*9.1.4.1. Conventional sources*

Rotating anodes were initially developed for biological scattering experiments on muscle samples and have the advantage of higher intensity compared with sealed-tube generators. They usually have a copper target providing radiation at a fixed wavelength of 1.542 Å. Alternative targets, such as silver or molybdenum, provide lower intensities at short wavelengths, but have not found general applications to macromolecules. It is also possible to use a chromium target, giving a longer wavelength of 2.29 Å. Historically, rotating anodes were first used with nickel filters to give monochromatic Cu $K\alpha$ radiation. Current systems are equipped with either graphite 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. Considerable progress has been made in the technologies for rotating anodes in recent years, as well as in the development of high-intensity sealed-tube sources for home laboratories (Bloomer & Arndt, 1999).

*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 four decades have seen great advances in their application to macromolecular crystallography (Helliwell, 2004; Hendrickson, 2000). Synchrotron radiation (SR) is now used for the great majority 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.

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 currently under construction at a number of sites, is not yet possible to assess.

Present beamlines produce radiation of extremely high quality for macromolecular data collection. At third-generation sources complete data sets can be collected from cryogenically vitrified 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, *i.e.* single-wavelength, the crystal has to be rotated during exposure to bring successive reflections into the diffraction condition.

*9.1.5.2. The screenless rotation method and 2D detectors*

In the early days of protein crystallography, a number of geometries were used for X-ray cameras, notably the Weissenberg and precession methods. In addition, single-counter diffractometers were used with four-circle goniostats. However, in practice only the screenless rotation geometry (Arndt & Wonacott, 1977) survives today. This requires a 2D detector, which was initially in the form of photographic film. It is of significance that typical film sizes were of the order of 10 × 10 cm with up to 2000 × 2000 scanned pixels; a similar effective area has proved effective for image plates and charge-coupled devices (CCDs).

However, automation of protein-data collection needed 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. A major advance occurred in the late 1980s with the widespread introduction of imaging plates (Amemiya, 1995), scanned on-line both at synchrotron beamlines and on 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 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 of 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 is 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

 

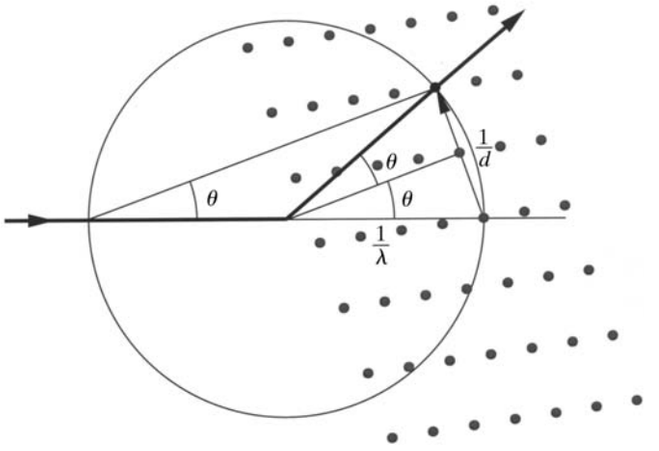


**Figure 9.1.6.1**
The Ewald-sphere construction. A reciprocal-lattice point lies on the surface of the sphere if the following trigonometric condition is fulfilled: $1/2d = (1/\lambda)\sin\theta$. After a simple rearrangement, it takes the form of Bragg's law: $\lambda = 2d\sin\theta$. Therefore, when 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.

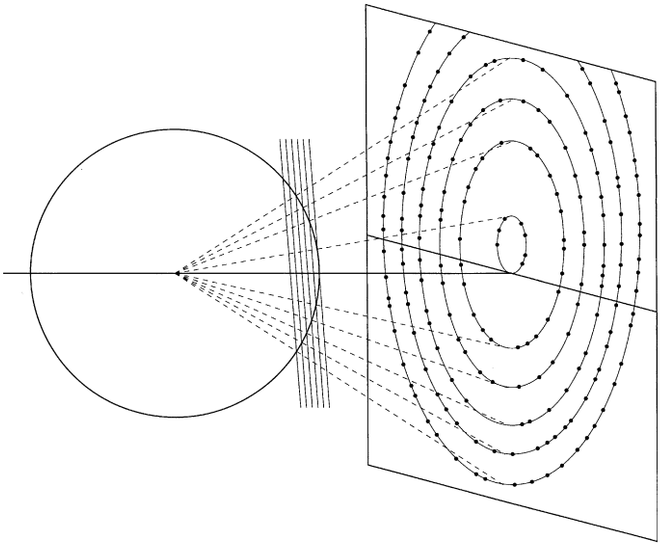

**Figure 9.1.6.2**
The plane of reflections in the reciprocal sphere that is approximately perpendicular to the X-ray beam gives rise to an ellipse of reflections on the detector.

recorded in minutes rather than hours and has transformed approaches to data collection.

While the CCD has revolutionized data-collection times, further advances are expected from the use of solid-state pixel detectors. Such detectors record individual X-ray quanta and have essentially zero read-out time. The most advanced of these, the PILATUS 1M device, is a hybrid pixel array detector (Broennimann *et al.*, 2006; Hülsen *et al.*, 2006), first installed at the Swiss Light Source.

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.

### 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). A more recent review of the method is provided by Dauter (2005).

#### *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 with 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 the centre of the Ewald sphere, form a cone that intersects the sphere on the circle formed by the set of points. In most experiments, the detector is placed perpendicular to the direct beam and the cone of diffracted rays forms an ellipse of spots on its surface (Fig. 9.1.6.2). If a major axis of the crystal lies nearly parallel to the beam, then the ellipses will approximate a set of circles around the centre of the detector. All reflections within each circle will have one index in common, corresponding to the unit-cell axis lying along the beam. For non-centred unit cells, this index will increase by one in successive circles. The gaps between the circles depend on the spacing between the members of the set of reciprocal-lattice planes and are inversely proportional to the real cell dimension related to these planes.

Still exposures were used extensively in the early applications of the rotation method for estimation of crystal alignment. The geometric location of the spots with respect to the origin allows accurate determination of the unit-cell parameters and the crystal orientation. This approach has been superseded in modern