

Considerations in the Choice of a Wavelength Range for White-Beam Laue Diffraction

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Abstract

The white-beam Laue-diffraction method is a useful tool for rapid measurement of crystallographic intensities with synchrotron radiation. Considerations of the signal-to-noise ratio to be expected from scattering of X-rays within a limited wavelength range suggest that it will pay to limit that range to something like an octave. This rule-of-thumb has the added advantage that there will be significantly fewer diffraction spots that are overlapping harmonics of one another. To maximize the number of reflections recorded in a single stationary-crystal exposure, one should choose this octave of wavelengths in a region where the curvature of the Ewald sphere is greatest, that is at the longest wavelength allowable after other considerations are taken into account.

Introduction

The white-beam Laue method, making use of synchrotron radiation, is a powerful tool in the rapid measurement of diffraction data. It has especially proven its usefulness in dynamic studies of changing states in crystals of biological macromolecules [see, for example, Hajdu & Johnson (1990), Helliwell *et al.* (1989) and Moffat (1989)]. The method makes use of a finite but possibly broad spectrum of X-rays in the wavelength region that is useful for crystallography. The wide selection of available wavelengths excites multiple reflections in a stationary crystal. This can be described either in direct crystal space or in reciprocal space. In the first case, one can say that for a wide range of incident wavelengths, many Bragg planes in a stationary crystal are oriented to satisfy Bragg's law so X-rays will be reflected. In reciprocal space, one describes the limits of the wavelength distribution as two limiting Ewald spheres, and the diffraction limit of the crystal d_{\min} as a sphere centered at the reciprocal lattice origin. The volume enclosed within the limiting sphere and between the two Ewald spheres contains the reciprocal lattice points that will represent diffraction maxima for that

wavelength range and that crystal setting. This situation is shown schematically in Fig. 1.

In general one will want to apply the Laue method to a particular crystal or class of crystals, and therefore the diffraction limit, or diameter of the $1/d_{\min}$ sphere, will be determined by the specimen. Also, one may be limited to a particular X-ray source, therefore limiting the spectrum of the usable X-rays. However, it is worth some thought to see how a spectrum could be tailored to produce the best possible X-ray source for a Laue-diffraction experiment.

Just how important these considerations can be was revealed to us in our own recent work. We have reported (Singer, Carty *et al.*, 1992; Singer, Smalås, Carty, Mangel & Sweet, 1992) both static and dynamic crystal structure analyses of the enzyme bovine pancreatic trypsin. Many diffraction data were recorded on X-ray film, and the individual intensities were integrated by methods that have become conventional. A part of this data-reduction process

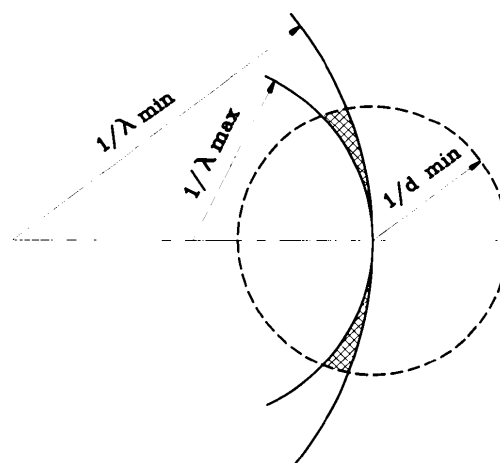


Fig. 1. Schematic representation of the volume of the reciprocal lattice that might be measured for a given minimum d spacing and for a range of wavelengths. The cross-hatched area represents a cross-section of the volume in question. The proportions in this diagram are correct for $d_{\min} = 2.0$, $\lambda_{\min} = 0.75$ and $\lambda_{\max} = 1.50$ Å.

is to predict which reflections will lie within the volume described in Fig. 1. At the end we can compare these predictions with the data that are measured successfully. We were dismayed to discover that the ratio of observed to predicted data was so low, and interested to see that the ratio was lowest for the data recorded at the shortest wavelengths. In Fig. 2 we reproduce a plot of a typical X-ray spectrum from a focused beamline at the National Synchrotron Light Source at Brookhaven National Laboratory, and curves that show the numbers of reflections that were predicted to occur and actually were measured within reasonable limits of error, as a function of wavelength.

We will analyze these results firstly in terms of choosing the magnitude of the optimal range of wavelengths and secondly in choosing the precise range.

Results

In general, elastic scattering of X-rays varies in intensity roughly* as λ^2 . In Fig. 3 we show a diagram displaying

* James (1967) gives the total energy scattered from a crystal (for polarized incident radiation with scattering in the plane perpendicular to the polarization) as

$$Q = \frac{N^2 \lambda^3}{\sin 2\theta_0} |F|^2 \left(\frac{e^2}{mc^2} \right)^2.$$

Arndt (1984) points out that for small diffraction angles $\sin 2\theta_0 = 2\sin\theta_0 \cos\theta_0 \simeq \lambda/d$. This approximation is appropriate when one considers alternative measurement of particular sets of reflections that would be related to particular d spacings. Therefore, Q will vary approximately as λ^2 .

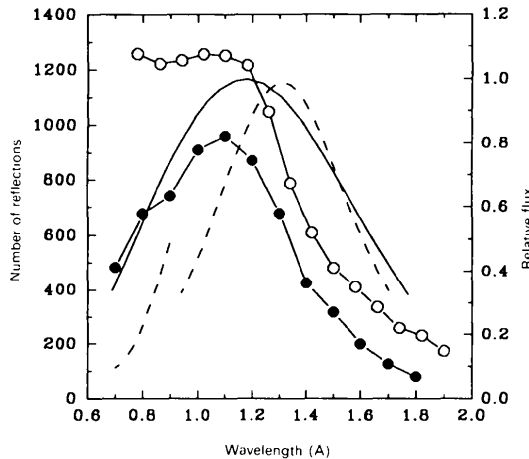


Fig. 2. Wavelength-dependent quantities for a Laue experiment at wiggler beamline X25 of the National Synchrotron Light Source. Solid line: the spectrum at the specimen from a polychromatic focused beam at beamline X25 in relative photons $s^{-1} mm^{-2}$ (Berman, 1991; Berman, Hastings, Oversluizen & Woodle, 1992). Dashed line: the action spectrum, or wavelength-normalization curve, determined by the data-reduction program *LAUENORM* (Helliwell *et al.*, 1989) for integrated Laue data from this beamline. Open circles: numbers of observable reflections calculated to appear on a single Laue film. No spatial overlaps nor harmonic overlaps have been included in this count. Closed circles: numbers of reflections observed ($I > 2\sigma_I$) for a pack of five films.

weak reflections, with a signal-to-background ratio (S/B) of approximately 1.0, lying on top of the accompanying elastically scattered background. In the diagram, the scattered intensity increases as λ^2 , as if the incident X-ray spectrum were flat. One can see immediately that the weak short-wavelength reflections will suffer badly if they must be measured on top of the long-wavelength background. To get a feel for the nature of this background, we could write an analytical expression for it, given that one has a flat incident X-ray spectrum. We define a wavelength range $\lambda_{\min} - \lambda_{\max}$, and, following Cruickshank, Helliwell & Moffat (1987), will describe the magnitude of the range by M , where $\lambda_{\max} = M\lambda_{\min}$. Then we integrate λ^2 over this range to calculate the total background B ,

$$B = b \int_{\lambda_{\min}}^{\lambda_{\max}} \lambda^2 d\lambda = \frac{b}{3} (M^3 \lambda_{\min}^3 - \lambda_{\min}^3) = \frac{b \lambda_{\min}^3}{3} (M^3 - 1).$$

One can see that the total background rises quickly with increasing wavelength range, so one will always have to suffer reduction in S/B over monochromatic data collection.

In addition one can compare the S/B ratio for equivalent reflections measured at the wavelength extremes. Since the diffracted intensity is proportional to λ^2 , we can evaluate S/B for both cases,

$$\frac{I_{\lambda_{\min}}}{B_{\text{total}}} = a \frac{\lambda_{\min}^2}{\lambda_{\min}^3 (M^3 - 1)} = \frac{a}{\lambda_{\min} (M^3 - 1)}$$

$$\frac{I_{\lambda_{\max}}}{B_{\text{total}}} = a \frac{M^2 \lambda_{\min}^2}{\lambda_{\min}^3 (M^3 - 1)} = \frac{a M^2}{\lambda_{\min} (M^3 - 1)}.$$

The S/B advantage of the λ_{\max} data over the λ_{\min} data is the ratio of these two, or M^2 .

In a real experiment, there are several other considerations to an understanding of the anticipated S/B ratio. In

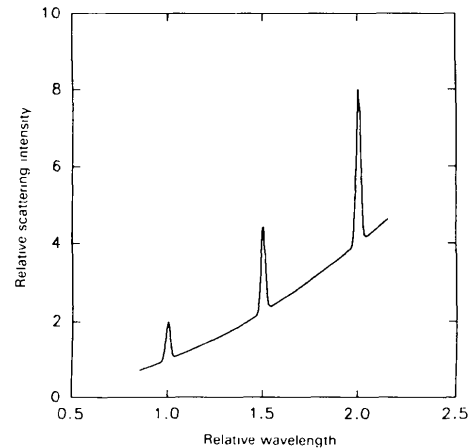


Fig. 3. Schematic representation of weak reflections, that is with a signal-to-background ratio of approximately 1, displayed on top of the background that would be expected as a function of wavelength.

the first case, the spectrum for a real X-ray source, shown in Fig. 2, is not flat. On the contrary, it falls off sharply both at short and long wavelengths. One could multiply this curve by the one in Fig. 3 to obtain a more realistic display of the scattering power of both reflections and background. The situation can be even worse than this for some X-ray detectors used in Laue crystallography. Photographic film absorbs, and thus detects, only a fraction of the photons in a normal Laue pattern, such as one that might be produced by the spectrum in Fig. 2. The film absorption efficiency is modest for the longer wavelengths, approximately 0.65 at 1.5 Å. Then the absorption coefficient decreases rapidly, roughly according to λ^3 . The fraction of radiation absorbed at 0.75 Å is approximately 0.13. Consequently, when X-ray film is used as a detector, the action spectrum would be something like the measured spectrum of the source multiplied by λ to a power higher than two. Fortunately, modern X-ray detection films, such as X-ray storage phosphors, absorb nearly all of the radiation in the range useful for crystallography, so this effect can be ignored when these detectors are employed.

A peripheral issue, discussed in detail in the references cited above, is the number of reflections that are superimposed on one another as wavelength harmonics of one another. This problem is minimized when the wavelength range is as small as practicable. Cruickshank, Helliwell & Moffat (1987) show especially that the number of reflections (spots on the detector) that are two reciprocal lattice points superimposed rises abruptly as M becomes greater than 2. Therefore, we recommend that a limited range of wavelengths, probably on the order of a factor of two, or an octave, should be chosen for Laue experiments where one wants to produce integrated intensities.

A further issue is choice of the actual wavelength range to use. One can calculate the volume of the concave lens-shaped volume of reciprocal space carved out by the three spheres in Fig. 1, which is given by

$$V_R = \left(\frac{\pi}{4}\right) d^{*4} (\lambda_{\max} - \lambda_{\min}) = \left(\frac{\pi}{4}\right) d^{*4} \lambda_{\min} (M - 1)$$

for the case of d_{\min} substantially greater than $\lambda_{\min}/2$ or $\lambda_{\max}/2$ (Moffat, Schildkamp, Bilderback & Volz, 1986). Therefore, for a given ratio of wavelengths, M , the accessible volume is precisely linear with λ_{\min} . The volume will be largest for the smallest radii of Ewald spheres; longer wavelength will produce a larger number of reflections. In this sense, then, longer wavelengths are good for Laue crystallography.

Absorption of X-rays and crystal damage always plague biomolecular crystallographers. As Arndt (1984) sug-

gested, much of the usefulness of synchrotron radiation for this work derives from the availability of shorter wavelengths. He pointed out that specimen damage resulting from X-ray absorption, which varies as λ^3 , is lessened at shorter wavelengths. This somewhat counterbalances the λ^2 -dependent decrease in scattering. However, in the Laue experiment, especially if exposure times are short and there is a possibility of cooling a specimen to prevent radiation damage, the longer-wavelength choice may be better. On the other hand, systematic errors in the data owing to absorption become increasingly severe at long wavelengths. An operational upper limit for wavelength before these errors become unmanageable is probably in the range 1.5–1.6 Å.

Finally, all these considerations suggest that an optimal choice of wavelength range for Laue crystallography is in the range 0.75–1.5 Å. To the extent that one can tailor the spectrum of the source, one would like sharp cut-offs at each end, and a shape to the spectrum that approximates λ^{-2} , counterbalancing the fall-off in scattering at short wavelengths.

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