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X-ray Collimation for Protein Crystallography*

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Abstract

Native protein crystals frequently exhibit a very low mosaicity: the mosaic blocks are large and their relative mis-orientation is small. With suitable collimation it is possible to make use of this perfection so as to obtain diffraction profiles with a very narrow width and, accordingly, to improve the spot-to-background ratio and to increase the recorded intensity.

1. Introduction

Evidence has been accumulating for some time that many crystals of proteins are much closer to perfect than is normal with crystals of smaller organic or inorganic molecules. Diffraction studies with conventional X-ray tube sources are usually carried out with a primary X-ray beam which has a considerable cross-fire (typically about 5 mrad), in order to maximize the diffracted intensity from the weakly scattering sample. This cross-fire leads to a wide rocking curve which masks the very low mosaicity of these crystals. At synchrotron radiation beam lines the collimation is usually better, but here data collection is usually carried out by screenless rotation photography (Wilson *et al.* 1983), when a small mosaic spread is neither very obvious nor very useful. Diffractometer measurements with synchrotron radiation have indeed demonstrated very narrow diffraction profiles (J. C. Phillips, personal communication 1977), but have been confined to only a few different crystals. Measurements made with a television diffractometer (Arndt and Thomas 1982) have confirmed that native protein crystals quite commonly have mosaic spreads which cannot be greater than a few tenths of a mrad.

In drawing attention to the relative perfection of many protein crystals we must emphasize that this perfection covers two phenomena which may be present to different extents in different crystals. In the first part of this paper we are concerned with the size of the mosaic blocks which influences the size of the coherently diffracting region of the crystal. In the second part of the paper we are concerned with the relative mis-orientation of these blocks which affects the width of the experimental rocking curve.

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Our main purpose here is to examine how collimation conditions can be optimized to take advantage of both aspects of the crystal perfection to achieve the best diffraction-spot-to-background ratios in the three commonly utilized techniques of single-counter diffractometry, area-detector diffractometry and screenless rotation photography.

Earlier analyses of collimation conditions by one of the present authors (Arndt and Willis 1966; Arndt and Sweet 1977), and by others, apply only to crystals with very small coherently diffracting regions.

2. Diffraction from a Crystal with Zero Mosaicity

Let us consider a crystallite of linear dimension r set exactly at the Bragg angle for a particular set of lattice planes at a distance R from a point source. The peak diffracted intensity will be obtained when the entire crystallite diffracts in phase; this is the condition for Fraunhofer diffraction which requires that the maximum value of r is of the order $(R\lambda)^{\frac{1}{2}}$.

The intensity of the diffracted beam is proportional to r^2/R^2, that is to R^{-1}, while the intensity of the radiation absorbed by the crystal and the incoherent background will both be proportional to R^{-2}, so that the diffracted intensity per unit radiation damage and the reflexion-to-background ratio will both be proportional to R. These conclusions remain valid until r exceeds the size of the 'mosaic blocks'; they are also true for an extended focus, since what matters is the curvature of the wave-front due to a single photon. A stationary crystal can, of course, only utilize a small part of an extended focus but, if the crystal is rotated, our conclusions apply to every part of the rocking profile.

Table 1. Radius of coherently diffracting region of crystal r as a function of source-to-crystal distance R

f_{max} and W are X-ray tube focus and total power for a constant cross-fire of 2 mrad and for a constant diffracted intensity

R (mm)	r (μm)	f_{max} (mm)	W (kW)
200	5·5	0·1	1
1000	12	1·7	4
4000	23	7·7	20

Table 1 shows the values of r as a function of R for 1·5 Å radiation and the X-ray tube power W required to give equal diffraction spot intensities at the different distances. If it is desired to measure reflexions from a 100 Å axis crystal out to 2 Å, the maximum width of the spot profile for which high-angle reflexions are completely resolved is about 2 mrad: Table 1 also gives f_{max}, the maximum permissible size of the foreshortened X-ray tube focus to achieve this width with a 0·3 mm specimen crystal. Inspection of the f_{max} and W values shows that existing commercially available rotating-anode tubes would permit the use of unorthodox collimation distances with a gain in intensity and with an improvement in the spot-to-background ratio of 20 : 1 as compared with the normal conditions represented by the data for $R = 200$ mm.

Haubold (1975) has described the use of the Jülich 100 kW X-ray generator with a collimation distance of 5000 mm and has analysed the reduction in counting time obtained with this arrangement in diffuse X-ray scattering investigations on single crystals.

3. Nonzero Mosaicity

The full advantages of diffraction experiments carried out with plane rather than curved wave-fronts accrue only with macroscopic mosaic blocks. It remains true, however, even for smaller blocks, that the signal-to-background ratio can be improved by reducing the cross-fire in the primary X-ray beam until the angular width of the diffraction profile is determined essentially by the mosaic spread of the specimen.

A distinction must be drawn between equatorial and non-equatorial moving-crystal measuring techniques. On single-detector four-circle diffractometers all measurements are made in the equatorial plane, that is, for any given reflexion the crystal is rotated about an axis (the ω-axis), which is normal to the scattering vector. Consequently, the width of the diffraction profile is influenced almost entirely by the cross-fire of the incident beam in the equatorial plane to the almost complete exclusion of the effect of cross-fire in the plane orthogonal to the former. The ideal form of collimation for such a diffractometer is, therefore, one in which the incident beam is fan-shaped. The rays should be parallel in the equatorial plane; in the orthogonal plane the cross-fire should be approximately equal to the mosaic spread so as to give all mosaic blocks the opportunity of diffracting simultaneously. From the point of view of maximizing the diffracted intensity, it is only the magnitude of the cross-fire which matters and not whether the primary beam is diverging, as with pin-hole collimation, or converging, as with some focusing mirrors. However, the signal-to-background ratio is improved by making the size of the beam a minimum at the detector by using a mirror focused on the detector and by slit-limiting the detector aperture accordingly.

With area-detector diffractometers the rays of the incident beam should be parallel in both planes. Reflexions are now measured mostly out of the equatorial plane and the widths of their ω profiles are affected by the cross-fire in both planes.

In screenless rotation photography the situation is quite different. Any given photograph corresponds to a crystal rotation which is many times larger than the angular width of any one reflexion, so that there is little opportunity of optimizing the signal-to-background ratio by reducing the angular widths. X-ray film has the highest detective quantum efficiency for small diffraction spot images (Arndt 1968). The primary X-ray beam should, therefore, be focused on the film plane in both directions.

4. Focusing Monochromators

Curved-crystal monochromators can convert a beam of a given angular divergence into one of the same convergence; they cannot produce a parallel monochromatic beam from a divergent one since the deviations of all rays after reflexion from any point of the crystal must be twice the Bragg angle. For single-crystal experiments the required convergences are a few mrad; these monochromators must, therefore, be placed at large distances from the source. For asymmetrically cut crystals the ratio of source-to-monochromator to monochromator-to-focus distance can be made as great as 10 : 1, leading to a corresponding de-magnification of the image of the tube focus in the detector plane. This arrangement has been employed by Witz

(1969) who obtained virus crystal oscillation photographs using two curved quartz monochromator crystals at right angles, but this arrangement was very difficult to adjust. A better arrangement today might be to use a germanium monochromator for focusing in the horizontal plane in conjunction with a grazing-incidence mirror for focusing in the vertical plane, as is commonly done at synchrotron beam lines (see e.g. Holmes and Rosenbaum 1980). However, in general, curved crystals focus less precisely than do specular-reflexion mirrors.

5. Focusing Mirrors

X rays can be focused by reflexion at curved grazing-incidence mirrors. This is a case of a true specular, total external reflexion, and so the angular aperture of the reflected beam can be different from that of the incident beam: elliptical mirrors can produce line foci and parabolic mirrors a parallel beam. Following the method of Franks (1955) it has been the normal practice to use two mirrors with orthogonal curvatures in tandem and to approximate the surface with a cylindrical one. Optical flats are bent elastically by the application of appropriate couples. The method has been discussed by Harrison (1968), Witz (1969), Holmes and Rosenbaum (1980) and in an unpublished but often quoted paper by W. Phillips and I. Rayment.*

Cylindrical surfaces are obtained either by applying symmetrical couples at the two ends of a rectangular plate or by clamping the base of a triangular plate and applying a force to the apex of the triangle (Lemonnier *et al.* 1978; Milch 1983). Other curvatures, such as elliptical or parabolic surfaces, are produced in the first method by making the couples different at the two ends of the plate (Mokveld and Van Heyningen 1984), and in the second method by appropriately modifying the taper, or the width, of the tapering plate along its length (Milch 1983).

For most purposes the cylindrical approximation is adequate (Milch 1983). Thus, the deviation from parallelism in the beam reflected from a cylindrical mirror 60 mm long is considerably smaller than the cross-fire due to the finite size of the X-ray tube focus. However, the slight extra complication of the design of a mirror bender with asymmetrical couples is well justified when the mosaic blocks of the specimen are large and the wave-front due to a single photon must be as planar as possible.

The critical angle for X rays varies approximately with the square root of the density of the reflector and always has a magnitude of a few mrad. The mirrors are set to make the glancing angle of incidence at the upstream end equal to this critical angle. The length of the mirror then determines both the width of the reflected parallel beam and the angle which the mirror subtends at the source. It has been customary to choose the shortest mirror lengths which will give a beamwidth equal to the size of the specimen. (With Cu Kα radiation, 60 mm nickel-coated mirrors give a beamwidth of about 0·35 mm.) However, it is possible to get a several-fold gain in intensity by employing longer mirrors and reducing the width of the resultant beam by a plane concentrating monochromator (Fankuchen 1937), in which the external plane is ground at an angle to the reflecting planes. In principle, it is possible to use this arrangement in both planes, but in practice it is inconvenient for the final beam to emerge at an inclination to the horizontal plane. The arrangement adopted

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in the authors' laboratory embodies a first horizontal mirror 60 mm long followed by a 200 mm vertical mirror and a final silicon monochromator, to produce a parallel beam approximately 0·3 mm square in cross section.

Both collimating and point-focusing mirror systems require the use of X-ray sources with effective dimensions of about 0·1 mm. The limited power which can be dissipated in the X-ray tube target under these conditions is compensated by the relatively large solid angle which the mirrors subtend at the source.

6. Conclusions

There is considerable scope for the improvement of conventional collimation systems, especially for structure determination of crystals with low mosaicity. It should be possible, with conventional X-ray tubes, to equal, or even to exceed, the quality of the diffraction patterns currently obtained at synchrotron beam lines.

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