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(*d*) the cross fire, that is, the maximum angle between rays in the beam;

(*e*) 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 Å. 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 Å, which is a convenient limit.

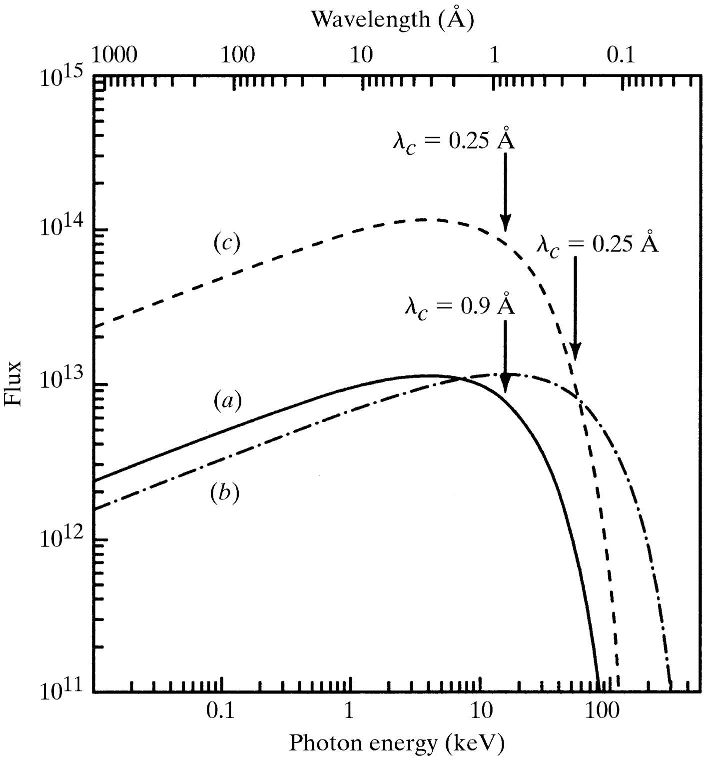

Fig. 6.1.2.6. Spectral distribution and critical wavelengths for (*a*) a dipole magnet, (*b*) a wavelength shifter and (*c*) a multipole wiggler at the ESRF. From Buras & Tazzari (1984).

The next shortest, useful characteristic X-rays are, in practice, those from a molybdenum target (0.71 Å), 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 Å 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 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 cryocrystallographic methods (Part 10), radiation damage is no longer as severe a problem as it once was.