

6. RADIATION SOURCES AND OPTICS

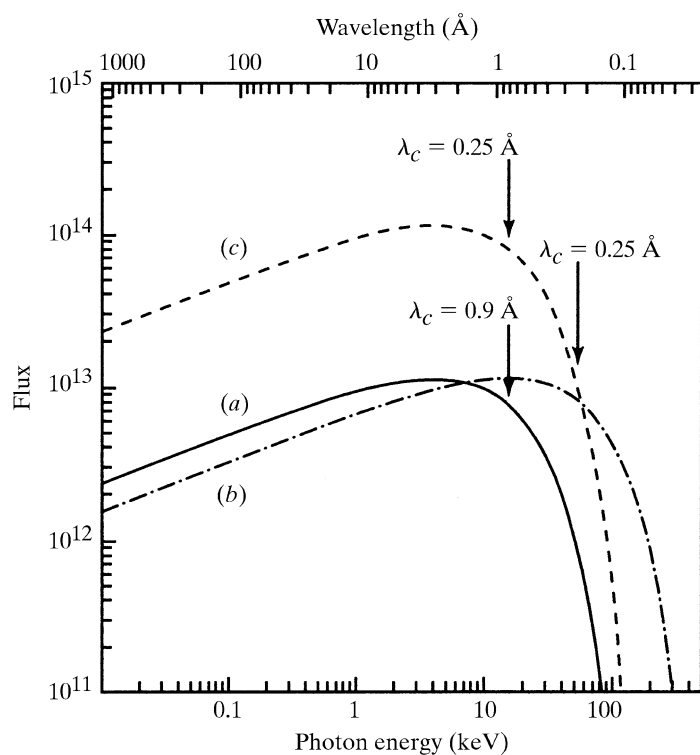


Figure 6.1.2.6 Spectral distribution and critical wavelengths for (a) a dipole magnet, (b) a wavelength shifter and (c) a multipole wiggler at the ESRF. From Buras & Tazzari (1984).

(Hofmann, 1978). Typically, the bandwidth of the lines, $\delta\lambda/\lambda$, is ~ 0.01 to 0.1 , and the photon flux per unit bandwidth from the undulator is many orders of magnitude greater than that from a bending magnet. Undulators at the ESRF have a fundamental wavelength of less than 1 \AA .

The spectra for a bending magnet and a wiggler are compared with that from a copper-target rotating-anode tube in Fig. 6.1.2.3.

6.1.3. Properties of the X-ray beam

We must now consider the properties of the X-ray beam necessary for the gathering of intensity data from single crystals of biological macromolecules. The properties of the beam with which we are concerned are:

- the size of the beam appropriate for the sample dimensions;
- the X-ray wavelength and its spectral purity;
- the intensity in photons s^{-1} ;
- the cross fire, that is, the maximum angle between rays in the beam;
- the temporal structure of the beam, that is, its stability or constancy, and for generators other than X-ray tubes, the duration and frequency of intensity pulses.

These properties cannot be considered in isolation since the requirements depend on the particular crystal under investigation (size, unit-cell dimensions, mosaic spread and resistance to radiation damage), on the geometry of the X-ray camera or diffractometer and on the detector used.

6.1.3.1. Beam size

The best signal-to-noise ratio in the diffraction pattern is secured when the sample crystal is just bathed in the X-ray beam, which is often taken to be about 0.2 to 0.3 mm in diameter.

Unfortunately, many crystals are plate- or needle-shaped and present a greatly varying aspect to the beam. To date, no-one has described data-collection instruments in which the incident-beam dimension is changed automatically as the crystal is rotated; the next best thing is a versatile collimation system that makes use of interchangeable beam-limiting apertures.

6.1.3.2. X-ray wavelength

For X-ray tube sources, the main component of the beam is the characteristic radiation of the tube target. The vast majority of macromolecular structure determinations have been carried out with copper $K\alpha$ X-rays of wavelength 1.54 \AA . These are reasonably well matched to the linear absorption coefficients of biological materials. Diffractometers and cameras are usually designed to permit data collection out to Bragg angles of about 30° , that is, to a minimum spacing of 1.54 \AA , which is a convenient limit.

The next shortest, useful characteristic X-rays are, in practice, those from a molybdenum target (0.71 \AA), but are rarely used in macromolecular crystallography.

The advantages of shorter wavelengths are a reduced absorption correction, smaller angles of incidence on the film, image plate or area detector, and, probably, a slightly smaller amount of radiation damage for a given intensity of the diffraction pattern. The disadvantage is a lower diffracted intensity, which is approximately proportional to the square of the wavelength. Crystal monochromators and specularly reflecting X-ray mirrors have a lower reflectivity for shorter wavelengths; most X-ray detectors, other than image plates and scintillation counters, are less efficient for harder X-rays (see Part 7).

At synchrotron beam lines where there is no shortage of X-ray intensity, it is now customary to select X-ray wavelengths of about 1 \AA for routine data collection. Here, of course, it is possible to choose optimum wavelengths for anomalous-dispersion phasing experiments. This possibility is one of the major advantages of synchrotron radiation. The selection of a narrow wavelength band from the white radiation continuum (*Bremsstrahlung*) of an X-ray tube by means of crystal monochromators is not of practical importance: a tungsten-target X-ray tube operated at 80 kV produces about 1×10^{-5} 8 keV photons per steradian per electron incident on the target within a wavelength band, $\delta\lambda/\lambda$, of 10^{-3} ; a copper-target X-ray tube at 40 kV produces about 5×10^{-4} $K\alpha$ photons per steradian per electron, that is, about 50 times more X-rays.

6.1.3.3. Spectral composition

Any X-rays outside the wavelength band used for generating the desired X-ray pattern contribute to the radiation damage of the sample and to the X-ray background. In the interests of resolving neighbouring diffraction spots in the pattern, one would require the wavelength spread, $\delta\lambda/\lambda$, in the incident radiation to be less than 5×10^{-3} . For the Cu $K\alpha$ doublet $(\lambda_{\alpha_2} - \lambda_{\alpha_1})/\lambda \simeq 2.5 \times 10^{-3}$ and the doublet nature of the line usually does not matter. On the other hand, the value of $(\lambda_{\alpha} - \lambda_{\beta})/\lambda$ is 0.1 , so the $K\beta$ component must be eliminated by means of a β -filter (a 0.15 mm -thick nickel foil for copper radiation) or by reflection from a crystal monochromator to avoid the appearance of separate $K\beta$ diffraction spots. The dispersion produced by a crystal monochromator is small enough to be ignored in most applications.

In synchrotron beam lines, the bandpass is usually determined by the divergence of the beam and is of the order of 10^{-4} . This is a

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smaller bandpass than is required for most purposes, and intensity can be gained by widening the bandpass by the use of an asymmetric-cut monochromator in spatial expansion geometry (Nave *et al.*, 1995; Kohra *et al.*, 1978). The intensity outside the monochromator bandpass is usually totally negligible.

6.1.3.4. Intensity

The intensity of the primary X-ray beam should be such as to allow data collection in a reasonably short time; increased speed is one of the main factors which has led to the popularity of synchrotron-radiation data collection as compared to data collection using conventional sources. Moreover, the radiation damage to the sample per unit incident dose is smaller at high intensities. This does not mean that ever more intense beams are necessary for today's protein-crystallography problems; very often, the speed of data collection is limited by the read-out time of the detector; the counting-rate capabilities of present-day X-ray detectors make it impossible to use in full the intensities available at some beam lines. With the widespread use of cryo-crystallographic methods (Part 10), radiation damage is no longer as severe a problem as it once was.

No doubt, the day will come when available intensities will be so high that instantaneous structure determination will become a possibility, but this will require major advances in X-ray detectors, probably in the form of the development of large pixel detectors (*e.g.* Beuville *et al.*, 1997).

There is still some scope for increasing the intensity of X-ray beams from conventional sources, which offer the advantage of making measurements in the local laboratory instead of at some remote central facility.

6.1.3.5. Cross fire

The cross fire is defined as the angle or the half-angle between extreme rays in the beam incident on a given point of the sample. In the absence of focusing elements, such as specularly reflecting mirrors, the X-ray beam diverges from the source. A diverging beam can be turned into a converging one by reflection from a curved mirror or crystal. A crystal with constant lattice spacing can change the sign, but not the magnitude, of the angle between rays, whatever the curvature of the crystal; the deviation produced by a reflection anywhere on the surface of the crystal must always be twice the Bragg angle. It is possible to change a divergent beam into a convergent one with a different cross fire by specular reflection at a mirror or by means of a crystal in which the lattice spacing varies from point to point along the length of the plate. Such variable-spacing reflectors may be either artificial-crystal multilayers (Schuster & Göbel, 1997) or, less commonly, natural crystals whose spacing is modified by variable doping or by a temperature gradient along the crystal plate (Smither, 1982). In the neutron-scattering community, graded-spacing multilayer monochromators are usually referred to as 'supermirrors' (see Section 6.2.1.3.3 in Chapter 6.2).

The construction of curved mirrors and curved-crystal monochromators is discussed below.

As a general rule, the better the collimation of the incident X-ray beam, that is, the smaller the cross fire and the more nearly parallel the beam, the cleaner the diffraction pattern and the lower the background. Synchrotron-radiation sources permit a certain prodigality in X-ray intensity and the beams from them are thus usually better collimated than beams from conventional sources.

The useful degree of collimation depends on the crystal under investigation. The aim in the design of an instrument for data collection from single crystals must be to make the widths of the angular profiles of the reflection small, but these widths cannot be reduced beyond the rocking-curve width determined by the mosaic spread of the sample, however small the cross fire. The mosaic spread of a typical protein crystal is often quoted as being about 10^{-3} rad or about 3.4 minutes of arc. However, there are many crystals with much larger mosaicities; for such samples, the intensity of the X-ray beam, expressed as the total number of photons which strike the crystal, can be increased by permitting a larger cross fire.

The mosaic spread must be understood as the angle between individual domains of the mosaic crystal. These domains may be as large as 100 μm , that is, they may have dimensions not so very much smaller than those of the macroscopic crystal. The individual rocking-curve widths may be as small as 10 seconds of arc (50 μrad). Fourme *et al.* (1995) have discussed the implications of this degree of perfection if the collimation is improved to a stage where it can be exploited.

The way in which the cross fire influences the angular widths of the reflections depends on the instrument geometry. In a single-counter four-circle diffractometer, all reflections are brought onto the equator and the crystal is rotated about an axis perpendicular to the equatorial plane. The cross fire should, therefore, be small parallel to the equatorial plane, *i.e.* usually in the horizontal plane. The cross fire in the plane containing the rotation axis affects the angular width of the reflections much less, and it could thus be made larger in the interest of a high intensity.

The situation is different if the diffractometer is fitted with an electronic area detector, such as a CCD or other TV detector or a multi-wire proportional chamber (see Part 7). Here, the widths of reflections in upper levels are affected by the cross fire in the plane containing the crystal rotation axis, and the divergence or convergence of the beam in this plane should also be kept small.

With recording on photographic film or image plates, each exposure, or 'shot' or 'frame', corresponds to a crystal rotation that is usually many times larger than the angular width of a reflection. It is then less important to keep the cross fire small in the plane perpendicular to the rotation axis of the crystal.

In many collimation arrangements, the cross fire can be chosen independently in the two planes. In the absence of monochromators or mirrors, the cross fire is determined by beam apertures, which can be rectangular slits; it is, of course, simpler to employ circular holes, which give the same cross fire in both planes.

6.1.3.6. Beam stability

The synchrotron beam decays steadily after each filling of the ring as the number of stored positrons or electrons decays. Even with an X-ray tube operated from voltage- and current-stabilized supplies, the X-ray intensity changes with time as a result of contamination and roughening of the target surface. It is, thus, highly desirable to have a method of monitoring the beam incident on the sample, for example, by means of an ionization chamber built into the collimator (Arndt & Stubbings, 1988).

It should be noted that when the collimator contains focusing elements, the intensity at the sample can vary by several hundred per cent, depending on the exact alignment of the focusing mirrors or crystals and on the exact dimensions of the electron focus on the tube target.

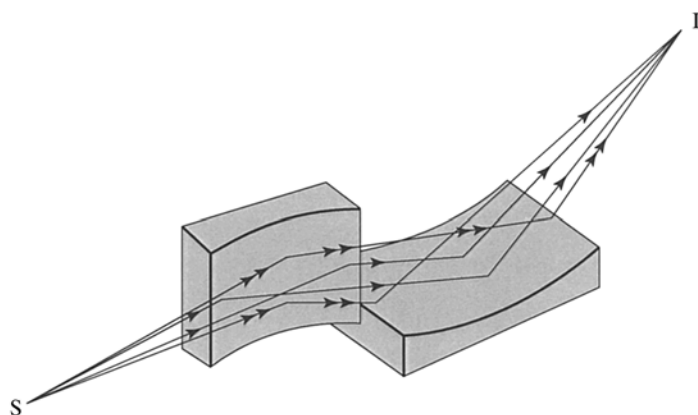


Figure 6.1.4.1

Production of a point focus by successive reflections at two orthogonal curved mirrors. Arrangement due to Kirkpatrick & Baez (1948) and to Franks (1955).

Intensity changes can be caused by mechanical movement of collimating components. Among these may be such unsuspected effects as flexing of the target surface with changes in cooling-water pressure.

The response of an incident-beam monitor may itself vary as a result of changes in temperature, barometric pressure, or humidity.

Synchrotron radiation from storage rings has a regular time-dependent modulation brought about by the rate of passage of bunches of electrons or positrons in the ring. For the great majority of measurements, this time structure has no effect, but at very high intensities, the counting losses are greater than they would be from a steady source.

6.1.4. Beam conditioning

The primary X-ray beam from the source is conditioned by a variety of devices, such as filters, mirrors and monochromators, to produce the appropriate properties for the beam incident on the sample.

6.1.4.1. X-ray mirrors

It is usually necessary to focus the X-ray beam in two orthogonal directions. This can be achieved either by means of one mirror with curvatures in two orthogonal planes or by two successive reflections from two mirrors which are curved in one plane and planar in the other; the two planes of curvature must be at right angles to one another. In the arrangement adopted by Kirkpatrick & Baez (1948) and by Franks (1955), the two mirrors lie one behind the other (Fig. 6.1.4.1) and thus produce a different degree of collimation in the two planes. Instead of this tandem arrangement, the mirrors can lie side-by-side, as proposed by Montel (1957), to form what the author calls a 'catamegonic roof' (Fig. 6.1.4.2). The mirrors are then best made



Figure 6.1.4.2

The 'catamegonic' arrangement of Montel (1957), in which two confocal mirrors with orthogonal curvatures lie side-by-side.

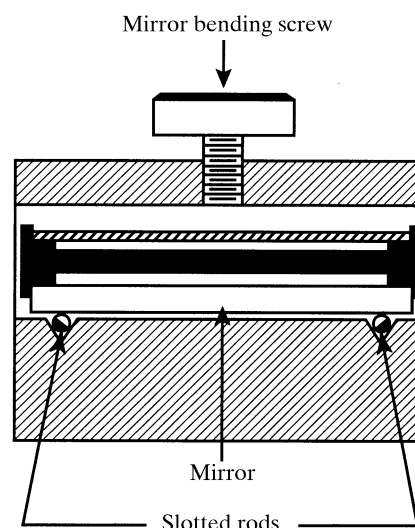


Figure 6.1.4.3

Mirror bender (after Franks, 1955). The force exerted by the screw produces two equal couples which bend the mirror into a circular arc. The slotted rods act as pivots and also as beam-defining slits.

from thicker material, and the reflecting surfaces are ground to the appropriate curvature. The same arrangement has been used by Osmic Inc. (1998) for their Confocal Max-Flux Optics, in which the curved surfaces are coated with graded-spacing multilayers.

Flat mirror plates can be bent elastically to a desired curvature by applying appropriate couples. Fig. 6.1.4.3 shows the bending method adopted by Franks (1955). A cylindrical curvature results from a symmetrical arrangement that produces equal couples at both ends. With appropriate unequal couples applied at the two ends of the plate, the curvature can be made parabolic or elliptical. Precision elliptical mirrors have been produced by Padmore *et al.* (1997); unequal couples are applied in this way. Cylindrically curved mirrors can be produced by applying a force at the tip of a triangular plate whose base is firmly anchored (Fig. 6.1.4.4). Lemonnier *et al.* (1978) first used this method for making curved-crystal monochromators. Milch (1983) described X-ray mirrors made in this way; the effect of the linear increase of the bending moment along the plate is compensated by the linear increase of the plate section so that the curvature is constant. An elliptical or a parabolic curvature results if either the width or the thickness of the plate is made to vary in an appropriate way along the length of the plate. Arndt, Long & Duncumb (1998) described a monolithic mirror-bending block in which the mirror plates are

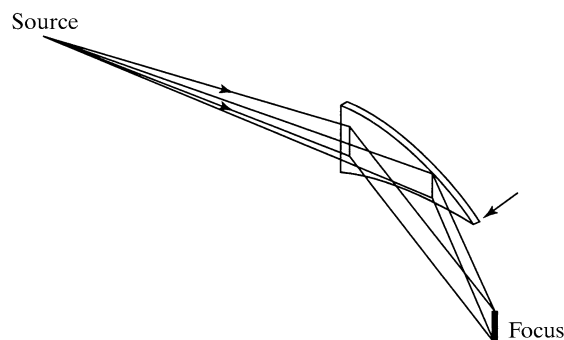


Figure 6.1.4.4

Triangular mirror bender as described by Lemonnier *et al.* (1978) for crystal plates and by Milch (1983) for glass mirrors. The base of the triangular plate is clamped and the bending force is applied along the arrow.