monochromators, a focusing mirror, or multilayer optics. The latter provide substantially enhanced intensity. Rotating anodes remain the source of choice in most structural biology laboratories. An important choice for the user is in the selection of optimal collimator aperture: this should roughly match the crystal sample dimensions. For large crystals, especially if the cell dimensions are also large, it may be preferable to use collimator settings smaller than the crystal in order to resolve the diffraction spots on the detector. The fine-focus tubes currently being developed may affect the choice of home source over the next years (Arndt, Duncumb *et al.*, 1998; Arndt, Long & Duncumb, 1998).

#### 9.1.4.2. *Synchrotron storage rings*

The radiation intensity available from rotating anodes is limited by the heat load per unit area on the target. In the early 1970s, it was realized that synchrotron storage rings produced X-radiation in the necessary spectral range for studies in structural molecular biology (Rosenbaum *et al.*, 1971), and the last three decades have seen great advances in their application to macromolecular crystallography (Helliwell, 1992). Synchrotron radiation (SR) is now used for more than 70% of newly determined protein-crystal structures.

The general advantages of SR are:

(1) High intensity: third-generation sources provide more than 1000 times the intensity of a conventional source.

(2) A highly parallel beam allowing the resolution of closely spaced spots from large unit cells.

(3) Short wavelengths, less than 1 Å, essentially eliminating the problems of correcting for absorption.

(4) Tunability of the wavelength, allowing its optimization for single- or multiple-wavelength applications; this is simply not possible with a conventional source.

(5) The ability to use a white, non-monochromated beam, the so-called Laue technique discussed in Chapter 8.2.

(6) Collection of complete images generated from a single circulating bunch of particles in the ring, only relevant for time-resolved experiments (Chapter 8.2).

SR beamlines take a number of forms. The source may be a bending magnet or an insertion device, such as a wiggler or an undulator. The properties of different beamlines thus vary considerably, and it is vital to choose an appropriate beamline for any particular application. The beamline capabilities are, of course, affected by the detector as well as the source itself. As far as the user is concerned, the primary questions regard the intensity, the size of the focal spot, the wavelength tunability and the detector system.

The present consensus for new synchrotron beamlines for macromolecular crystallography is that they should be on sources with an energy of at least 3 GeV and should receive radiation from tunable undulators. Together, these provide high and tunable intensity over the range required for most crystallographic experiments, including multiwavelength anomalous dispersion (MAD). The impact of free-electron lasers, which are likely to be built within the next decade, is not yet possible to assess.

Present beamlines produce radiation of extremely high quality for macromolecular data collection. At third-generation sources, such as the European Synchrotron Radiation Facility (ESRF) or the Advanced Photon Source (APS), complete data sets can be collected from cryogenically frozen single crystals in minutes.

### 9.1.5. Goniostat geometry

#### 9.1.5.1. *Overview*

The diffraction condition for a particular reflection is fulfilled when the corresponding reciprocal-lattice point lies on the surface of the Ewald sphere. If a stationary crystal is irradiated by the X-ray beam, only a few reflections will lie in the diffracting position. To record intensities of a larger number of reflections, either the size of the Ewald sphere or the crystal orientation has to be changed. The first option, with the use of non-monochromatic, or 'white', radiation, is the basis of the Laue method (Chapter 8.2). If the radiation is monochromatic, with a selected wavelength, the crystal has to be rotated during exposure to bring successive reflections into the diffraction condition.

Several different ways of rotating the crystal have been used in crystallographic practice. These range from rotation about a single axis to use of a three-axis cradle, depending on the detector and application.

#### 9.1.5.2. *Film methods: the precession and Weissenberg methods*

The first data-collection techniques involved photographic methods with visual estimation of the intensities, and the geometry of the original cameras involved simple rotation of the crystal. The basis of the screenless rotation method is discussed in Section 9.1.6 and Chapter 11.1. Two further developments of film methods involved rotation coupled to translation of the film (the Weissenberg technique) or precession photography, with more complex coupling of parallel precession of the crystal and film. Both methods involved isolating the diffraction from single layers of reflections through the use of screens. The intensities from the films were estimated by eye. This was an extremely time-consuming and inaccurate procedure and was only applicable for small cells. The original Weissenberg camera was not extensively used for protein data.

A key feature of the precession camera (Buerger, 1964) was that it provided an undistorted representation of individual layers of the reciprocal lattice, which were easy to index by eye, and it was an excellent tool for teaching prospective crystallographers. A disadvantage was that it required extremely accurate orientation of the crystal on the goniometer. The precession camera became an important tool for many years in most structural biology laboratories for defining the symmetry and lattice dimensions of new crystals and for screening derivatives, but it has largely been superseded by 2D detectors.

Volume C of *International Tables for Crystallography* (1999) presents a full and proper discussion of the precession and Weissenberg geometries.

#### 9.1.5.3. *Single-counter diffractometers*

A great advance in automation came with the development of single- and later three- and five-counter diffractometers. The most common type was the four-circle diffractometer (Arndt & Willis, 1966). Single-scintillation-counter detectors are capable of measuring the intensity of only one individual reflection at a time. Therefore, in this technique, it is necessary to set the counter at the appropriate $2\theta$ angle and to orient the diffracting plane so that the vector normal to it bisects the angle between the source and the detector. This can be achieved by the use of three axes of the Eulerian $\omega$, $\chi$, $\varphi$ cradle or of the $\omega$, $\kappa$, $\varphi$ cradle. Such systems lent themselves readily to automated computer control, with accurate intensities and standard uncertainties output directly to storage devices at the rate of one reflection every one to five minutes. A full discussion of four-circle diffractometers and their associated geometry is given in *IT* C (1999).

Single-counter diffractometers are still widely used for small molecules. They were also applied in the 1960s and 1970s to the first protein structures, albeit at limited resolution. Their use is

greatly limited for macromolecules since only a single reflection can be collected at a time, despite the fact that many simultaneously lie in a diffracting position. The overall exposure time is very large and the radiation damage is likely to be considerable.

Single-counter diffractometers are so rarely used in present-day macromolecular crystallography that they are not discussed further here. Their applications are limited to specialist techniques, such as multibeam methods for direct phase determination.

#### 9.1.5.4. *2D detectors*

The solution for macromolecules has been a return to screenless rotation geometry (Arndt & Wonacott, 1977) with a 2D detector, at first in the form of photographic film with automated scanning optical densitometers to provide a digitized image of the film and to transfer it to disk. While much faster than single-counter methods, this approach still suffered from severe problems, as it was highly labour intensive and the film had a substantial chemical fog background and a rather low dynamic range. It did have one great advantage: excellent spatial resolution. In addition, the physical size of X-ray film was well matched to that of the diffraction pattern to be measured. It is significant that typical film sizes were of the order of $10 \times 10$ cm with up to $2000 \times 2000$ scanned pixels, and a similar effective area is the target of recent developments of imaging plates and charge-coupled devices (CCDs).

The further automation of protein-data collection required efficient 2D detectors (Part 7). The first were multiwire proportional counters, which found widespread use in the early 1980s (Hamlin, 1985). These finally proved to be limited by a combination of spatial resolution and dead time of the read-out. An alternative was the TV detector, but this never achieved high popularity and has largely fallen into disuse. A major step occurred in the late 1980s with the widespread introduction of imaging plates (Amemiya & Miyahara, 1988; Amemiya, 1995), scanned either off-line or, more conveniently, on-line (Dauter *et al.*, 1990) at both synchrotron beamlines and at laboratory rotating-anode sources. This represented a revolution in macromolecular data collection, making it technically straightforward to save full 2D images with sufficient positional resolution and dynamic range to computer disk automatically. The limiting factor of the imaging plate has proved to be the slow read-out time of the order of several seconds to minutes. At high-intensity sources in particular, *e.g.* third-generation SR sites, exposure times per image can fall to one second or less, and with an imaging plate the bulk of the time is spent reading the detector image rather than collecting data. Typical data-collection times with imaging plates remained in the order of several hours, even with the use of SR. This is a much smaller problem with rotating-anode sources, where exposure times dominate the duty cycle.

For high-intensity SR sites, the detector of choice has become the CCD (Gruner & Ealick, 1995). The spatial resolution is comparable with that of imaging plates, but the read-out time can be as low as one to two seconds. This means that complete data can be recorded in minutes rather than hours, and this is already transforming approaches to data collection. Further advances in detector technology are to be expected with the introduction of solid-state pixel systems with yet shorter read-out times and improved spatial properties. Again, these will prove to be most advantageous at high-intensity SR sites.

Almost all current 2D detectors are used in conjunction with a goniostat, providing rotation of the crystal about a single axis during exposure. Indeed, the majority of instruments have only a single rotation axis. The remainder are based on the kappa ($\omega$, $\kappa$, $\varphi$) cradle to select different initial orientations of the sample in the beam; the sample is nevertheless subsequently rotated about a single axis for data collection.

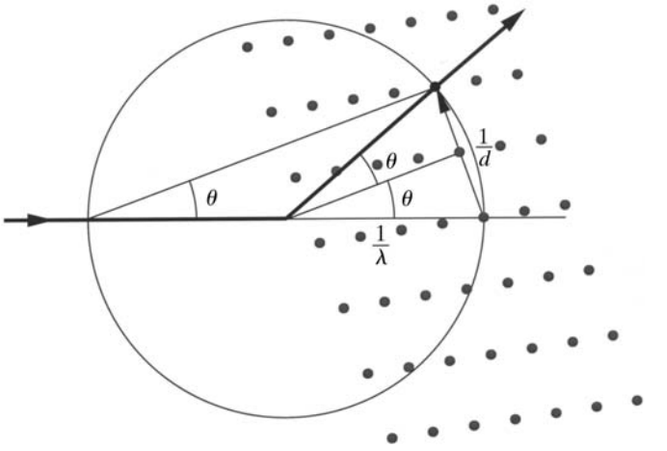


Fig. 9.1.6.1. The Ewald-sphere construction. A reciprocal-lattice point lies on the surface of the sphere, if the following trigonometric condition is fulfilled: $1/2d = (1/\lambda)\sin\theta$. After a simple rearrangement, it takes the form of Bragg's law: $\lambda = 2d\sin\theta$. Therefore, when a reciprocal-lattice point with indices *hkl* lies on the surface of the Ewald sphere, the interference condition for that particular reflection is fulfilled and it gives rise to a diffracted beam directed along the line joining the centre of the sphere to the reciprocal-lattice point on the surface.

### 9.1.6. Basis of the rotation method

#### 9.1.6.1. *Rotation geometry*

The physical process of diffraction from a crystal involves the interference of X-rays scattered from the electron clouds around the atomic centres. The ordered repetition of atomic positions in all unit cells leads to discrete peaks in the diffraction pattern. The geometry of this process can alternatively be described as resulting from the reflection of X-rays from a set of hypothetical planes in the crystal. This is explained by the Ewald construction (Fig. 9.1.6.1), which provides a visualization of Bragg's law. Monochromatic radiation is represented by a sphere of radius $1/\lambda$, and the crystal by a reciprocal lattice. The lattice consists of points lying at the end of vectors normal to reflecting planes, with a length inversely proportional to the interplanar spacing, $1/d$. In the rotation method, the crystal is rotated about a single axis, with the rotation angle defined as $\varphi$. A seminal work giving an excellent background to this field by a number of contributors was edited by Arndt & Wonacott (1977).

#### 9.1.6.2. *Diffraction pattern at a single orientation: the 'still' image*

For a stationary crystal in any particular orientation (a so-called 'still' exposure), only a fraction of the total number of Bragg reflections will satisfy the diffracting condition. The number of reflections will be very limited for a small-molecule crystal, possibly zero in some orientations. Macromolecules have large unit cells, of the order of 100 Å, compared with the wavelength of the radiation, which is about 1.0 Å. In geometric terms, the reciprocal space is densely populated by points in relation to the size of the Ewald sphere. Thus, more reflections diffract simultaneously but at different angles, since many reciprocal-lattice points (reflections) lie simultaneously on the surface of the Ewald sphere in any crystal orientation. This is the great advantage of 2D detectors for large cell dimensions.

The real crystal is a regular and ordered array of unit cells. This means that reciprocal space is made up of a set of points organized in regular planes. For a still exposure, any particular plane of points in the reciprocal lattice intersects the surface of the Ewald sphere in the form of a circle. The corresponding diffracted rays, originating from



references