of the distances between atoms. Such radiation is readily available

(1) in the X rays produced by bombarding a target composed of an element of intermediate atomic number (for example, between Cr and Mo in the Periodic Table) with fast electrons, or from a synchrotron source,*
(2) in neutrons from a nuclear reactor or spallation source, or
(3) in electrons with energies of 10–50 keV.

Each of these kinds of radiation is scattered by the atoms of the sample, just as is ordinary light, and if we could recombine this scattered radiation, as a microscope can, we could form an image of the scattering matter. This recombination of radiation scattered by atoms

* Synchrotron radiation is an intense and versatile source of X rays that is emitted by high-energy electrons, such as those in an electron storage ring, when their path is bent by a magnetic field. The radiation is characterized by a continuous spectral distribution (which can, however, be "tuned" by appropriate selection), a very high intensity (many times that of conventional X-ray generators), a pulsed time structure, and a high degree of polarization.

is, however, found to be more complicated than that necessary for viewing through a microscope, and it is the major subject of this book.

X rays are scattered by the electrons in an atom,** neutrons are scattered by the nuclei and also, by virtue of their spin, by any unpaired electrons in the atom, and electrons are scattered by the electric field of the atom, which is of course a consequence of the combined effects of both its nuclear charge and its extranuclear electrons. However, neither X rays nor neutrons of the required wavelengths can be focused by any known lens system, and high-energy electrons cannot (at least at present) be focused sufficiently well to show individually resolved atoms. Thus, the formation of an atomic-resolution image of the object under scrutiny, which is the self-evident aim of any method of crystal structure determination—and is a process that we take for granted when we use our eyes or any kind of microscope—is not directly possible when X rays, neutrons, or high-energy electrons are used as a probe. Unfortunately, the atoms that we wish to see are too small to be seen without these short-wavelength radiation sources.

When, however, X rays or neutrons are diffracted by crystalline materials, a measurable pattern of diffracted beams is obtained and these results can be analyzed to give a three-dimensional map of the atomic arrangement within the crystal and hence the molecular structures involved. In order for the reader to understand the process involved it is necessary to consider diffraction in general, and easier to start with the effects of visible light on masks that are readily visible. Scattering of light by slits will serve as a preliminary model for the scattering of X rays by atoms. When the dimensions of both the slits and the wavelength of visible light are reduced by several orders of magnitude, analogous results can be obtained for atoms and X rays.

** When X rays hit an atom, its electrons are set into oscillation about their nuclei as a result of perturbation by the rapidly oscillating electric field of the X rays. The frequency of this oscillation is equal to that of the incident X rays. The oscillating dipole so formed acts, in accord with electromagnetic theory, as a source of radiation with the same frequency as that of the incident beam. This is referred to as "elastic scattering" and is the type of scattering discussed in this book. When there is energy loss, resulting in a wavelength change on scattering, the phenomenon is described as "inelastic scattering." This effect is generally ignored by crystallographers interested in structure and will not be discussed in this book.

Diffraction of visible light by single slits

The pattern of radiation scattered by any object is called the *diffraction pattern* of that object. Diffraction occurs whenever the wavefront of a light beam is obstructed in some way. We are accustomed to think of light as traveling in straight lines and thus casting sharply defined shadows, but that is only because the dimensions of the objects normally illuminated in our experience are much larger than the wavelength of visible light. When light from a point source passes through a narrow slit or a very fine pinhole, the light is found to spread into the region that normally would be expected to be in shadow. In explanation of this effect, each point on the wavefront within the slit or pinhole is considered to act as a secondary source, radiating in all directions. The secondary wavelets so generated interfere with each other, either reinforcing or partially destroying each other, as originally described by Francesco Maria Grimaldi, Christiaan Huygens, Thomas Young, and Augustin Jean Fresnel (Grimaldi, 1665; Huygens, 1690; Young, 1807;

de Sénarmont et al., 1866). As these waves combine, the extent of interference will depend on their relative phases and amplitudes. It is assumed that any phase change on scattering is the same for each atom and therefore this change is generally ignored.† There are, however, exceptions to this assumption, for example when the wavelength of the radiation can cause changes in the atom (see Chapter 10).

The phenomenon of diffraction by a regular two-dimensional pattern may be illustrated by holding a woven fabric handkerchief taut between your eyes and a distant point source of light, such as a street light. Instead of just one spot of light, as expected, a cluster of lights will be seen. The same phenomenon can also be demonstrated with a fine sieve (see the cover of this book). The narrowly and regularly spaced threads of the fabric or wires of the sieve are considered to produce this diffraction effect. The larger the spacing between the wires of the sieve, the closer diffraction spots are found around the central spot.

Keeping in mind that we are interested in scattering (diffraction) by atoms, we begin with a discussion of diffraction by slits because these involve visible light and therefore help with the description of the various principles of diffraction. Two examples of the diffraction of light when it passes through a single slit are given in Figure 3.1; in one,

† The reader will remember that an electromagnetic wave has a constant velocity *in vacuo* (the speed of light) and consists of successive crests and troughs. Two crests are a wavelength apart, and this distance, which is inversely proportional to the frequency of the radiation, defines the properties of the electromagnetic wave (such as color red or blue, X ray or infrared, etc.). The wave has an amplitude (the maximum value measured from its mean value), which is related to the square root of the intensity of the beam. It also has a "relative phase," which is the distance of the crest of the wave measured from a chosen origin of the wave or with respect to the crest of another wave (see Figure 1.2). It was shown by John Joseph Thomson that when radiation is scattered by an electron, there is a phase change of 180° in the sense that the electric field in the scattered wave at a given point is opposed to that of the direct (incident) wave at that same point (Thomson, 1906). This is discussed in detail by Reginald William James (1965).

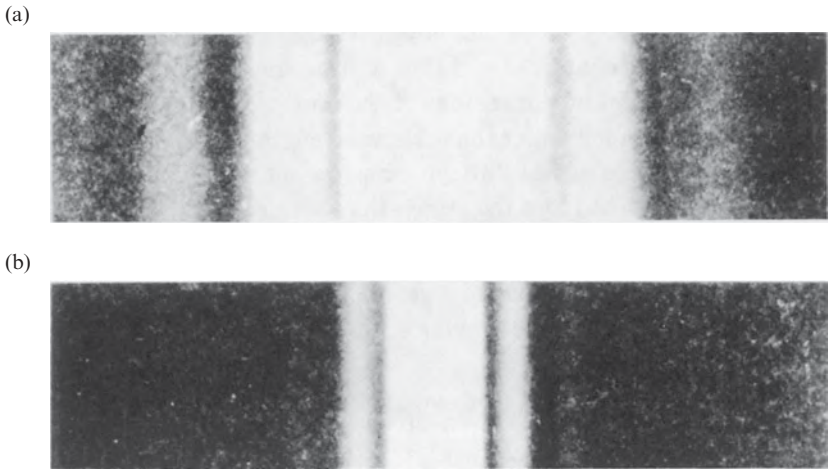

Fig. 3.1 Diffraction patterns of single narrow slits.

The diffraction patterns of two single slits of different width, both illuminated with light of the same single wavelength.

(a) The diffraction pattern of a narrow slit.
(b) The diffraction pattern of a slit 2.2 times wider than that used in (a). The diffraction pattern is now narrower by a factor of 2.2.

Note that the wider slit gives the narrower diffraction pattern.

From *Fundamentals of Optics* by Francis A. Jenkins and Harvey E. White, 3rd edition (1957) (Figure 16A). Copyright © 1957, McGraw-Hill Book Company. Used with permission of McGraw-Hill Book Company.

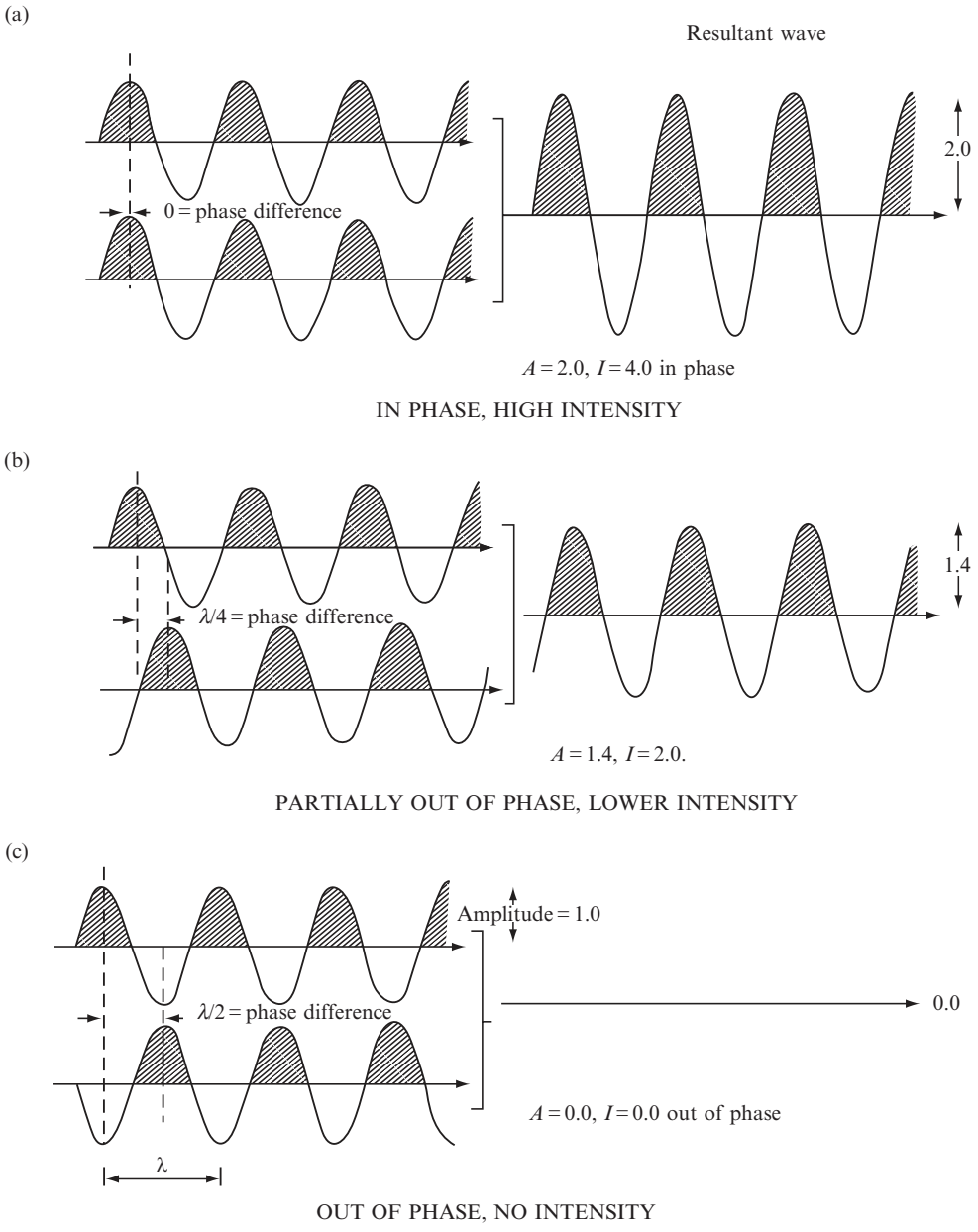

Fig. 3.2 Interference of two waves. Summation of waves.

Three examples are shown of what happens when two parallel waves of the same wavelength and equal amplitude add. In each example, the two separate waves are shown on the left and their sum or resultant wave on the right. The different examples are characterized by varying phase differences. The *relative phase* of a wave is the position of a crest relative to some arbitrary point (see Figure 1.2). This position (relative phase) is usually expressed as a fraction of the wavelength, and often this fraction is multiplied by 360° or 2π radians, so that the phase will be given as an angle. Thus a phase difference of $\lambda/4$ may be given as 1/4, 90°, or $2/\pi$ radians. The resultant wave has the same wavelength, λ, as the original two waves. The intensity, I, of the resultant wave is proportional to the square of its amplitude, A, obtained on wave summation.

Figure 3.1a, the slit is narrow and the diffraction pattern is wide, while in the other, Figure 3.1b, the slit has a greater width but the diffraction pattern is narrower. This implies that there is a reciprocal relation between the angular spread of the scattering or diffraction pattern in a particular direction and the corresponding dimension of the object causing the scattering. The smaller the object, the larger the angular spread of the diffraction pattern. What is actually involved is the ratio of λ (the wavelength of the radiation used) to the minimum dimension, a, of the scattering object (for example, the width of the slit); the larger the value of λ/a (wavelength divided by slit width), the greater the spread of the pattern. Therefore Figure 3.1b might equally well be a view of the same slit as in Figure 3.1a, illuminated with radiation of wavelength about 2.2 times shorter than that used in Figure 3.1a. It is, in fact, possible to produce this change of scale by any change in a and λ whose combined effect is to decrease the value of λ/a by a factor of 2.2.

The phenomenon of interference between two waves traveling in the same direction and the importance of phase differences between these two parallel waves are illustrated in Figure 3.2. The amplitude of the wave resulting from the interaction of two separate waves traveling in the same direction with the same wavelength, and a constant phase difference, depends markedly on the size of this phase difference. Figure 3.2 shows how such waves may be summed‡ for three examples of different phase differences (zero, a quarter, and half a wavelength). The intensity of the resulting beam is proportional to the square of the amplitude of the summed waves in each case.

‡ The displacements from the mean (zero), parallel to the vertical axis (the ordinates), are directly summed at many points along the horizontal axis (the abscissae) to give the resultant wave.

The variations in intensity seen in Figure 3.1 arise from the interference of the secondary wavelets generated within the slit, as shown in Figure 3.3. In the direction of the direct beam, the waves scattered by the slit are totally in phase and reinforce one another to give maximum intensity. However, at other scattering angles, as illustrated in Figure 3.3, the relative phases of the waves cause interference between waves traveling in the same direction so that the intensity falls off as a function of scattering angle; this leads to an overall intensity contour of the diffraction peak, and we term this "the envelope." At most scattering angles the different scattered waves are neither completely in phase nor completely out of phase, so that there is partial reinforcement and thus an intermediate intensity of the diffracted beam. The result is illustrated in the single-slit diffraction pattern (the envelope) shown on the right of Figure 3.3.

(a) Phase difference zero. In this case there is total reinforcement, and the waves are said to be "in phase" or to show "constructive interference". If the original waves are of unit amplitude, the resultant wave has amplitude 2, intensity 4.
(b) Phase difference $\lambda/4$. Partial reinforcement occurs in this case to give a resultant wave of amplitude 1.4, intensity 2.
(c) Phase difference $\lambda/2$. The waves are now completely "out of phase" and there is destructive interference, which gives no resultant wave (that is, a wave with amplitude 0, intensity 0).

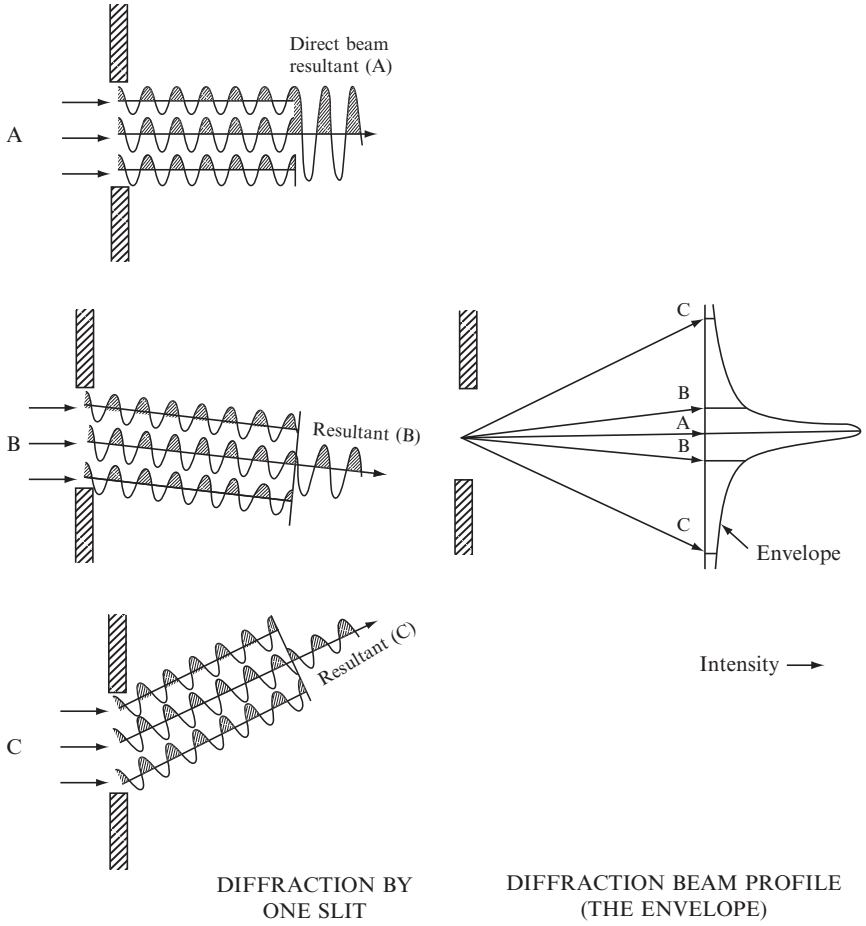

Fig. 3.3 Diffraction by a single slit.

Diffraction from a single slit is diagrammed by the superposition of waves generated within the area of the slit. The variation in intensity with increasing angle is shown by the different amplitudes of the resultant waves (A, B, and C) at different angles. Left-hand side: diffraction by a single slit; right-hand side: diffracted beam profile (the envelope), showing the location of A, B, and C on this envelope.

Diffraction of light by regular arrays of slits

In order to consider what happens when a crystal that has a periodic internal structure diffracts radiation, we now describe diffraction by a series of equidistantly aligned slits. Reinforcement of the diffracted beam occurs at angles at which the path difference between two parallel waves is an integral number of wavelengths; for example, when the two waves are out of phase by three wavelengths ($n = 3$), there will be reinforcement at a specific scattering angle and the wave will be described as the "third order of diffraction" (see Figure 3.4). As shown in Figure 3.5, the diffraction pattern of a single slit is modified by interference effects when increasing numbers of slits are placed

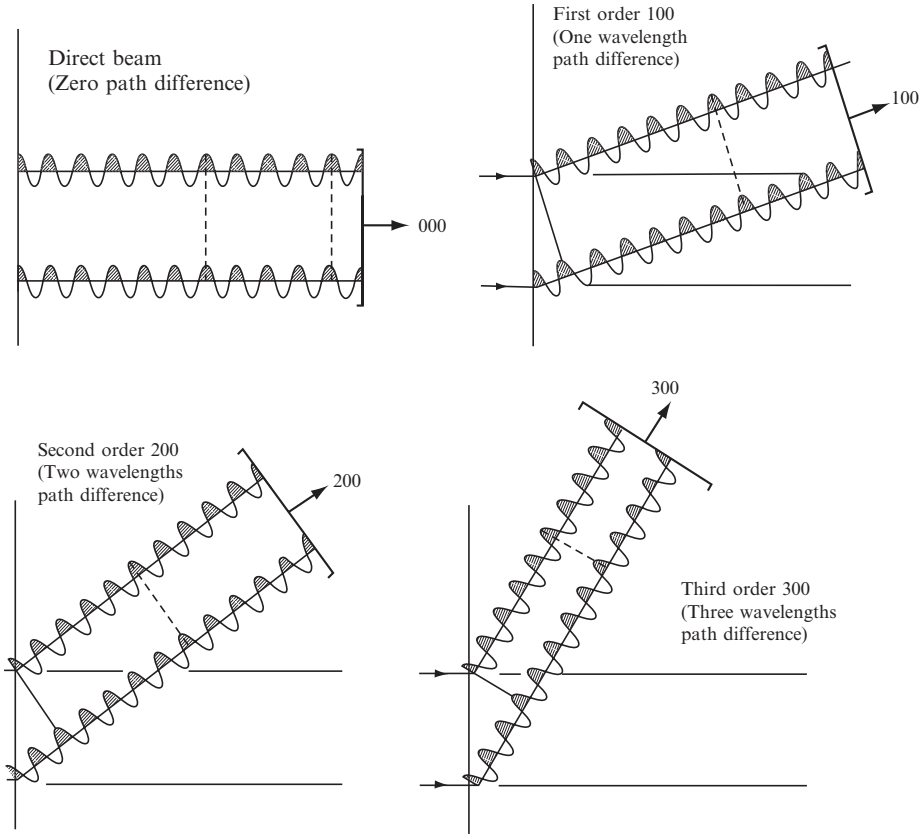

Fig. 3.4 Orders of diffraction.

First, second, third, and higher orders of diffraction are obtained as scattered waves differ by one, two, three, and more wavelengths. Readers should satisfy themselves that with a smaller spacing a between scattering objects, the angle at which a given order of diffraction occurs is proportionally increased.

side by side in a regular manner to form a one-dimensional grating. Sometimes rays proceeding in a specific direction after scattering are in phase and sometimes they are not. The important point to note is that the *diffraction pattern from a grating of slits is a sampling of the single-slit pattern in narrow regions that are representative of the spacings between the slits* (see Figure 3.5). With even as few as 20 slits in the "grating" (see Figure 3.6), the small subsidiary maxima vanish almost completely and the lines in the diffraction pattern are sharp. The overall diffraction pattern of a series of slits is thus composed of an "envelope" and a series of "sampling regions" within the envelope. This envelope represents the diffraction pattern of a single slit (see Figure 3.3). The "sampling regions" result from interference of waves scattered from equivalent points in different slits; the spacing of these sampling regions in the diffraction pattern (see Figure 3.6) is inversely related to the spacing of the slits.

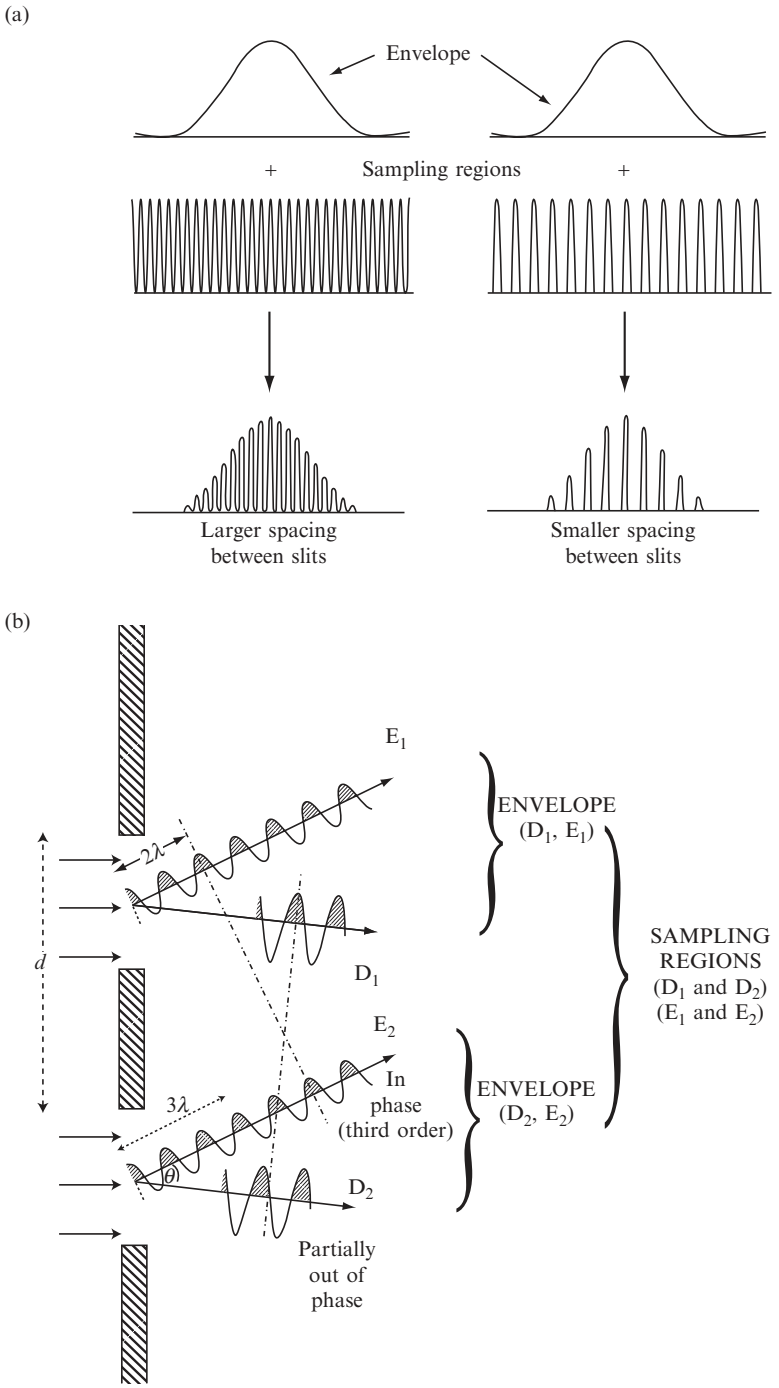

Fig. 3.5 Diffraction by two slits.

Figure 3.7 shows schematically how a two-dimensional regular arrangement of simple scattering objects, in this case holes in an opaque sheet (drawn as black spots on the left), produces a two-dimensional diffraction pattern (drawn as lines or spots on the right). Each of the one-dimensional gratings in Figures 3.7a and b produces (in two dimensions) a pattern of scattered light (the diffraction pattern) consisting of lines (representing the maxima of light, as seen on the right). These lines are perpendicular to the direction of the original grating because interference effects between light scattered from adjacent holes reduce the scattered light intensity effectively to zero in all directions except that perpendicular to the repeat direction of the original grating. Hence lines of diffracted light are formed. In Figure 3.7c, a combination of both kinds of one-dimensional gratings that were shown in (a) and (b) are present at once. This gives a regular two-dimensional grating. The lattice of the diffraction pattern in Figure 3.7c is necessarily, then, the "reciprocal" of the lattice of the original scattering objects (the crystal lattice) shown on its left; see Figure 3.7d. This will now be described.

The reciprocal lattice

In addition to the lattice of the crystal structure in real or crystal space (discussed earlier), there is a second lattice, related to the first, that is of importance in diffraction experiments and in many other aspects of solid state physics. This is the *reciprocal lattice*, introduced by Josiah Willard Gibbs in 1884, long before X-ray diffraction was known (Gibbs, 1901; Ewald, 1921). Its definition in terms of the crystal lattice vectors is shown in Appendix 3. In the reciprocal lattice a point, (*hkl*), is drawn at a distance $1/d_{hkl}$ from the origin (the direct beam, (000)), and in the direction of the perpendicular distance between the (*hkl*) crystal lattice planes (Figure 3.7d). The relationship between these two important lattices (the crystal and reciprocal lattices) is a particularly simple one if the fundamental translations of the crystal lattice are all perpendicular to one another; then the

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(a) An overview of the envelope profile (equivalent to diffraction by a single slit or an atomic arrangement) and the sampling regions (equivalent to the diffraction of a series of equidistant slits or a crystal lattice). The envelope is accessed at the sampling regions only.

(b) When diffraction occurs from two slits, there are two effects to consider:

(1) The variation in intensity with angle as a result of interference of the waves generated within each slit separately. Interference between D_1 and E_1 and between D_2 and E_2 gives the "envelope," as obtained for a single slit (see also Figure 3.3). This is the equivalent of diffraction by a single slit.

(2) The interference of scattered waves at a given angle with those at the same angle from the adjacent slit (D_1 with D_2 from the next slit, E_1 with E_2 from the next slit, etc.). At angles of constructive interference, when the two resultant waves are in phase, "sampling" of the "envelope" occurs, as shown in part (a). At certain other angles, no diffraction is observed. This sampling is the result of the distance between the two slits.

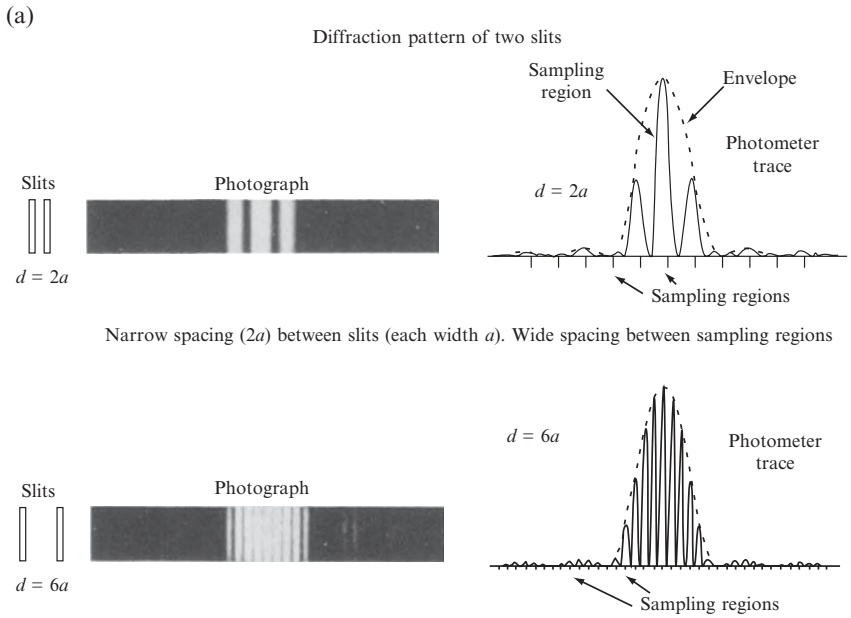

Fig. 3.6 Diffraction patterns from equidistant parallel slits.

(a) The effect of varying the distance, d, between two slits of constant width, a, is shown. On the left is a diagram of the slits with spacings of $2a$ and $6a$, respectively, between them. In the center is shown a photograph of the diffraction pattern. On the right, a photometer tracing of the diffraction pattern for the combination of the two slits is drawn as a solid line, and the diffraction pattern for a single slit, referred to in the text as the "envelope," is drawn as a dashed line. The envelope in both cases has the same shape because it represents the diffraction pattern of a single slit of the same width. The regions of the "envelope" that are sampled are indicated by short vertical lines at the lower edge of the drawings on the right. When there is a relatively narrow spacing between the slits ($d = 2a$), the distance between sampling regions is relatively large, as shown in the upper diagram. When there is a relatively wide spacing between the slits ($d = 6a$), the distance between sampling regions has decreased; that is, there is an inverse relationship of the spacing of the sampling regions to the spacing of the slits.

fundamental translations of the reciprocal lattice are parallel to those of the crystal lattice, and the lengths of these translations are inversely proportional to the lengths of the corresponding translations of the crystal lattice. With nonorthogonal axes, the relationships between the crystal lattice and the reciprocal lattice are not hard to visualize geometrically; a two-dimensional example is given in Figure 3.7d. As we shall see shortly, the fundamental importance of the reciprocal lattice in crystal diffraction arises from the fact that *if a structure is arranged on a given lattice, then its diffraction pattern is necessarily arranged on the lattice that is reciprocal to the first*.§

§ This may be stated alternatively as follows. The diffraction pattern of a molecular crystal is the product of the diffraction pattern of the molecule (also called the molecular transform) with the diffraction pattern of the crystal lattice (which is also a lattice, the reciprocal lattice, described above). The result is a sampling of the molecular transform at each of the reciprocal lattice points. The diffraction pattern of a single molecule is too weak to be observable. However, when it is reinforced in a crystal (containing many billions of molecules in a regular array) it can be readily observed, but only at the reciprocal lattice points.

Diffraction of X rays by atoms in crystals

It is a principle of optics that the diffraction pattern of a mask with very small holes in it is approximately equivalent to the diffraction pattern of the "negative" of the mask—that is, an array of small dots at the positions of the holes, each dot surrounded by empty space. This equivalence is discussed lucidly by Richard Feynman (Feynman et al., 1963). In a crystal, the *electrons in the atoms act, by scattering, as sources of X rays, just as the wavefront in the slits in a grating may be regarded as sources of visible light.* There is thus an analogy between atoms in a crystal, arranged in a regular array, and slits in a grating, arranged in a regular array. In diffraction of X rays by crystals, as of visible light by slits in a grating, the intensities of the diffraction maxima show a variation in different directions and also vary significantly with angle of scattering.

Most unit cells contain a complex assembly of atoms, and each atom is comparable in linear dimensions to the wavelength of the X rays or neutrons used. Figure 3.8a shows a typical X-ray diffraction photograph, taken by the "precession method," which records the reciprocal lattice without distortion. Considerable variation in intensity of the individual diffracted beams is evident; this is a result of the arrangement of atoms (and their accompanying electron density) in the structure. The analogy with Figures 3.3, 3.5, and 3.6 holds; that is, *the X-ray photograph is merely a scaled-up sampling of the diffraction pattern of the contents of a single unit cell.* The "envelope," which is shown by the

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(b) Diffraction patterns are shown for gratings containing 1, 2, 5, and 20 equidistant slits, illuminated by parallel radiation of the same wavelength. The diffraction pattern for a grating composed of 20 (or more) slits consists only of sharp lines, the intervening minor maxima having disappeared; similarly, the diffraction pattern for a crystal composed of many unit cells contains sharp diffraction maxima.

Summary of key points:

(1) The size and shape of the envelope are determined by the diffraction pattern of a single slit.

(2) The positions of the regions in which the envelope is sampled are determined by the spacing between the slits.

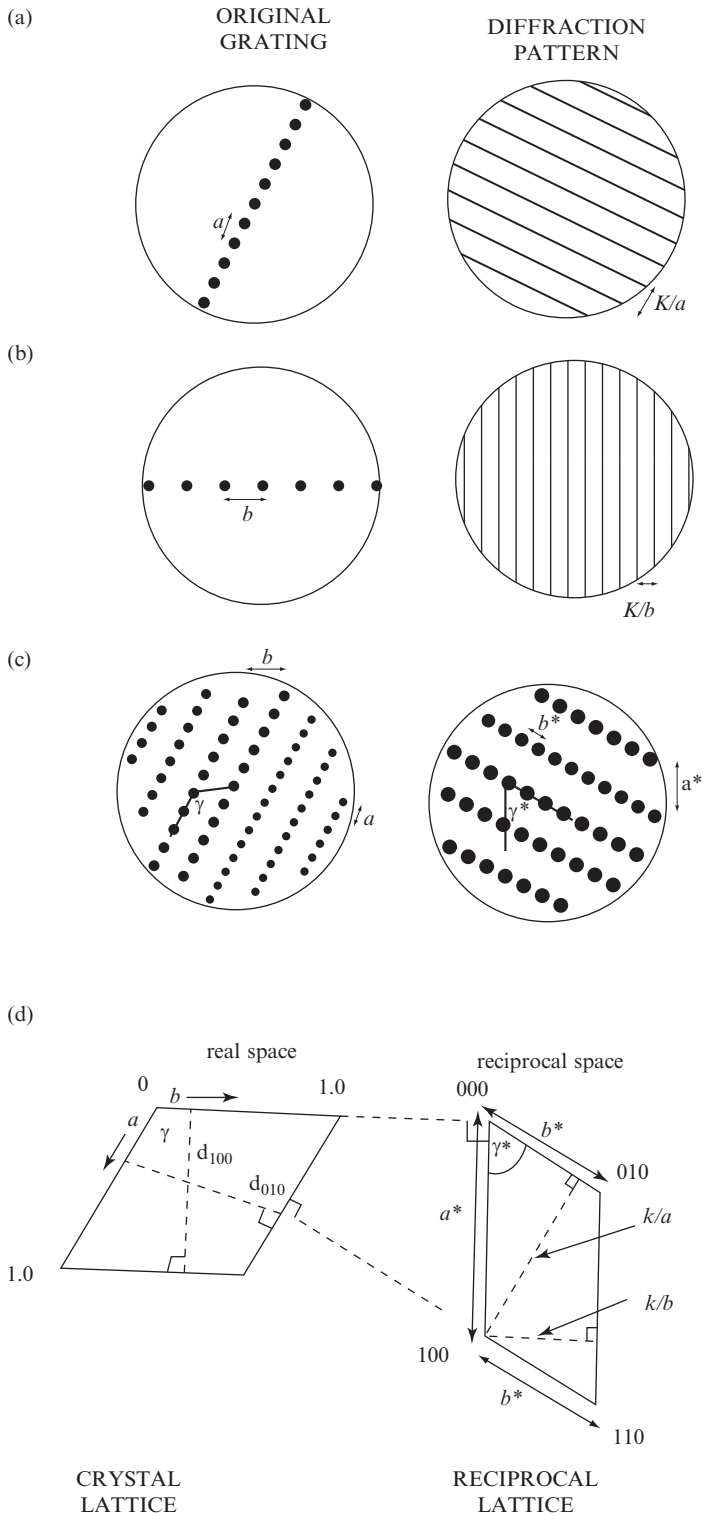

Fig. 3.7 Diagrams of diffraction patterns from one- and two-dimensional arrays. Relation between the crystal lattice and reciprocal lattice.

(a) (b)

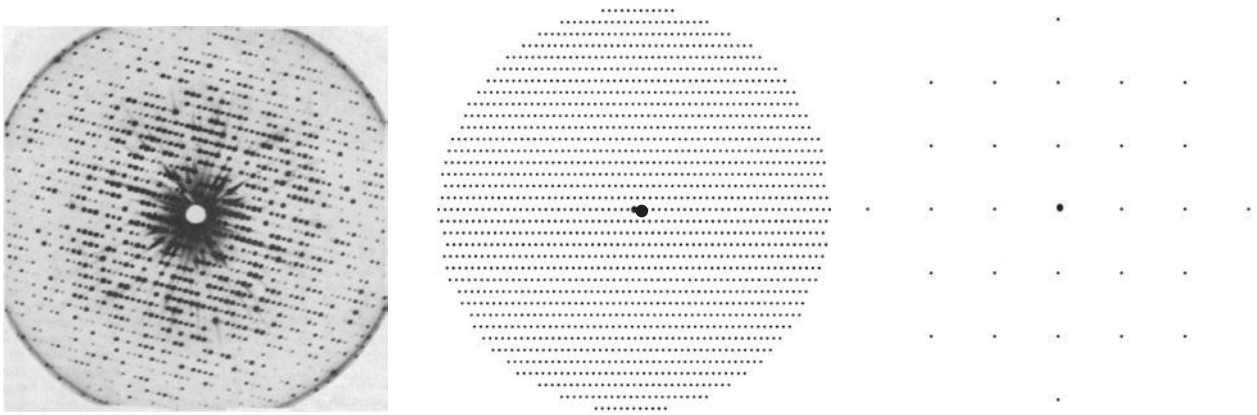

Fig. 3.8 X-ray diffraction photographs taken by the precession method.

(a) The precession method gives an undistorted representation of one layer of the reciprocal lattice. An X-ray precession photograph of a crystal of myoglobin is shown here. The direct X-ray beam, which might otherwise "fog" the film, has been intercepted, hence the white hole in the middle of the photograph. The radial streaks, found for very intense Bragg reflections, occur because the X rays are not truly monochromatic (one wavelength) but contain background radiation of varying wavelength but lower intensity. As a result the spot appears somewhat smeared out (that is, for each Bragg reflection, sin θ/λ is constant but since λ varies for the background "white radiation," sin θ must also vary, giving rise to a streak rather than a spot on the film). Note the regularity of the positions of spots in this photograph but the wide variation in intensity (from a very black spot to one that is almost or apparently absent). The positions of the spots (diffracted beams) give information on unit cell dimensions; the intensities of the spots give information on the arrangement of atoms in that unit cell.

Photograph courtesy Dr. J. C. Kendrew.

(b) A comparison of diagrams of the diffraction patterns of myoglobin (large unit cell, monoclinic, a = 64.5 Å, b = 30.9 Å not shown, c = 34.7 Å, $\beta = 106.0^\circ$) on the left and potassium chloride (small unit cell, cubic, a = 6.29 Å) on the right. The larger the unit cell, the nearer together the diffraction spots if the wavelength of the radiation is the same for both. Variations in Bragg reflection intensities are not shown in these diagrams. Note that many Bragg reflections are measured when the unit cell is large.

variation in intensities of the individual diffracted-beam spots, is the diffraction pattern of the scattering matter (the electrons of the atoms) in a single unit cell. The "sampling regions," which are the positions of the diffracted-beam spots, are arranged on a lattice that is "reciprocal" to the crystal lattice. Measurements of the distances between these will lead to the dimensions of the unit cell, and they sample the diffraction

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(a, b, c) On the left is shown the grating used and, on the right, the corresponding diffraction pattern (such as might be obtained by holding the original grating in front of a point source of light). **a** and **b** are direct lattice vectors in the crystal or grating, and **a*** and **b*** are vectors in the diffraction pattern (a and b are the spacings of the original gratings, and a^* and b^* are spacings in the diffraction pattern). The reciprocal relationships of a and b to the spacings of certain rows in the diffraction pattern are shown. These are diagrams, and no intensity variation is indicated. The black dots on the left-hand side represent holes that cause diffraction, giving the pattern on the right-hand side, in which black lines or spots represent appreciable intensity for diffracted light.

Adapted from H. Lipson and W. Cochran. *The Crystalline State. Volume III. The Determination of Crystal Structures.* Cornell University Press: Ithaca, New York; G. Bell and Sons: London (1966) (Lipson and Cochran, 1966).

(d) The relationships of **a** and **b** in the crystal lattice to **a*** and **b*** in the corresponding *reciprocal lattice* are shown.

pattern of a single unit cell. A comparison of the reciprocal lattices of the protein myoglobin (Figure 3.8a) with that of potassium chloride (with a much smaller unit cell) is shown in Figure 3.8b.

Some diffraction patterns of individual and assembled molecules are illustrated in Figures 3.9 and 3.10, which have been prepared using a special optical device that permits photographs to be made of the diffraction patterns of arrays of holes cut in an opaque sheet. By an appropriate choice of the optical components, the effective ratio of the wavelength of the light used to the sizes of these holes can be made similar to the ratio of X-ray wavelengths to the sizes of atoms. One can, then, simulate X-ray diffraction photographs of crystals by making patterns of holes in opaque sheets that are similar, except for scale, to the patterns of arrangement of the atoms in the crystals.

The relationship between the diffraction pattern of a single "molecule" and various "samplings" that can be produced by regular arrangements of such molecules are shown in Figure 3.9. The left-hand side of each part of the figure shows different arrangements of molecules and the right-hand side shows the corresponding diffraction patterns. This figure also shows, from the dimensions of the unit cell, that the lattice of the diffraction pattern is reciprocal to that of the "crystal". Figure 3.9b shows the diffraction pattern of two "molecules" side by side (horizontally in the orientation shown here) and illustrates the interference arising from the interaction of the scattering by the two molecules, exactly analogous to the interference caused by the presence of two adjacent slits that gives rise to Figure 3.6a. Figure 3.9c shows the pattern arising from four "molecules" arranged in a parallelogram; now there is interference parallel to each of the two axes of the incipient crystal lattice. Figure 3.9d shows the diffraction pattern of an extended regularly spaced row of the molecules—that is, from a one-dimensional crystal; there is sharpening of the diffraction effects parallel to the direction of ordering, but no interference at all in other directions. Figure 3.9e shows the pattern obtained by placing two lengthy rows

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(c) Four molecules arranged in a parallelogram.
(d) Many molecules horizontally side by side (a one-dimensional crystal). Only part of the row is shown.
(e) Two rows of molecules arranged on an oblique lattice. Only parts of the rows are shown.

In comparing (e) with (d), note again the analogy with the relation of the one-slit and two-slit patterns of Figures 3.1 and 3.6.

(f) Two-dimensional crystal of molecules. Only part of the crystal and part of the diffraction pattern are shown. Compare this with Figure 3.8a.

From C. A. Taylor and H. Lipson. *Optical Transforms. Their Preparation and Application to X-ray Diffraction Problems*. Plate 26. G. Bell and Sons, London (1964). Published with permission.

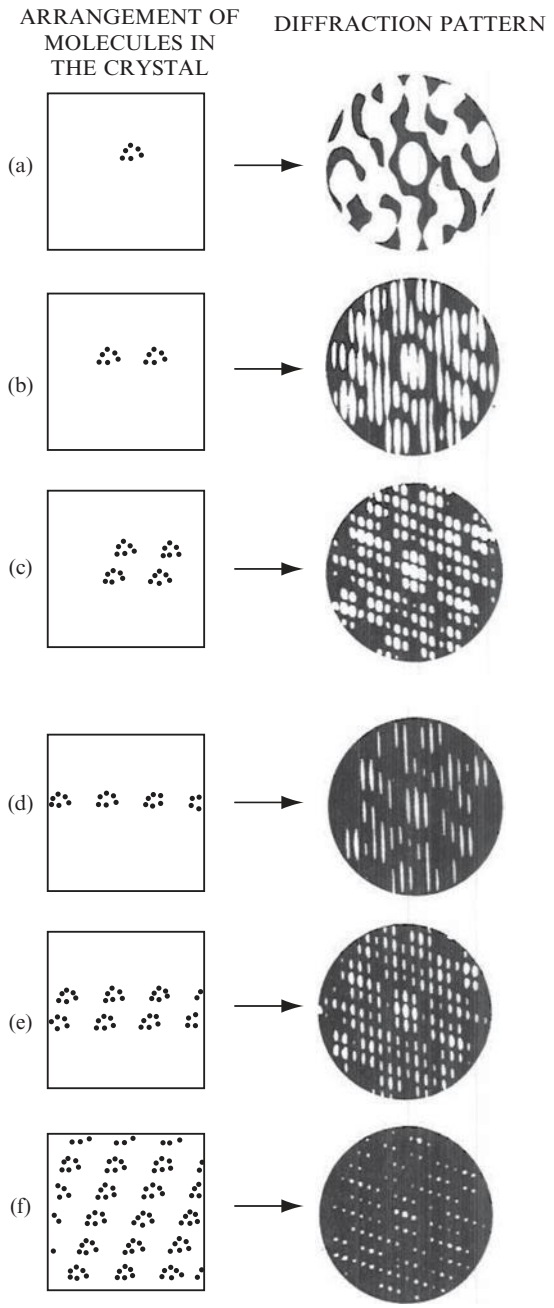

Fig. 3.9 The effect of different lattice samplings on the diffraction pattern.

This shows the relationship between the diffraction pattern of a "molecule" and various regular arrangements of such molecules. The optical mask is on the left (black points as holes) and its diffraction pattern is on the right.

(a) A single molecule.
(b) Two molecules horizontally side by side.

In comparing (b) with (a), note the analogy with the one-slit and two-slit patterns of Figures 3.1 and 3.6.

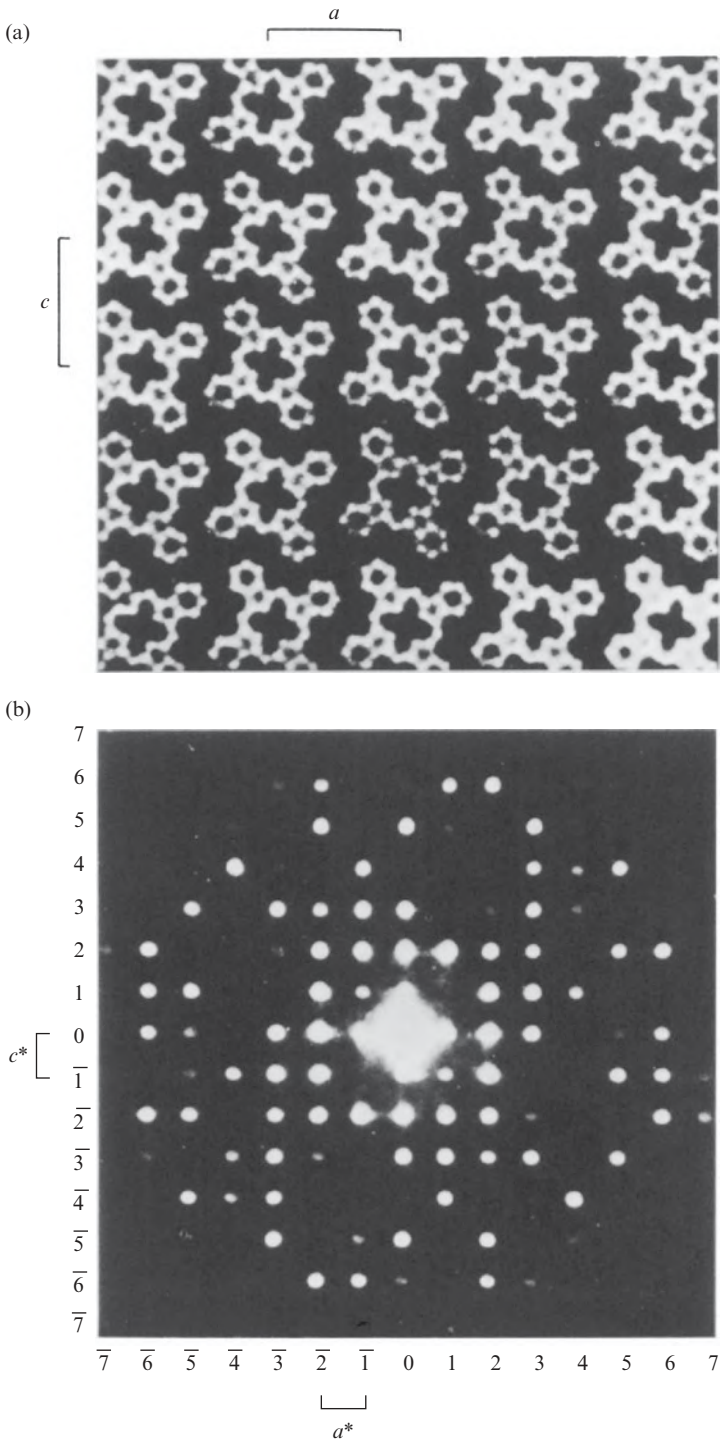

Fig. 3.10 The optical diffraction pattern of an array of templates resembling the skeleton of a phthalocyanine molecule.

side by side and, finally, Figure 3.9f shows the pattern obtained from a two-dimensional crystal of these "molecules." The resemblance to the precession photograph in Figure 3.8a is good. Figure 3.9a is being sampled at reciprocal lattice points to give Figure 3.9f.

In Figure 3.10a arrays of holes, each of which has the shape of the skeleton of a phthalocyanine molecule, are shown, together with the optical diffraction pattern obtained (with visible light) from these arrays (Figure 3.10b). Note that the intensity variation in the optical diffraction pattern (shown as intensities in Figure 3.10b) parallels that found in the corresponding pattern obtained by the diffraction of X rays (listed in Figure 3.10c).

Diffraction and the Bragg equation: Two ways of analyzing the same phenomenon

Von Laue, who, with Friedrich and Knipping, discovered the diffraction of X rays by crystals in 1912, interpreted the observed X-ray diffraction

(c) Relative intensities for the phthalocyanine crystal

$h \rightarrow$	0	1	2	3	4	5	6	7
7	6	0	2	7	0	6	0	0
6	25	52	45	11	4	0	3	0
5	36	1	0	58	0	1	2	0
4	3	17	0	14	0	38	0	9
3	15	1	2	14	4	4	2	1
2	72	85	21	16	0	8	27	1
1	61	0	64	30	2	2	1	3
0		94	72	10	0	2	17	1
−1	61	29	55	0	2	7	10	5
−2	72	46	23	3	0	0	18	14
−3	15	37	14	10	2	21	2	0
−4	3	13	0	10	18	2	1	0
−5	36	0	18	3	19	0	0	0
−6	25	5	35	5	2	0	1	0
−7	6	0	2	0	14	2	0	0
$l \uparrow$								

From C. W. Bunn. *Chemical Crystallography: An Introduction to Optical and X-ray Methods*. 2nd edition. Plate XIV. Oxford at the Clarendon Press: Oxford (1961).

(a) The array used to obtain the optical diffraction pattern. This models a crystal structure of phthalocyanine.
(b) The optical diffraction pattern obtained from (a).
(c) Relative $h0l$ intensities measured from the X-ray diffraction pattern of a phthalocyanine crystal. Qualitative comparison of these values with the intensities of the corresponding spots in the optical diffraction pattern shown in (b) indicates that the model used is a surprisingly good one. *Note:* Intensities for $h0l$ and $-h0-l$ (not listed below) are equal.

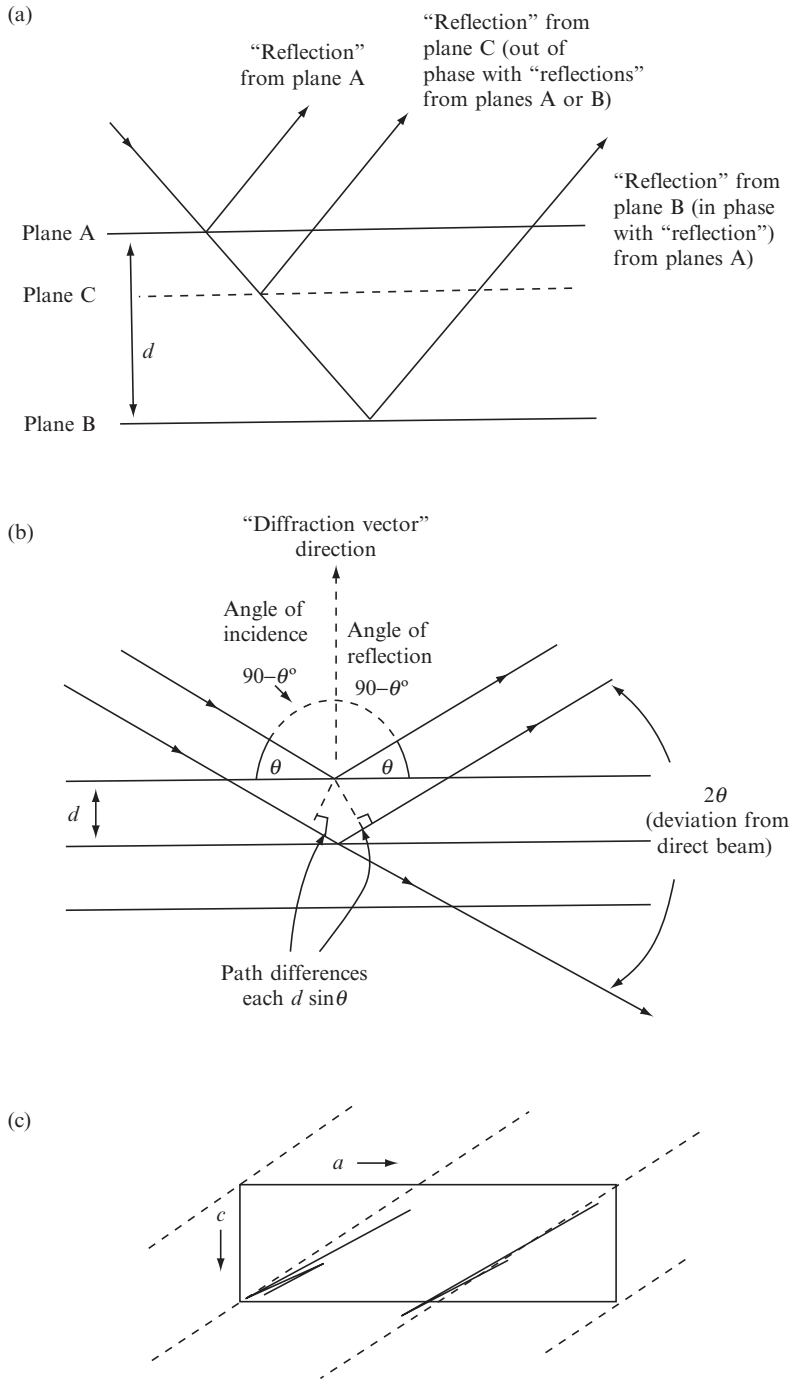

Fig. 3.11 Diagram of "reflection" of X rays by imaginary planes through points in the crystal lattice.

patterns of crystals in terms of a theory analogous to that used to treat optical diffraction by gratings, extended to three dimensions. On the other hand, William Lawrence Bragg, who worked out the first crystal structures with his father, William Henry Bragg, during the summer of 1913, showed that the angular distribution of scattered radiation could be understood by considering that the diffracted X-ray beams behaved *as if they were reflected* from planes passing through points of the crystal lattice (Bragg, 1913). This "reflection" is analogous to that from a mirror, for which the angle of incidence of radiation is equal to the angle of reflection, as shown in Figure 3.11a. Waves scattered from adjacent crystal lattice planes will be just in phase (i.e., the difference in the paths traveled by these waves will be an integral multiple of the wavelength, $n\lambda$) only for certain angles of scattering, as shown in Figure 3.11. From such considerations Bragg derived the famous equation that now bears his name:

$$n\lambda = 2d \sin\theta \quad \text{The Bragg equation} \tag{3.1}$$

In this equation λ is the wavelength of the radiation used, n is an integer (analogous to the order of diffraction from a grating, so that $n\lambda$ is the total path difference between waves scattered from adjacent crystal lattice planes with equivalent indices), d is the perpendicular spacing between the lattice planes in the crystal, and θ is the complement $(90^\circ - \theta)$ of the angle of incidence of the X-ray beam (and thus also the complement of the angle of scattering or "reflection"). Since it appears as if reflection has occurred from these crystal lattice planes, so that the direct beam is deviated by the angle 2θ from its original direction, diffracted beams are commonly referred to as "reflections." Because the Bragg equation is easily visualized, it is commonly presented in elementary discussions in diagrams such as those in Figures 3.11a and b; in Appendix 4 we show how it can be related to diffraction by a crystal lattice (as described above).

The Bragg equation can be derived by considering the path difference between waves scattered from adjacent parallel crystal lattice planes; the path difference must be an integral number of wavelengths

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(a) Constructive and destructive interference as waves are "reflected" from imaginary planes, spacing d, in a crystal. Constructive interference of planes A and B (the unit-cell repeat distance d apart), and partial destructive interference of plane C with A and B.

(b) Diffraction geometry. Since the path difference of waves scattered by two adjacent planes is $2d \sin\theta$, this must equal $n\lambda$ for total reinforcement to occur to give a diffracted beam (as illustrated in Figures 3.3, 3.4, and 3.5).

(c) Planes (2 0 1) in a crystal that has many atoms in its structure (see Figure 9.3d); the planes lie perpendicular to the plane of the paper. Note that the planes intersect the unit-cell edges once in the **c** direction and twice in the **a** direction. The 201 Bragg reflection is intense in this structure.

if constructive interference (reinforcement) is to occur. The equation is satisfied, and thus diffraction maxima occur, when and only when the relation of wavelength, interplanar spacing, and angle of incidence is appropriate. If a nearly monochromatic beam of X rays is used with a single-crystal specimen, diffraction maxima will be observed only for special values of the angle of incidence of the beam of X rays, and not necessarily for other arbitrary angles. If the crystal is rotated in the beam, it may be in a position (at certain rotation angles) to form additional diffracted beams. Therefore rotation of the crystal increases the number of observed Bragg reflections available for measurement. We use the term "Bragg reflections" for the diffracted beams to remind the reader that they will only occur when the angle of incidence of the X-ray beam is such as to satisfy Eqn. (3.1) for some set of crystal lattice spacings present in the crystal. This means that λ, d, and θ must all be such that the Bragg equation holds. The chance of this happening for a perfect crystal is low. However, real crystals have a mosaic spread (as if composed of minute blocks of unit cells, each block being misaligned by a few tenths of a degree with respect to its neighbors), and the X rays used are never truly monochromatic, so that, in practice, a Bragg reflection can be observed over a small range of θ and therefore some Bragg reflections are observed in almost any orientation of a single crystal. With a powdered crystalline specimen many different orientations of tiny crystallites are present simultaneously, and for any set of crystal planes, Eqn. (3.1) will be satisfied in some of the crystallites so that the complete diffraction pattern will be observed for any orientation of the specimen with respect to the X-ray beam. It is also possible to get a diffraction pattern from a stationary single crystal by the use of a wide range of wavelengths simultaneously. This was, in fact, the way in which von Laue, Friedrich, and Knipping did their original experiment; the technique is known as the *Laue method*, and is now currently used for studies of biological macromolecules with high-energy X rays (see Moffat et al., 1984).

The Bragg equation says nothing about the intensities of the diffraction maxima that will be observed when it is satisfied. If, however, a particular set of crystal lattice planes happens to coincide, in orientation and position, with some densely populated planar or nearly planar arrays of atoms in a crystal, and if there are no intervening densely populated planes, the corresponding diffraction maximum will be an intense one because the scattering from all atoms is approximately in phase. In an example cited in Chapter 9 (Figure 9.3d) involving a planar organic molecule, the "reflection" with indices $h = 2$, $k = 0$, $l = 1$ (written 2 0 1, i.e., second order in h, direct for k, and first order for l) is very intense because the molecules lie nearly parallel to the crystal lattice plane with indices (2 0 1) and are separated by a spacing very nearly the same as the interplanar spacing of this crystal lattice plane. This is shown in Figure 3.11c.

Summary

To explain what happens when a crystal diffracts X rays, we first examined optical analogies with slits and then with templates resembling two-dimensional crystals. The pattern of radiation scattered by any object is called the diffraction pattern of the object. For diffraction from a slit, the wider the slit the narrower the diffraction pattern for a given wavelength of radiation. The diffraction pattern of many parallel and equidistant slits consists of a sampling of the single-slit pattern in regions that are representative of the spacings between the slits.

For a series of several slits, the diffraction of light of a given wavelength leads to the information that:

(1) The size and shape of the "envelope" of the intensity variation is determined by the characteristic diffraction pattern of a *single slit*. This intensity variation tells us the shape and size of the diffracting object.
(2) The spacings between the "sampling regions" in this "envelope" are inversely related to the *spacings between the slits*. Thus the differences between diffracting objects are revealed by the distances between diffraction maxima.

These principles may be extended to three dimensions and to crystals, in which the electrons in the atoms act as scatterers for X rays, just as the areas within the slits behave as if they were scatterers for visible light. The diffraction pattern of a crystal is arranged on a lattice that is reciprocal to the lattice of the crystal. The analogy with the optical example holds; the X-ray photograph is merely a scaled-up "sampling region" of the diffraction pattern of a single unit cell, with the "envelope" being the diffraction pattern produced by scattering from the electrons in the atoms of the unit cell, and the "sampling regions" arranged on the lattice reciprocal to the crystal lattice. In an analogous manner, diffraction of X rays of a given wavelength by a series of unit cells in a crystal gives an envelope, related to the arrangement of atoms in the unit cell, and sampling regions, related to the unit-cell dimensions.

This phenomenon of X-ray diffraction by crystals can be considered in terms of a theory analogous to that of diffraction by gratings and extended to three dimensions (von Laue) or be considered in terms of reflection from planes through points in the crystal lattice (Bragg). While these two treatments are equivalent, we have chosen to emphasize the first approach because it provides more insight into the process of structure analysis by diffraction methods.

4 Experimental measurements

The analysis of a crystal structure by X-ray or neutron diffraction consists of three stages:

(1) *Data collection*. This involves experimental measurement of the directions of scatter of the diffracted beams so that a unit cell can be selected and its dimensions measured. The intensities of as many as possible of the diffracted beams (Bragg reflections) from that same crystal are then recorded. These intensities depend on the nature of the atoms present in the crystal and their relative positions within the unit cell.

(2) *Finding a "trial structure."* This is the deduction by some method (such as one of those described in Chapters 8 and 9) of a suggested atomic arrangement (a "trial structure"). This is listed as atomic coordinates that have been measured with respect to the unit-cell axes. The intensity of each Bragg reflection corresponding to this trial structure can then be calculated (see Chapter 5) and its value then compared with the corresponding experimentally measured intensity in order to determine whether the trial structure is "good," meaning that it is essentially correct.

(3) *Refinement of the trial structure.* This involves modification (refinement) of a good trial structure until the calculated and measured intensities agree with each other within the limits of any errors in the observations (see Chapter 11). This is usually done by a least-squares refinement, although difference electron-density maps may also prove useful. The result of the refinement is information on the three-dimensional atomic coordinates in this particular crystal, together with atomic displacement parameters.

This chapter is concerned with the first of these stages, the experimental measurements. This is a rapidly changing area of science as more powerful and precise equipment and detection devices become available. The *experimental data* that may be derived from measurements of an X-ray or neutron diffraction pattern include:

(1) The *overall appearance* of the Bragg reflections at the detection system. Ideally these diffraction maxima should be sharp,

well-resolved peaks. Blurred, double spots or arcs may indicate disorder or poor *crystal quality*.

(2) The *angles or directions of scattering* (including 2θ, the angular deviation from the direct beam).* These can be used to determine the order, *hkl*, of each Bragg reflection and lead to a selection of *a unit cell* and a measurement of its *size* and *shape*.

(3) The *intensities*, $I(hkl)$, of the diffracted beams, which may be analyzed to give *the positions of the atoms* within the unit cell.

* See Figure 3.11 for a diagrammatic definition of θ.

The result is a set of values—$2\theta, h, k, l, I(hkl)$—and some measure of the precision, $\sigma(I)$, for each Bragg reflection. The diffraction pattern is uniquely characteristic of the atomic identities and arrangement in the particular crystal under study, and will only be the same for other crystals of the same material grown under the same conditions and having the same unit-cell dimensions and atomic composition. This means that a diffraction pattern can serve as a "fingerprint," and can be used for identifying material.

The experimental setup

The apparatus that is used to measure an X-ray diffraction pattern has the same configuration as that used in the very first diffraction experiment in 1912. The overall setup, illustrated in Figure 4.1, consists of:

(1) *The crystal that has been selected for study*. It is checked to ensure that it is a single crystal and is mounted in the measurement apparatus so that the incident radiation can pass through it and be diffracted by it.

(2) *An incident beam of radiation*. This is a fine pencil-like beam of X rays or a larger beam of neutrons directed at the crystal. The source of such radiation may be an X-ray tube, a synchrotron source, or neutrons from a nuclear reactor or spallation source. The beam may be monochromatic (one wavelength) or polychromatic (many wavelengths, known as "white radiation").

(3) *A system to detect the diffraction pattern*. This is usually an image plate or a charge-coupled device that can detect, measure, and electronically record the directions and intensities of Bragg reflections. Measurements may be serial (one Bragg reflection at a time) or may involve as much as possible of the entire diffraction pattern. The apparatus that aligns the incident beam, crystal, and detector, ready for measurement, is a diffractometer.

There have been many improvements to these components of the setup through the years and they are now significantly more efficient and "user-friendly." Advances in their design have now made it possible to study the crystal structures of extremely large biological

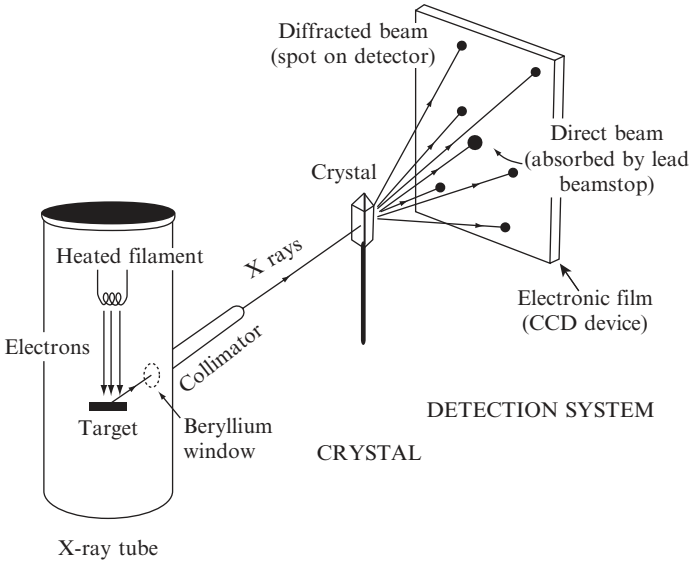

Fig. 4.1 The diffraction experiment.

The experimental setup used by von Laue, Friedrich, and Knipping to measure X-ray diffraction intensities in 1912. The important components of the experimental equipment consist of an X-ray source that provides a finely collimated beam of radiation, a crystal that can scatter this radiation, and a detection device that can detect the diffraction pattern and measure the directions and intensities of the diffracted beams. Currently this same arrangement of equipment is used by X-ray crystallographers, but each component is now much more sophisticated.

macromolecules (Blundell and Johnson, 1976; Helliwell, 1992; McRee, 1993; Drenth, 1999).

Selection of a suitable crystal

A crystal whose structure is to be determined should be a single crystal, not cracked or a conglomerate. This may be checked by examining it under a microscope, with polarized light, since most crystals are birefringent[**] (Blundell and Johnson, 1976; Wahlstrom, 1979; Hartshorne and Stuart, 1950). In the polarizing microscope two Nicol prisms each transmit only plane-polarized light, that is, light vibrating in a specific direction. One prism, the polarizer, produces plane-polarized light and the other prism, the analyzer, is only able to transmit light if the two prisms are in the same orientation. They are set perpendicular to each other so that no light can pass through. An optically isotropic crystal placed between the prisms will not change this, but if the crystal is birefringent and is rotated on the stage, it will show sharp extinction of light at four rotation positions 90° apart. These extinctions occur when the vibration directions of the Nicol prisms are the same as those of the

[**] One crystal form of the enzyme citrate synthase is cubic (Rubin et al., 1983) and shows no birefringence when a test tube containing crystals is shaken.

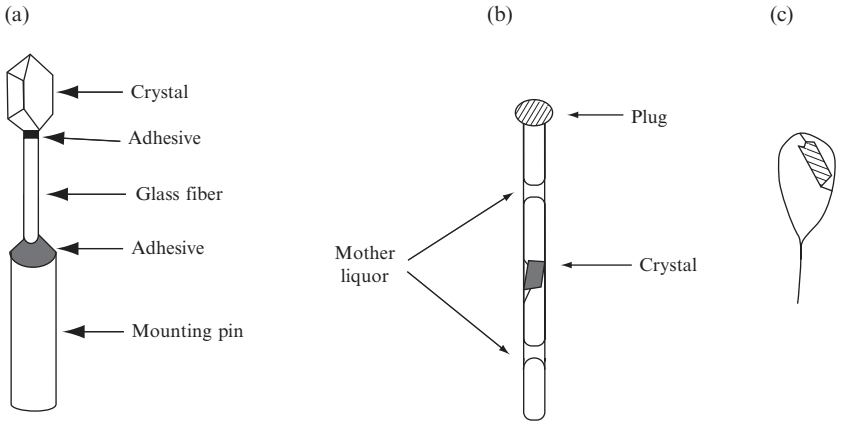

Fig. 4.2 Mounting a crystal.

Methods for mounting crystals. (a) A crystal mounted on a glass fiber, as used for a small-molecule crystal that does not decompose on exposure to air. (b) A crystal that does not diffract if it dries out is mounted in a sealed capillary tube with its mother liquor. (c) A protein crystal frozen on a thin film of solvent in a loop.

crystal under examination. Generally, if multiple crystals are present, only one part of the crystal will extinguish, and others will extinguish on further rotation of the crystal (Bunn, 1961). In this way one can check that a crystal is single.

If the crystal is too large, and therefore will not be fully bathed by the incident X-ray beam, it may be possible to cut it safely with a razor blade or with a solvent-coated fiber. Ideally one can try to find a crystal that can be shaped, often by grinding, until it is approximately spherical so that corrections for absorption of X rays are simplified. Some crystals, however, are too soft, fragile, or sensitive even for a delicate cutting and must be used as they have grown. For example, crystals of macromolecules contain 30–70% water, sometimes more, and they break very readily because the forces between such large molecules are weak in view of the macromolecular size; therefore attempts to cut the crystals may destroy them (Bernal and Crowfoot, 1934; McPherson, 1982; Bergfors, 2009).

The ultimate test of how good a crystal is comes from an inspection of the diffraction pattern obtained. Crystals are mounted on an aligning device (such as a goniometer head, see Figures 4.2 and 4.3), so that they can be positioned in the direct X-ray or neutron beam, ready for diffraction. The centering of the crystal in the beam is checked by rotating and viewing it through a microscope to make sure the center of the crystal is fixed in space during the rotation, and therefore does not move out of the incident beam during data collection.

A crystal to be studied is generally attached to a glass fiber with glue or some similar material. If the crystal is unstable, it is put into a thin-walled glass capillary tube (generally by gentle suction or simple capillary action) and the capillary is then sealed. An appropriate atmosphere is then maintained in the capillary to ensure stability of the crystal;

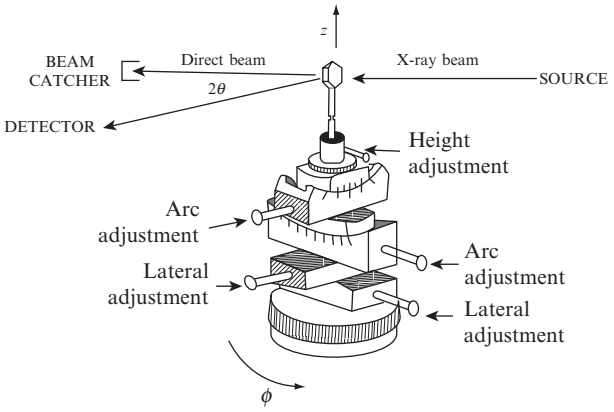

Fig. 4.3 Centering a crystal.

A goniometer head is used for orienting and centering a crystal in the incident X-ray beam. The goniometer arcs and lateral adjustments provide the means for the crystallographer to orient the crystal so that, in spite of reorientations of the centering device during data collection, the crystal is always centered in the incident X-ray beam. The angle ϕ and the position z define the orientation and height of the crystal.

for example, protein crystals require a small amount of mother liquor to prevent drying out and disordering or collapse of the crystalline structure. The fiber or capillary is fixed onto a brass pin by shellac or glue and this pin is then attached to the diffraction equipment, as shown in Figure 4.2. For biological macromolecules, such as enzymes, it is currently more usual to capture the crystal in a tiny loop (made of rayon, nylon, or plastic and attached to a tiny rod). The crystal is mounted or positioned for cryocrystallography in the thin film that forms when the small loop is immersed in real or synthetic mother liquor, as shown in Figure 4.2c; the crystal in the loop is then flash-cooled in liquid nitrogen. The aim of this cooling is to reduce radiation damage caused by the X rays, but it can sometimes cause the crystal to crack or form ice on its surface; therefore it may be necessary to soak the crystal in a cryoprotectant solution, such as glycerol, prior to cooling. Cooling will also increase the maximum resolution of the diffraction data and the value of $I(hkl)/\sigma(I)$. The crystals are then kept at a low temperature (just above the boiling point of nitrogen) for data measurement. If its quality is still poor the crystal can be annealed by warming the crystal, and then flash-cooling it for a second time (Harp et al., 1998). Newer methods of crystal mounting continue to be designed and reported on in the literature.

Radiation damage usually occurs as a result of free-radical formation and heating effects; it will continue after X-ray exposure has stopped. It is generally believed that such radiation damage can be reduced by the use of incident monochromatic X rays, or by lowering the temperature with appropriate attention to the solvent. If a small group of Bragg reflections is measured at regular intervals throughout a sequential measurement process, it will be possible to determine the amount of

crystal decay as a function of time. In practice, each Bragg reflection is affected in a unique manner, depending on the nature of the atomic movement during damage, but an average fall-off in intensity will give some (but not precise) information that is suitable for use in correcting intensities for radiation damage. A neutron beam generally does not cause any radiation damage to the crystal.

Unit-cell dimensions and density

The dimensions of the unit cell ($a, b, c, \alpha, \beta, \gamma$) can be found from the angles, 2θ, of the deviation of given diffracted beams from the direction of the incident beam, because each value of 2θ at which a diffraction maximum is observed is a function only of the cell dimensions and of the known wavelength of the radiation used, see Appendix 1. The spatial orientation of these diffracted beams allows indexing so that the determination of cell dimensions is simplified; however, it is also possible to determine unit-cell dimensions from powder photographs.

The density of the crystal can be measured by flotation, but generally the value is now assumed to be the same as that of crystals with a similar composition. Most crystals of organic compounds have a density near $1.3\,\mathrm{g\,cm^{-3}}$, otherwise described as $18\,\text{Å}^3$ per atom, excluding hydrogen atoms. For macromolecular crystals, which may have a high water content, the Matthews coefficient (V_M, volume per dalton of protein), calculated as the unit-cell volume, V, divided by the molecular weight, MW, times the number of asymmetric units in the unit cell, Z, should lie in the range 1.7 to $3.5\,\text{Å}^3$ per dalton (average near 2.3) (Matthews, 1968; Kantardjieff and Rupp, 2003):

$$\text{Matthews coefficient } V_\mathrm{M} = V/\{Z \text{ times MW}\} \text{ cubic Å per dalton} \quad (4.1)$$

If the nature of the atomic contents of the crystal is uncertain, it still may be necessary to measure its density. Experimentally, this is done by mixing two miscible liquids in which the crystal is insoluble (one more dense, one less dense than the crystal) in such proportion that the crystal remains suspended in the mixture (it neither sinks nor rises to the surface of the resulting mixture). The density of the liquid mixture (with the same density as that of the crystal) is then found by weighing a known volume in a "specific gravity bottle" or "pycnometer." For macromolecules, a "density gradient column" is prepared by layering an organic liquid (in which the protein is insoluble) on another that is miscible with the first. This column can be calibrated by measuring the equilibrium positions along the column of drops of aqueous solutions of known density. Some protein crystals are then added to the column and their equilibrium positions read; these positions can be directly converted to densities using the previously prepared chart. As seen in Appendix 1, the density of the crystal, combined with its unit-cell dimensions, will give the weight of the contents of the unit cell. If the elemental analysis of the crystal is known, then the number of each type

of atom in each unit cell can be determined. Then a decision can be made whether or not to proceed with a structure analysis.

The Bragg reflections to be measured

The Bragg equation (Eqn. 3.1) is only satisfied for a few diffracted beams if the crystal is stationary. Therefore it is usual to oscillate the crystal in order to obtain more diffraction data. The maximum number of Bragg reflections that can be accessed, N, will depend on appropriate oscillation of the crystal, the wavelength λ of the radiation, the volume V of the unit cell, and the number n of crystal lattice points in the unit cell, according to the formula

$$N = (4\pi/3)(8V/n\lambda^3) \tag{4.2}$$

How do we tell which Bragg reflections can be measured with a selected arrangement of the diffraction-measuring apparatus? There is a geometric construction that does exactly this. It is the Ewald sphere, named after Paul Ewald, who was involved in discussions with von Laue that led to the first crystal diffraction experiments in 1912 (Ewald, 1913). For the crystal under consideration, the Ewald sphere is a sphere of radius $1/\lambda$ (for a reciprocal lattice with dimensions $d^* = 1/d$), drawn with its diameter along the incident beam direction. This is shown in the diagrams of its construction in Figure 4.4. The origin of the reciprocal lattice is positioned at the point at which the incident beam emerges from the Ewald sphere. The reciprocal lattice is then rotated about its origin (in the same manner as that planned for data measurement). Whenever a reciprocal lattice point P touches the surface of the Ewald sphere, the conditions for a diffracted beam are satisfied. A Bragg reflection, with the *hkl* indices of that reciprocal lattice point P, will result. Thus, for a particular orientation of the crystal relative to the incident beam, it is possible to predict which reciprocal lattice points and thus which Bragg reflections will be observed. If radiation of a different wavelength is used, the radius $1/\lambda$, drawn in the Ewald sphere, can be adjusted accordingly, and the angles through which the crystal is rotated can be accounted for.

The incident radiation: X rays or neutrons

X rays are produced, as mentioned in Chapter 3, when a high voltage is applied between a cathode and an anode in an evacuated glass bulb; this voltage causes the cathode to emit fast-moving electrons,[†] and they are directed at the anode (a metal target), and are suddenly decelerated when they hit it. As a result of this impact, X rays are emitted. The intensity of this initial source of X rays is controlled by the applied voltage and amperage. A diagram of an X-ray tube is provided in Figure 4.5. X ray-tubes have a relatively low flux and the background

[†] The type of diffraction discussed in this book is referred to as "kinematical diffraction" and assumes that the incident beam is diffracted and leaves the crystal. In "dynamical diffraction," which is particularly evident in electron diffraction, the diffracted beams interact with the crystal and each other (Ewald, 1969). This repeated scattering makes analysis of the diffraction pattern much more complicated.

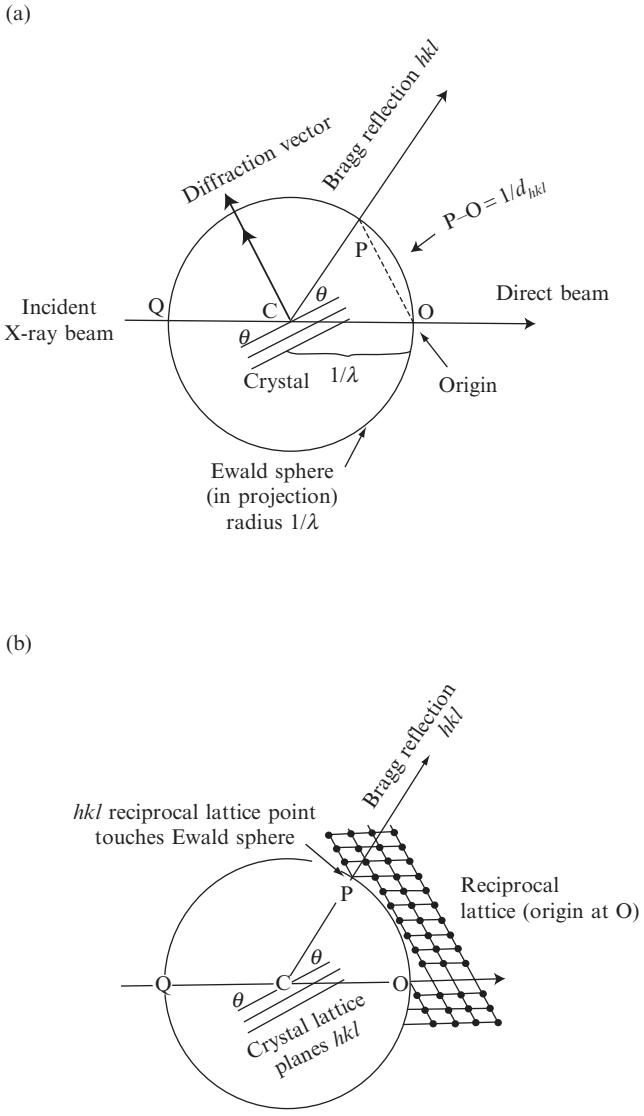

Fig. 4.4 The Ewald sphere (sphere of reflection).

(a) A sphere of radius $1/\lambda$ is drawn. (b) The origin of the reciprocal lattice, drawn on the same scale, is placed with its origin on the surface of the sphere, at O. When a reciprocal lattice point hits the surface of the Ewald sphere, a Bragg reflection will occur. To increase the likelihood of this happening the crystal is rotated in the diffractometer, an event that is represented in the Ewald construction by a similar rotation of the reciprocal lattice. If white radiation is used, it will be necessary to draw spheres at the two limits of radiation.

radiation is appreciable, unless filters or a monochromator are used. The greater the intensity of radiation from an X-ray tube, the more extensive the diffraction pattern (since weak Bragg reflections are made visible) and the better the signal-to-noise ratio. Since diffracted beams are much weaker in intensity than the direct (undiffracted) beam, it is

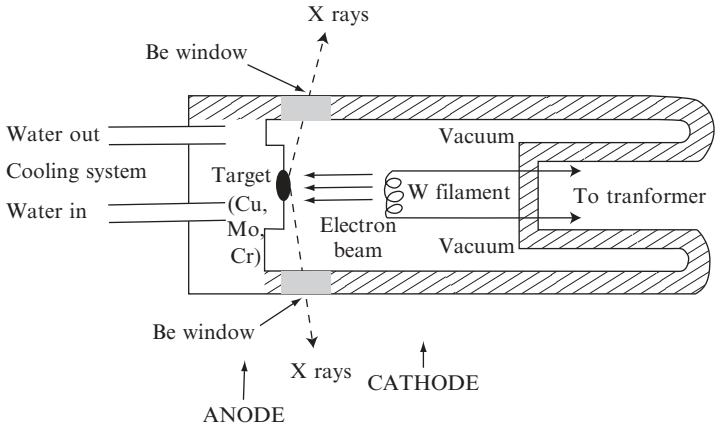

Fig. 4.5 An X-ray tube.

Diagram of the structure of an X-ray tube. Electrons are emitted from the tungsten filament (cathode) and are attracted to the target in the anode. On hitting the target (Cu, Mo, Cr, for example), X rays are emitted and exit the tube through beryllium windows.

necessary to intercept the direct beam by means of a small metal cup (a "beam stop") so that the detection system is not overloaded by the high intensity of the direct incident beam of X rays.

Two types of X rays are produced in the X-ray tube (see Figure 4.6a). The first has the label "Bremsstrahlung," which means "braking radiation" (in German), and is produced when accelerated electrons are suddenly decelerated by a collision with the electrical field of an atom in the metal target of an X-ray tube. This radiation, which generally serves as background, has a continuous spectrum. The kinetic energy of the fast electrons has been converted into radiation, including X rays. The second type of radiation, called "characteristic radiation," is produced when the fast electrons cause a change in the atom that they hit; this change is the ejection of an electron from an inner shell of an atom in the metal target anode. When another electron from an outer shell of the same atom moves to fill the void left by the ejected electron, an X ray photon will be emitted with a wavelength representative of the difference between the energy levels of the ejected electron and of the electron that takes its place. The X-ray spectrum obtained is therefore characteristic of the metal in the target anode. It is approximately monochromatic, and all but one narrow wavelength band can be selected and used for diffraction studies. Characteristic X rays from copper and molybdenum target anodes (wavelengths 1.54 Å and 0.71 Å, respectively) are most commonly used in X-ray diffraction experiments, but many other targets are available for use when necessary. The X rays are labeled by the shell of the ejected electron (K, L, M, etc.) and the number of shells that the replacement electron has passed through (α for one shell, β for two, etc.) (see Figure 4.6b). For example, Kα radiation corresponds to a transition from $n = 2$ to $n = 1$ (the innermost, highest-energy atomic level, where n is the principal quantum number

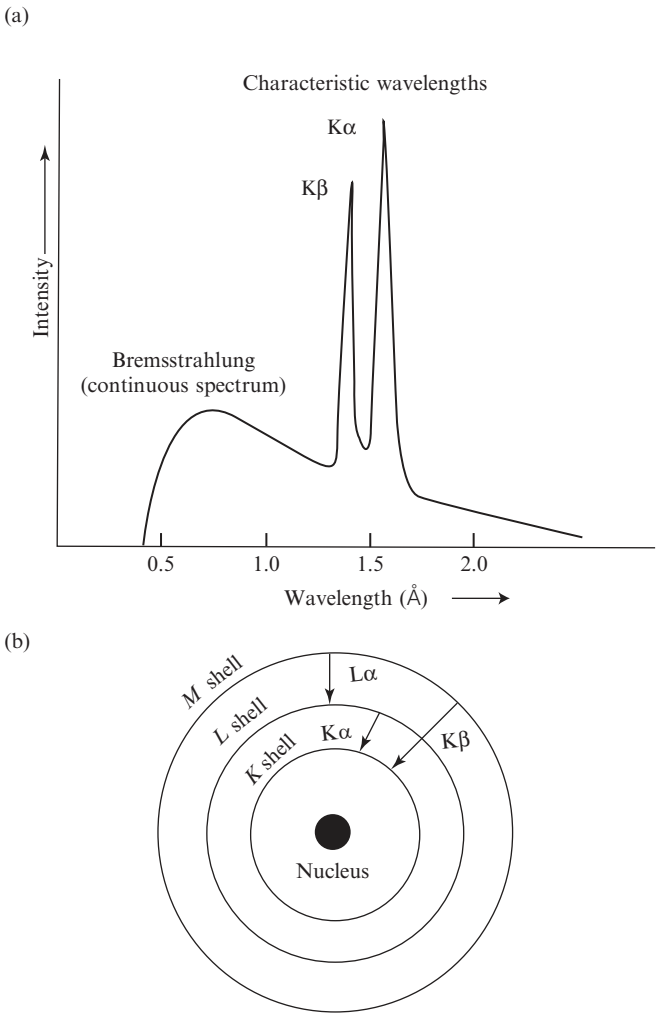

Fig. 4.6 Energy levels and X rays.

(a) The characteristic X-ray spectrum of copper radiation, produced with a copper target in the X-ray tube. (b) X rays are labeled by the shell of the ejected electron (K, L, M, etc.) and the number of shells the replacement electron has passed through (α for one shell, β for two, etc.).

of a shell). This means that when a K-shell vacancy is formed, it is filled by an electron from the adjacent L shell, and Kα radiation is emitted. Kβ radiation corresponds to a transition from $n = 3$ to $n = 1$; that is, a K-shell vacancy is filled by an M-shell electron, and so forth.

A monochromator, which transmits only a mechanically selectable small range of wavelengths (its bandpass) from a larger range, is used to tune X rays to a required wavelength. One type of monochromator selects (by slits) a single Bragg reflection from an appropriate crystal, such as one of graphite, silicon, germanium or copper, and this selected Bragg reflection becomes the new incident beam for diffraction studies.

Another type employs optical methods, that is, a combination of a collimating mirror, a diffraction grating, and a focusing mirror, to give the required spectral range; X rays can be focused by mirrors if the angle of incidence is extremely small (less than 0.1°). Sometimes two monochromators are used, acting in tandem.

A major problem when X rays are produced in a sealed tube (as just described) is that considerable heat is generated and must be eliminated, for example by cooling the tube with flowing water. It has been found that if the anode is rotated at high speed and the fast-electron beam is directed at its outer edge, this heat can be dissipated, and, as a result, it becomes possible to generate more intense X rays. This is the principle of the *rotating-anode generator*, and, because of the high flux of the X rays produced, it is possible to measure extensive diffraction data for crystalline biological macromolecules.

Synchrotron radiation, however, currently provides the most intense X rays suitable for diffraction studies. The emission of radiation is a property of accelerated charged particles. Electromagnetic radiation (which includes X rays) is emitted when accelerating electrons, traveling at near the speed of light, are forced, by a magnetic field, to travel in a circular orbit, as in an electron storage ring. The wavelength of this radiation will depend on the strength of the magnetic field, the speed of the electrons, and the size of the storage ring. These factors can be appropriately chosen and combined to give a good source of X rays. Synchrotron radiation has very high intensity (and therefore is good for single-crystal diffraction studies), and low divergence (so that there is good intrinsic collimation, a large signal-to-noise ratio, and a high resolution). It is also highly polarized (which is useful for distinguishing electronic from magnetic scattering) and is emitted in short pulses (which facilitates fast time-resolved studies). It is multiwavelength (white) radiation and, if a single wavelength is required, selection (tuning) with a monochromator is essential. Its range of wavelengths is wide, so that selection can be made of radiation near the absorption edge of an atom contained in the crystal; therefore anomalous-dispersion experiments, as described in Chapter 10, can be done.

Another type of radiation used in crystal diffraction studies consists of neutrons (Bacon, 1975; Dianoux and Lander, 2003; Willis and Carlile, 2009). Neutron diffraction can provide information that complements that from X-ray diffraction. Neutrons are uncharged particles, highly penetrating, but their beams are relatively weak, and, when not in nuclei, they decay with a mean lifetime of about 15 minutes. They were discovered by James Chadwick in 1932, and were subsequently shown to be diffracted by crystals (even though they are particles) (Chadwick, 1932; von Halban and Preiswerk, 1936; Mitchell and Powers, 1936).‡ This dual identity of neutrons is in line with the postulate of Louis Victor de Broglie in 1923 that particles and waves should have both particle-like and wavelike properties (de Broglie, 1923). Their wavelength can be calculated from his equation $\lambda = h/mv$, where λ is the wavelength, m is the mass of a neutron (1.67×10^{-24} g), v is its

‡ This was long after von Laue studied diffraction of X rays by crystals in 1912 and therefore decided that X rays are waves (Friedrich et al., 1912).

velocity, and h is Planck's constant ($6.626 \times 10^{-34}\,\mathrm{kg\,m^2\,s^{-1}}$) (Planck, 1901). The faster the neutron, the shorter its apparent wavelength.

X-ray diffraction probes the electron-density arrangement in the crystal, while neutron diffraction probes the positions of atomic nuclei in the crystal. Therefore, when the results of X-ray and neutron diffraction by a crystal are compared, a large amount of structural and chemical information, for example, on the asymmetry of the electron distribution around a particular atomic nucleus, is obtained. This will be described later in Chapter 12. Neutrons also have a spin of $1/2$ and therefore can also be used to probe the magnetic structure of a material.

Neutrons are generally produced at nuclear reactors, so that it is necessary to visit a national atomic energy center for neutron diffraction studies. A large number of neutrons are produced in a reactor by nuclear fission. They may also be produced at spallation sources. The word "spallation" describes the ejection of material on impact. Neutrons are obtained at a spallation source when short bursts of high-energy protons bombard a target of heavy atomic nuclei (such as mercury, lead, or uranium); each proton produces several high-energy neutrons in a pulsed manner. Slow neutrons with wavelengths of 1 to 2 Å are required for diffraction studies. Therefore fast neutrons produced by either of these two processes must be slowed down by moderators (such as heavy water) that reduce their kinetic energy and provide neutrons with wavelengths that are approximately the same as those used for X-ray diffraction studies. For further information on the practical aspects of neutron diffraction, there are several excellent texts (Bacon, 1975; Wilson, 2000; Willis and Carlile, 2009).

Equipment for diffraction studies

When X rays are used for crystal diffraction studies, it is found to be necessary, in order to get a large number of Bragg reflections, to oscillate or rotate the crystal, or to use polychromatic radiation (the Laue method). The general geometry of the detection system is shown in Figure 4.7. The relationship between the diffraction pattern and the crystal orientation is diagrammed in Figure 4.8. While the crystal lattice defines the crystal, the reciprocal lattice (Figure 4.9) represents the diffraction pattern, and this information is useful when interpreting the diffraction pattern in terms of Bragg reflections.

We first briefly describe the old film methods, as they are part of the literature on the subject and they illustrate some of the principles that the reader needs to know. Then we proceed to the more modern methods. The old methods mostly involve photographic film; this is a good X-ray detector, but has now been superseded by more efficient electronic devices. To take an oscillation or rotation diffraction photograph, a crystal, mounted on a goniometer head, is either rotated continuously in one direction (to give a rotation photograph) or oscillated back and forth through a small angle (to give an oscillation photograph). The

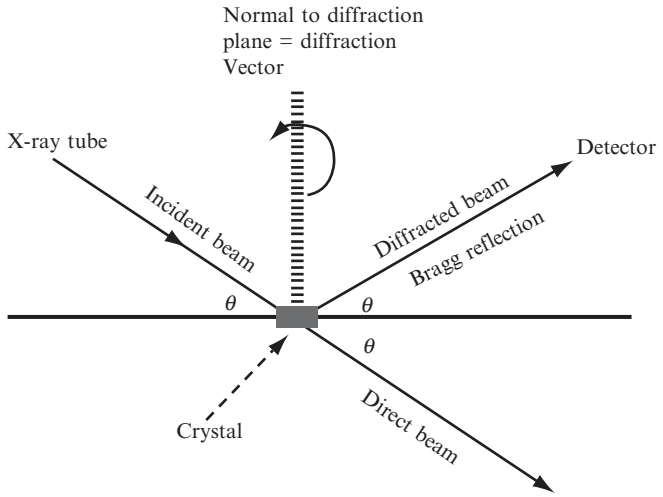

Fig. 4.7 Source, crystal, and detector.

Diagram of the relative arrangement of the X-ray source, the detector, and the crystal and their relationship to the diffraction vector. All are in the same plane.

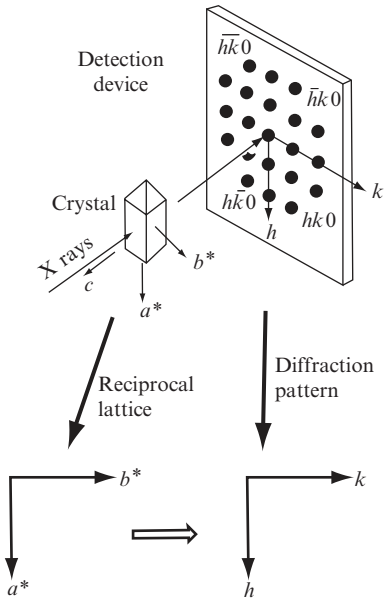

Fig. 4.8 The relation between the crystal orientation and the diffraction pattern.

The relative orientation of the reciprocal lattice of a crystal (expressed here as a^* and b^*), and its indexed X-ray diffraction pattern (expressed here as h and k). Note the relationship of a^* to h and b^* to k. From the positions of diffracted beams on the detection device it is possible to deduce the dimensions of the reciprocal lattice and hence of the crystal lattice; hence the indices h, k, and l of each Bragg reflection.

resulting diffraction pattern is recorded on photographic film placed around the crystal. If the axis of rotation or oscillation is perpendicular to the X-ray beam, the resulting photograph contains lines (layers) of Bragg reflections (see Figure 4.10). As can be seen in this figure, many of the Bragg reflections overlap each other, so that indexing them may difficult. Therefore the Weissenberg camera was invented, in which the camera is moved as the crystal is rotated or oscillated. Only one layer from an oscillation photograph is selected, by the positioning of a metal screen with a slit in it, between the film and the X-ray source (Weissenberg, 1924). The crystal is oscillated back and forth, while the slit ensures that only one layer of Bragg reflections (for example, a specific value for the h index) is recorded on the film. At the same time the camera moves in a direction parallel to the axis of crystal oscillation. The most important feature is that the motion of the camera is coupled to the oscillation of the crystal, which helps in interpreting the photograph. Bragg reflections on a Weissenberg photograph can therefore be more readily indexed than on an oscillation photograph.

An even more useful type of X-ray diffraction photograph is produced by a precession camera (Figures 3.8a and 4.11) (Buerger, 1964). It gives an undistorted view of one selected plane of the reciprocal lattice. This makes it particularly useful for measuring unit-cell dimensions and assigning a space group to the crystal. Here the camera motion is more complicated in order that the recorded image of the diffraction pattern may be simple. In fact, direct measurement of all reciprocal lattice parameters is possible from a series of precession photographs, with an appropriate scale factor taken into

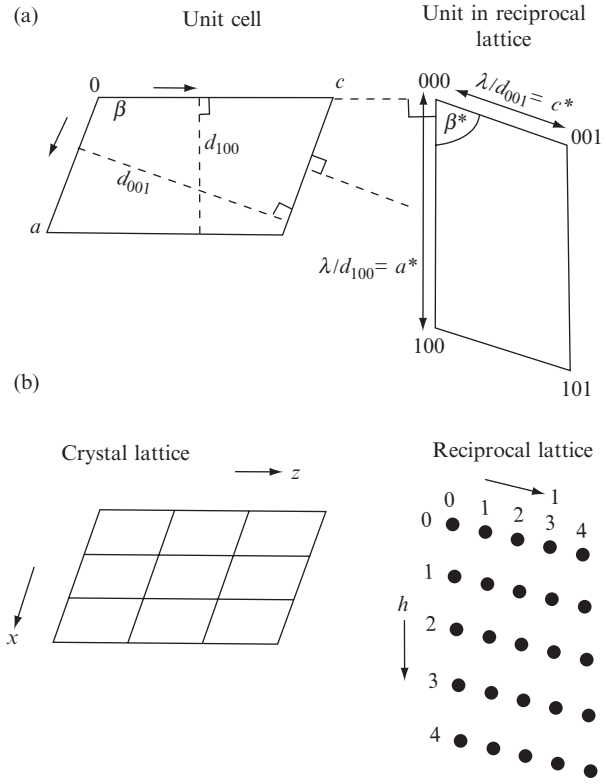

Fig. 4.9 The reciprocal lattice.

(a) The relationship between the unit cell of a crystal and its reciprocal lattice. (b) Indexing of a reciprocal lattice.

consideration. An axis of the crystal perpendicular to the required reciprocal lattice plane is inclined by an angle μ (typically 30°) to the direct incident X-ray beam, and this then precesses (like the motion of a toy spinning top) about the incident X-ray beam. The flat film holder, which has an annular screen that isolates a single plane of the reciprocal lattice, follows the precession motion, ensuring that the film is always parallel to the selected reciprocal lattice plane of the crystal being photographed. It does this in such a way that the direct beam always hits the center of the film. The photograph that results from this complicated set of motions is simple to interpret. This method is very useful for triclinic crystals and for macromolecular crystals.

Generally, crystal symmetry, crystal lattice constants, and diffraction data are currently measured with a diffractometer (Figure 4.12). The incident radiation may be X rays from a sealed tube, a rotating anode, or a synchrotron source, or it may be a neutron beam. A diffractometer requires a collimated incident beam and a beamstop to collect that part of the direct beam that has passed undeflected through the crystal. The

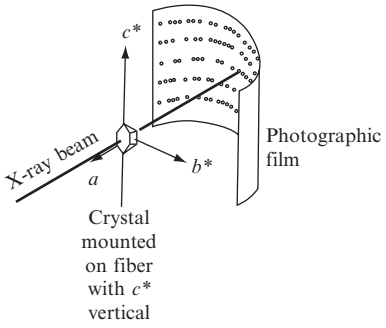

Fig. 4.10 Layer lines.

An X-ray diffraction photograph is obtained from a crystal mounted with the reciprocal lattice axis, c^*, vertical. On oscillation about this vertical axis the diffraction pattern shows layer lines, each with a constant value for the index l along them.

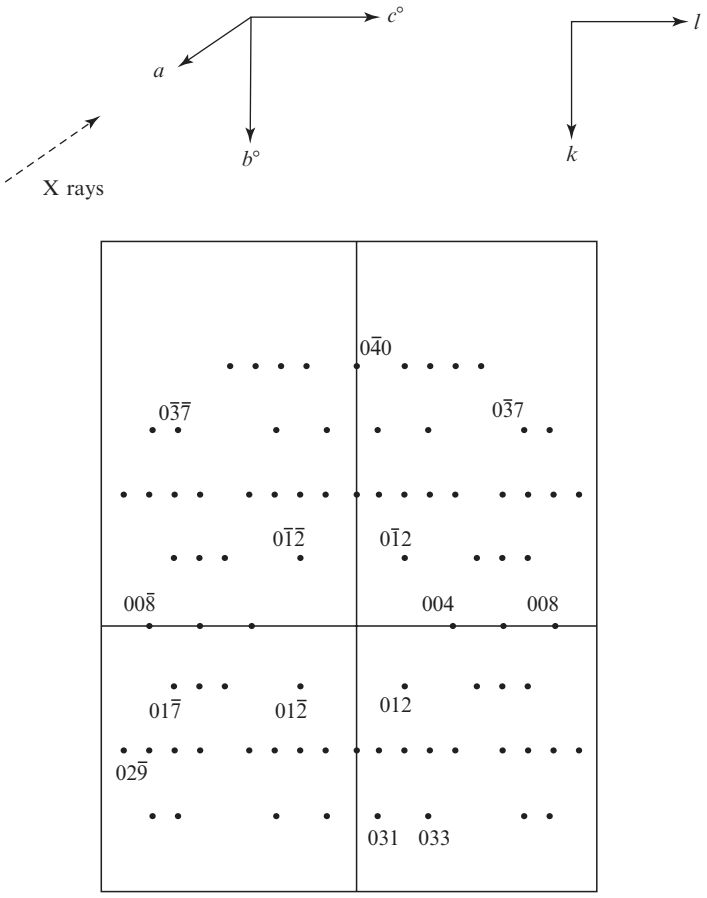

Fig. 4.11 Indexing a precession photograph.

The indexing of Bragg reflections on a precession photograph. Note the systematic absences—0k0 with k odd and 00l with l odd. By convention the positive direction of a is toward the X-ray source.

detection system is an image plate or a charge-coupled device, rarely photographic film. Many modern diffractometers do not require any orientation of the crystal, only centering of the crystal, so that no matter how the instrument is oriented the crystal is always centered in the incident beam. A goniometer head can, however, be used to align the crystal, if required. Protein crystals, mounted with mother liquor in a capillary, are also put in a centering device. While both imaging with film and digital signaling are employed for the detection of diffracted radiation, they operate in different ways. A film records light as the result of a series of chemical reactions, while charge-coupled devices convert light (caused when X-ray photons hit a phosphor) directly into a digital signal.

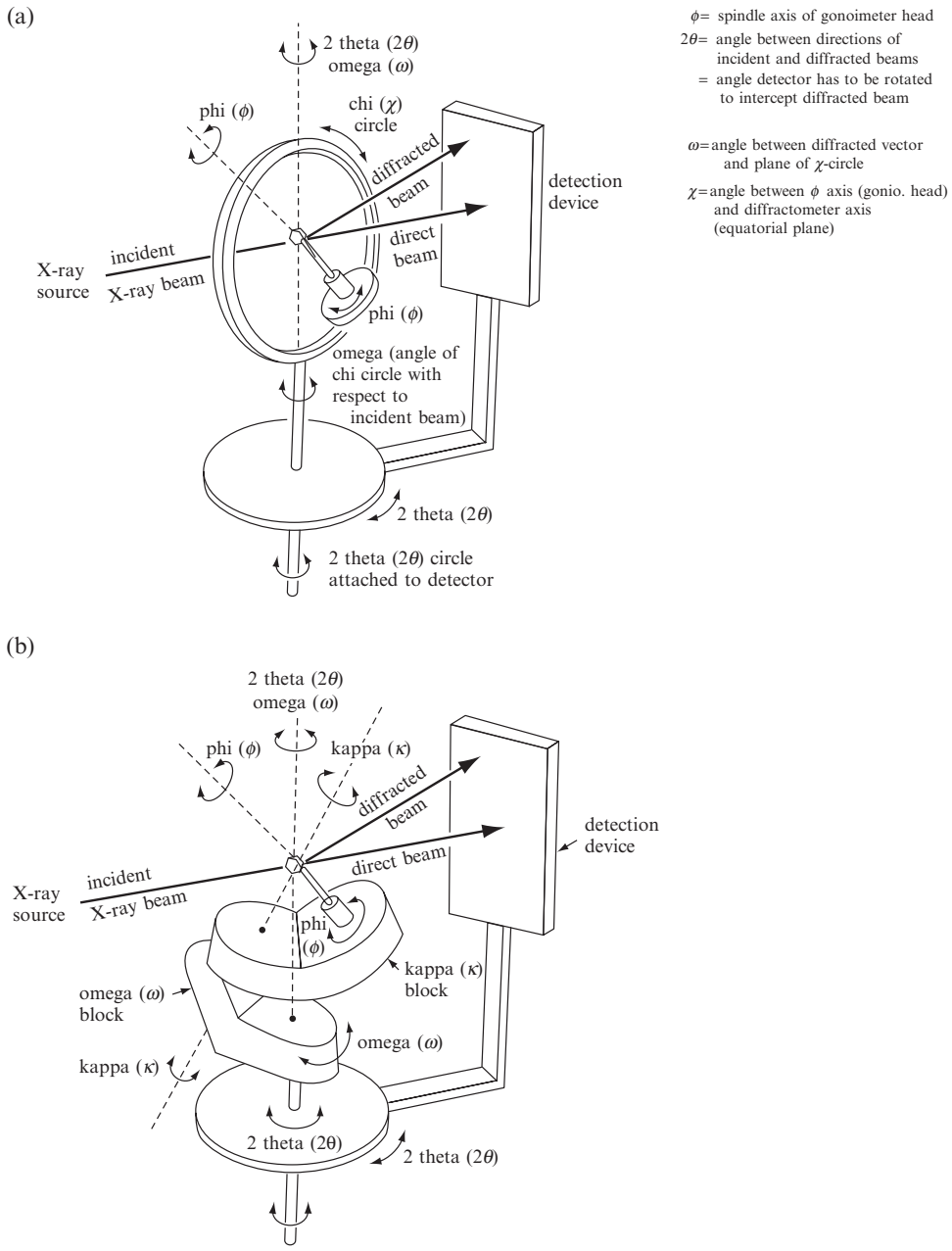

Fig. 4.12 An automatic diffractometer.

(a) A four-circle diffractometer. The crystal is mounted on a goniometer head, for which the spindle axis is ϕ. The goniometer head is attached to the χ circle. The angle χ is the angle between the ϕ axis of the goniometer head and the base of the diffractometer. The χ circle can be rotated about the ω axis, where ω is the angle between the diffraction vector and the plane of the χ circle. The detector is moved on the 2θ circle, where 2θ is the angle between the incident and diffracted X-ray beams. The detection device can be an image plate or a charge-coupled device. The setup for serial measurement is shown here. (b) A diffractometer with kappa (κ) geometry. The omega block rotates about the base plate while the kappa block rotates about the omega block as shown. This simulates the chi circle motions in the instrument in (a) but avoids clashes.

There are several types of diffractometers. Some move a detector to measure each Bragg reflection sequentially, and some employ a flat detection device, an "area detector," that measures a large number of Bragg reflections at one time. The source of radiation is usually fixed in space and, in a sequentially measuring diffractometer, the required angular settings for the crystal and detector with respect to the incident beam are calculated in advance once a few Bragg reflections have been located and identified. This type of diffractometer is composed of several mechanical circles that rotate the crystal or the detection system with respect to the X-ray beam, as shown in Figure 4.12a. In this "four-circle diffractometer" the crystal can be rotated around three axes (χ, ϕ, and ω) independently, and the detector can be rotated about a fourth angle (2θ, concentric with, but independent of, ω), in the equatorial plane parallel to the base of the instrument. The crystal is mounted on a goniometer head and can be rotated about the vertical ϕ axis (phi) of this mounting (see Figure 4.12a). The goniometer head is mounted on the χ circle, which tilts the crystal about the horizontal χ axis (chi). The 2θ circle is attached to the detector device. This is concentric with the ω circle that rotates the sample. The χ circle is mounted on top of the ω circle, and the ϕ circle is mounted on top of the χ circle. Usually the entire instrument is controlled by a computer and the data collection is then done automatically. There are also diffractometers that utilize the kappa (κ) geometry (Figure 4.12b). This type of diffractometer was designed specifically to reduce mechanical clashes during data collection. The ω, ϕ, and 2θ circles remain, but the χ circle is replaced by a κ block that sits on the ω block (which replaces the ω circle) and this controls the orientation of the crystal and its goniometer head.

If the measurement is to be sequential, the intensity of a Bragg reflection is measured with the detector and recorded, together with measurements of the background intensity near the Bragg reflection, and then a new set of angles is calculated and another intensity measurement made. One normally advances incrementally through the Miller indices, *hkl*. In this way a systematic scan of all desired Bragg reflections is done completely automatically. Alternatively, if the crystal is stationary and white radiation is used, an image plate or charge-coupled device will be positioned to receive and record as many as possible of the diffracted beams. For this Laue diffraction, the incident radiation is white radiation with a range of wavelengths. It has proved useful for studies of enzyme reactions (Hajdu et al., 1987). For example, a crystal of the enzyme glycogen phosphorylase *b* was mounted in a flow cell and substrate solution was passed over it. Laue photographs (stationary crystal, white radiation) were taken with synchrotron white radiation (over 10,000 Bragg reflections per second) at a series of times after initiation of the biochemical reaction. A comparison of electron-density maps from the various data sets showed the course of the reaction as a substrate was converted to product (by phosphorylation).

Detection systems

The intensities of the diffracted beams are measured by intercepting the beams with a detecting material or device that is sensitive to X rays. The intensity at the peak of the diffraction spot is measured, or, better, the peak profile is scanned. Measurements of background counts are also made, or calculated from the profile of the peak, and used to correct the recorded intensities. Measurements may be done electronically or photographically and may concentrate on one diffracted beam at a time (as is often done with a diffractometer) or on many diffracted beams at the same instant (as with electronic analogues of photographic film).

The simplest detection device for X rays is photographic film. This contains silver halide in an emulsion on its surface. When the film is developed, black metallic silver is deposited at the positions at which the diffracted beams hit the photographic film. The darkness of each spot so formed is a measure of the intensity of the diffracted beam. These intensities can be measured with a film scanner. Film is not used much nowadays, because of the development of electronic detection devices (with superior detection capabilities) and current problems in obtaining photographic film suitable for X-ray studies.

Electronic detectors of X rays that have an appreciable area for detection of the diffraction pattern, and offer the possibility of resolving and individually measuring the intensities of diffraction maxima at different points across this area, are now preferred. They consist of scintillation counters, television-enhanced scanning devices, image plates, and charge-coupled devices, and are the equivalent of electronic film. Position-sensitive detectors can measure the position at which a Bragg reflection hits the detection device. These various devices represent the development of improved ways of recording a diffraction pattern electronically in a computer-readable manner, and image plates and charge-coupled devices are the current instruments of choice for this. Whereas photographic film records photons through a series of chemical reactions, charge-coupled devices convert light directly into a digital signal. Scintillation counters make use of the ability of certain substances to emit visible light by fluorescence when X rays hit them. The intensity of the emitted light is measured by a photomultiplier tube. Similarly, television area detectors contain a phosphor that produces visible light when hit by X rays. The photon signal is intensified and then detected by a television photocathode. These methods of detection are now less used than image plates and charge-coupled devices. Neutrons, which lack any charge, and readily penetrate materials, are detected by gas or scintillator detectors; these are similar to the X ray detectors described above (Wilson, 2000).

An image plate is a storage phosphor on which a latent image is formed when X rays hit it. It contains plastic sheets with powdered

phosphor crystals, doped with divalent europium ions, on their surfaces. When X rays hit these sheets, the divalent europium ions are converted to metastable trivalent ions and the electrons that are liberated are stored ready for release when scanned by a laser beam of visible light. When trivalent europium ions are encountered, blue light (wavelength 3900 Å) is emitted that can be scanned and converted to a digital image. This latent image has to be read; it is exposed to laser light, which causes the emission of light of a different wavelength, which is then detected. The image plate can then be erased, ready for the next use, while the data from the scanning of the latent image, which are in a computer-readable form, are then ready for use in structure determination. The location of the direct beam is evident on the image, and from the positions of diffracted beams it is possible to determine the direction, as well as the intensity, of each Bragg reflection. Neutrons can only be detected if they have undergone some reaction that results in the emission of energetic charged particles; this means that a converter must be used. Neutron image plates contain elements such as gadolinium (which has a very high neutron, but not proton, capture cross-section, or stopping power) that absorb neutrons and act as a converter to enable the neutrons to emit electromagnetic radiation (such as gamma rays), which can be detected like the X rays in the description above.

Charge-coupled devices are used widely in X-ray diffraction equipment. They are two-dimensional grids of radiation-sensitive semiconductor capacitors that have the capability of transferring charge between their neighbors. They acquire a charge when hit by a photon, and electron–hole pairs are generated by the photoelectric effect. The total charge that is built up is a measure of the number of photons that have been detected (the radiation intensity), and it is collected in an array of electrodes. The charge and position of each pixel are transferred as a result of a differential voltage across the electrodes, and the data are read and digitized by a computer (see Ladd and Palmer, 2003). This gives an immediate computer listing of the intensity and position on the detection device, and therefore this device is closer to a direct detector than is an image plate.

When white radiation is incident on a crystal, as in the Laue method, it is necessary to know the wavelength of the radiation that causes a particular Bragg reflection. The time-of-flight neutron diffraction technique depends on the fact that neutrons with different energies (wavelengths) travel at different speeds. Therefore a measurement of the time of flight will reveal the wavelength of the diffracted beam (generally selected from a multiwavelength incident beam). The instant at which the diffracted beam hits the crystal and then impacts on the detection system is measured and recorded. This, with the known distance traveled, gives the velocity of the neutron and hence its wavelength. Therefore the wavelength of each diffracted neutron can be measured.

Preparing measured $I(hkl)$ for subsequent analysis

Since the intensity $I(hkl)$ of any radiation propagated as a wave is proportional to the square of its amplitude, $|F(hkl)|$ the intensity of the diffracted beam corresponding to the diffraction maximum for each set of planes hkl is proportional to $|F(hkl)|^2$. Modifications to $I(hkl)$ are necessary in order to correct for the geometry of measurement. Weak Bragg reflections are measured carefully, rather than being ignored. Of the various correction factors that are used, the Lorentz factor takes into account the time that it takes for a Bragg reflection, represented as a reciprocal lattice point with a finite size, to cross the surface of the sphere of reflection; the longer the time, the higher the intensity. The Lorentz factor equalizes the time taken to measure each Bragg reflection. The polarization factor depends on the state of polarization of the incident X-ray beam; X rays are polarized on scattering, with reduction of the intensity of the Bragg reflection. Corrections for absorption of X rays by the crystal are also made; ideally, the path lengths through the crystal of many component waves of each diffracted beam are computed, and the diminution in intensity resulting from absorption can then be determined. Semiempirical absorption corrections, based on the intensity variation as certain intense Bragg reflections are scanned while the crystal is rotated, are more generally used. If a crystal is strongly absorbing for the radiation used, it may be shaped (with a scalpel or razor blade) until it is approximately spherical so that absorption corrections may be more uniform. Generally it is better to avoid using a crystal larger than the primary beam, although this may be necessary for protein crystals that are damaged by the X-ray beam, so that one can move the crystal to an undamaged area during data collection. The aim is to keep the amount of matter exposed to radiation independent of the crystal orientation.

It is then possible to determine the absolute value (without phase) of the structure factor $F(hkl)$ from these measurements, as follows:

$$\begin{aligned} I(hkl) &= \mathrm{k}_1\{\lambda^3 V_\mathrm{c}\mathrm{Lp\ Abs}/\omega V^2\}|F(hkl)|^2 = \mathrm{K}\{\mathrm{Lp\ Abs}\}|F(hkl)|^2 \\ &= \mathrm{k}_2|F(hkl)|^2 \end{aligned} \tag{4.3}$$

where k_1, k_2, and K are constants, V_c is the volume of the crystal that is bathed in the incident beam, V is the volume of the unit cell, Lp consists of the Lorentz and polarization factors, Abs is an absorption correction, and ω is the angular velocity of the crystal. Thus, values of $\mathrm{k}_2|F(hkl)|^2$ and hence of $\mathrm{k}_2{}^{1/2}|F(hkl)|$ are immediately available once intensity measurements have been made. The values of Lp and Abs contain only known quantities and therefore can readily be computed for each Bragg reflection.

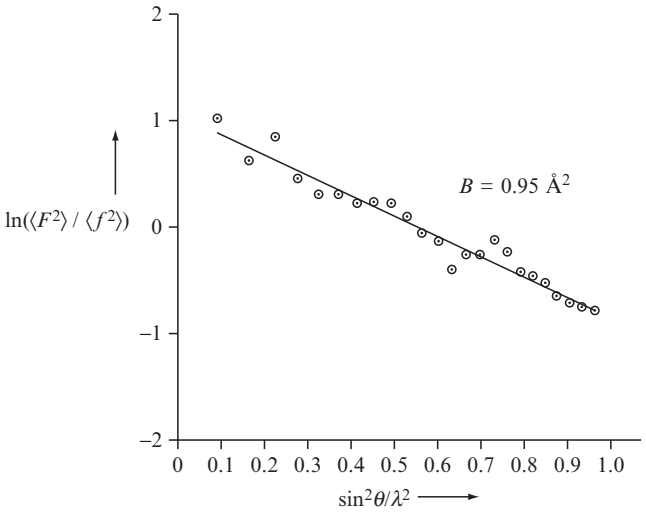

Fig. 4.13 Wilson plot.

A Wilson plot of $\sin^2\theta/\lambda^2$ versus the logarithm of a function of the measured structure factors, $F(hkl) = F$. The slope gives an overall measure of the displacement factors. The intercept gives the scale factor necessary to obtain intensities on an absolute scale.

If the value of $I(hkl)$, corrected for Lp and Abs, is called I_{corr}, we can say

$$I_{\text{corr}} = I(hkl)/\{\text{Lp Abs}\} = \text{K}|F(hkl)|^2 = \text{K}|F_{\text{novib}}|^2 \exp(-2B_{\text{iso}} \sin^2\theta/\lambda^2) \tag{4.4}$$

where $|F_{\text{novib}}|$ is the value of $|F(hkl)|$ for a structure composed of non-vibrating point atoms. The application of the Lp correction involves no knowledge of the structure. An estimation of Abs can be made from a knowledge of the shape, orientation, and composition of the crystal. The value of $|F(hkl)|$ so derived contains information on the atomic displacement factors, B. Thus $F = |F_{\text{novib}}| \exp(-B_{\text{iso}} \sin^2\theta/\lambda^2)$ (see Trueblood et al., 1996). It is possible to derive B_{iso} and K in Eqn. (4.4) from the experimental data by a "Wilson plot" (Wilson, 1942). It is assumed that, to a first approximation, the average intensity of Bragg reflections at a certain value of 2θ depends only on the atoms present in the cell, not on their positions—that is, that the arrangement of atoms in the crystal structure is random. By comparison of the averages of the observed intensities in ranges (shells) of $\sin^2\theta/\lambda^2$ with the theoretical values for a unit cell with the same atomic contents, approximate values for K and B_{iso} can be found from the Wilson plot (Figure 4.13). Values of the resulting scale factor K can then be used for preparation of a full list of values of $|F(hkl)|$ on an approximately absolute scale (relative to the scattering by one electron) for all Bragg reflections measured. The value of B_{iso} obtained from this graph will indicate the extent of disorder from unit cell to unit cell in the crystal structure.

The reader should note that the intensity, $I(hkl)$, is a simple function of the structure amplitude $|F|$. However, an inspection of Eqn. (4.4) shows that *each value of* $|F(hkl)|$, *and hence of the intensity*, $I(hkl)$, *of the diffracted beams contains*, with few exceptions, *a contribution from every atom in the unit cell*. The unraveling of these contributions makes the structure solution complicated.

Summary

The diffraction of a crystal by X rays results from the constructive and destructive interference of the X rays that have been scattered by each individual atom in the structure. Three types of experimental diffraction data may be obtained:

(1) The angle of scattering (2θ, the angular deviation from the direct undeviated beam), which is used to measure the spacings of the reciprocal lattice and hence the spacings of the crystal lattice. These spacings can be used to derive the size and shape of the unit cell.

(2) The orders of diffraction (hkl) of each diffracted beam.

(3) The intensities of the diffracted beams, $I(hkl)$, which may be analyzed to give the positions of the atoms within the unit cell. These atomic positions are usually expressed as fractions of the unit-cell edges.

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