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8.2. Laue crystallography: time-resolved studies

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8.2.1. Introduction

The term 'Laue diffraction' describes the process of X-ray scattering that occurs when a stationary crystal is illuminated by a polychromatic beam of X-rays. The process therefore differs from the now more conventional diffraction techniques in which a moving crystal is illuminated by a monochromatic beam of X-rays. Although Laue diffraction was widely used for structure analysis in the early days of crystallography by Pauling, Bragg, Wyckoff and others, by the 1930s it was superseded by arguably simpler and more readily quantifiable monochromatic techniques. With the advent of naturally polychromatic synchrotron sources in the 1970s, it was natural to (re-)examine the suitability of the Laue technique. The first synchrotron-based Laue experiments to be published appear to have been those of Wood *et al.* (1983) for a small inorganic crystal and of Moffat *et al.* (1984) for a macromolecular crystal; see also Helliwell (1984, 1985). These experimenters realized that the Laue technique afforded exposure times that were short even with respect to those obtainable with similar crystals at the same synchrotron source using conventional monochromatic techniques, and much shorter than those obtainable with laboratory X-ray sources. This advantage, along with the use of a stationary crystal, the large number of Laue spots evident in a single image and the clear distinction of the Laue spots from the underlying X-ray background, suggested that the Laue technique might be particularly applicable to time-resolved crystallography. In this form of crystallography, the total X-ray scattering from the crystal (both the Bragg scattering and the diffuse, non-Bragg scattering) varies with time as the position and/or extent of order of the atoms in the crystal changes in response to some structural perturbation.

It is one thing to propose that a venerable technique may be applicable to a new class of experiments; it is quite another to identify and overcome the complexities and disadvantages of that technique, and to demonstrate how experiments should be conducted and raw data accurately reduced to structure amplitudes. It took roughly 15 years and the efforts of many investigators before it could be stated that Laue crystallography is coming of age (Ren *et al.*, 1999).

The redevelopment of Laue diffraction has depended on three main advances: the use of very intense polychromatic synchrotron sources; the realization that the so-called energy-overlap or overlapping-orders problem in Laue diffraction was theoretically tractable, of limited extent and could be overcome experimentally; and the development of appropriate algorithms and suitable software to address the energy-overlap, spatial-overlap and wavelength-normalization problems. All are discussed below.

Since both Laue crystallography and its applications to time-resolved studies have recently been described at length, this article emphasizes only the key points and directs the reader to the primary and review literature for the details.

8.2.2. Principles of Laue diffraction

The principles of Laue diffraction have been reviewed by Amorós *et al.* (1975), Cruickshank *et al.* (1987, 1991), Helliwell *et al.* (1989), Cassetta *et al.* (1993), Moffat (1997), and Ren *et al.* (1999).

Assume that a stationary, perfect single crystal that diffracts to a resolution limit of $d^*_{\rm max}$ is illuminated by a polychromatic X-ray beam spanning the wavelength (energy) range from $\lambda_{\rm min}$ $(E_{\rm max})$ to $\lambda_{\rm max}$ $(E_{\rm min})$. All reciprocal-lattice points that lie between the Ewald spheres of radii $1/\lambda_{\rm min}$ and $1/\lambda_{\rm max}$, and within a radius $d^*_{\rm max}$ of the origin O where $d^*_{\rm max} = 1/d_{\rm min}$, the resolution limit of the crystal, are in a diffracting position for a particular wavelength λ, where $\lambda_{\rm min} \leq \lambda \leq \lambda_{\rm max}$ and will contribute to a spot on the Laue diffraction pattern (Fig. 8.2.2.1). All such points diffract simultaneously and throughout the exposure, in contrast to a monochromatic diffraction pattern in which each point diffracts sequentially and briefly as it traverses the Ewald sphere. A Laue pattern may alternatively be thought of as the superposition of a series of monochromatic still patterns, each arising from a different wavelength in the range from $\lambda_{\rm min}$ to $\lambda_{\rm max}$.

Each Laue spot arises from the mapping of a complete ray (a central line in reciprocal space, emanating from the origin) onto a point on the detector. In contrast, each spot in a monochromatic pattern arises from the mapping of a single reciprocal-lattice point onto a point on the detector. A ray may contain only a single reciprocal-lattice point hkl with spacing d^*, in which case the corresponding Laue spot arises from a single wavelength (energy) and structure amplitude, or it may contain several reciprocal-lattice points, such as $hkl, 2h2k2l \ldots nhnknl \ldots$, in which case the Laue spot contains several wavelengths (energies) and structure amplitudes. In the former case, the Laue spot is said to be single, and in the latter, multiple. The existence of multiple Laue spots is known as the energy-overlap problem: one spot contains contributions from several energies. It seems to have been thought by Pauling, Bragg and others that, as the wavelength range and the resolution limit $d^*_{\rm max}$ of the crystal increased, more and more Laue spots would be multiple and the energy-overlap problem would dominate. Cruickshank *et al.* (1987) showed that this was not so. Even in the extreme case of infinite wavelength range, no more than 12.5% of all Laue spots would be multiple. The energy-overlap problem is evidently of restricted extent. However, the magnitude

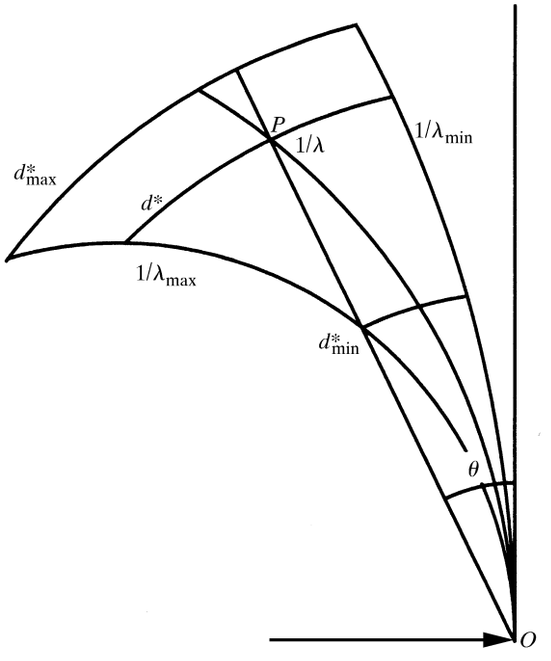

Fig. 8.2.2.1. Laue diffraction geometry. The volume element dV stimulated in a Laue experiment lies between d^* and $d^* + {\rm d}d^*$, between the Ewald spheres corresponding to λ and $\lambda + {\rm d}\lambda$, and between φ and $\varphi + {\rm d}\varphi$, where φ denotes rotation about the incident X-ray beam direction. The entire volume stimulated in a single Laue exposure lies between 0 and $d^*_{\rm max}$, between the Ewald spheres corresponding to $\lambda_{\rm min}$ and $\lambda_{\rm max}$, and between values of θ ranging from 0 to 2π.

of the energy-overlap problem varies with resolution: reciprocal-lattice points at low resolution are more likely to be associated with multiple Laue spots than to be single (Cruickshank *et al.*, 1987).

The extraction of X-ray structure amplitudes from a single Laue spot requires the derivation and application of a wavelength-dependent correction factor known as the wavelength normalization curve or λ-curve. This curve and other known factors relate the experimentally measured raw intensities of each Laue spot to the square of the corresponding structure amplitude. The integrated intensity of a Laue spot is achieved automatically by integration over wavelength, rather than in a monochromatic spot by integration over angle as the crystal rotates. If, however, a Laue spot is multiple, its total intensity arises from the sum of the integrated intensities of each of its components, known also as harmonics or orders *nhnknl* of the inner point *hkl* where *h*, *k* and *l* are co-prime.

Laue spots lie on conic sections, each corresponding to a central zone [*uvw*] in reciprocal space. Prominent spots known as nodal spots or nodals lie at the intersection of well populated zones and correspond to rays whose inner point *hkl* is of low co-prime indices. All nodal spots are multiple and all are surrounded by clear areas devoid of spots.

The volume of reciprocal space stimulated in a Laue exposure, V_v, is given by

$$V_v = 0.24\ d^{*4}_{\max}(\lambda_{\max} - \lambda_{\min}),$$

and contains N_v reciprocal-lattice points where $N_v = V_v/V^*$ and V^* is the volume of the reciprocal unit cell (Moffat, 1997). N_v can be large, particularly for crystals that diffract to high resolution and thus have larger values of $d^*_{\max}$. Laue patterns may therefore contain numerous closely spaced spots and exhibit a spatial-overlap problem (Cruickshank *et al.*, 1991). The value of N_v is up to an order of magnitude greater than the typical number of spots on a monochromatic oscillation pattern from the same crystal. Since the overall goal of a diffraction experiment is to record all spots in the unique volume of reciprocal space with suitable accuracy and redundancy, a Laue data set may contain fewer images and more spots of higher redundancy than a monochromatic data set (Clifton *et al.*, 1991). This is particularly evident if the crystal is of high symmetry.

Kalman (1979) provided derivations of the integrated intensity of a single spot in the Laue case and in the monochromatic case. Moffat (1997) used these to show that the duration of a typical Laue exposure was between three and four orders of magnitude less than the corresponding monochromatic exposure. The physical reason for this significant Laue advantage lies in the fact that all Laue spots are in a diffracting position and contribute to the integrated intensity throughout the exposure. In contrast, monochromatic spots diffract only briefly as each sweeps through the narrow Ewald sphere [more strictly, through the volume between the closely spaced Ewald spheres corresponding to $1/\lambda$ and $1/(\lambda + d\lambda)$]. The details are modified slightly for mosaic crystals of finite dimensions subjected to an X-ray beam of finite cross section and angular crossfire (Ren *et al.*, 1999; Z. Ren, unpublished results).

Exposure times are governed not merely by the requirement to generate sufficient diffracted intensity in a spot – the signal – but also to minimize the background under the spot – the noise. The background under a Laue spot tends to be higher than under a monochromatic spot, since it arises from a larger volume of reciprocal space in the Laue case. This volume extends from $d^*_{\min}$ (where $d^*_{\min} = 2\sin\theta/\lambda_{\max}$ and θ is the Bragg angle for that Laue spot) through the Laue spot at d^* to either $d^*_{\max}$ or $2\sin\theta/\lambda_{\min}$, whichever is the smaller (Moffat *et al.*, 1989). Since both the signal and the noise in a Laue pattern are directly proportional to the exposure time, their ratio is independent of that parameter. The ratio does depend on the wavelength range $(\lambda_{\max} - \lambda_{\min})$. Decreasing the wavelength range both generates fewer spots and increases the signal-to-noise ratio for each remaining spot by diminishing the background under it. This is analogous to decreasing the oscillation range in a monochromatic exposure.

The choice of appropriate exposure time in the Laue case is complicated, but the central fact remains: both in theory and in practice, Laue exposures are very short with respect to monochromatic exposures (Moffat *et al.*, 1984; Helliwell, 1985; Moffat, 1997). Satisfactory Laue diffraction patterns have been routinely obtained with X-ray exposures of 100 to 150 ps, corresponding to the duration of a single X-ray pulse emitted by a single 15 mA bunch of electrons circulating in the European Synchrotron Radiation Facility (ESRF) (Bourgeois *et al.*, 1996).

The advantages and disadvantages of the Laue technique, compared to the better-established and more familiar monochromatic techniques, are presented in Table 8.2.2.1.

8.2.3. Practical considerations in the Laue technique

The experimental aspects of a Laue experiment – the source and optics, the shutters and other beamline components, detectors, analysis software, and the successful design of the Laue experiments themselves – have been presented by Helliwell *et al.* (1989), Ren & Moffat (1994, 1995*a*,*b*), Bourgeois *et al.* (1996), Ren *et al.* (1996), Clifton *et al.* (1997), Moffat (1997), Yang *et al.* (1998), and Ren *et al.* (1999). Certain key parameters are under the experimenter's control, such as the nature of the source (bending magnet, wiggler or undulator), the wavelength range incident on the crystal (as modified by components of the beamline such as a mirror and attenuators), the choice of detector (active area, number of pixels and the size of each, dependence of detector parameters on wavelength, inherent background, and the accuracy and speed of readout), the experimental data-collection strategy (exposure time or times, number of angular settings of the crystal to be employed and the angular interval between them) and the data-reduction strategy (properties of the algorithms employed and of the software analysis package). A successful Laue experiment demands consideration of these parameters jointly and in advance, as described in these references. The goal is accurate structure amplitudes, not just speedily obtained, beautiful diffraction images.

For example, an undulator source yields a spectrum in which the incident intensity varies sharply with wavelength. Such a source should only be employed if the software can model this variation suitably in the derivation of the wavelength normalization curve. This is indeed so (at least for the *LaueView* software package) even in the most extreme case, that of the so-called single-line undulator source in which $\lambda_{\max}$ and $\lambda_{\min}$ may differ by only 10%, say by 0.1 Å at 1.0 Å (V. Šrajer *et al.* and D. Bourgeois *et al.*, in preparation).

As a second example, a Laue diffraction pattern is particularly sensitive to crystal disorder, which leads to substantial 'streaking' of the Laue spots that is predominantly radial in direction in each diffraction image and may be dependent both on direction in reciprocal space (anisotropic disorder) and on time (if, in a time-resolved experiment, disorder is induced by the process of reaction initiation or by structural evolution as the reaction proceeds). The software therefore has to be able to model accurately elongated closely spaced or partially overlapping spots, whose profile varies markedly with position on each detector image and with time (Ren & Moffat, 1995*a*). If the software has difficulty with this task, then either a more ordered crystal must be selected, thus diminishing the size of each spot and the extent of spatial overlaps, or a narrower wavelength range must be used, thus reducing the total number of spots per image and their average spatial density (Cruickshank *et al.*, 1991); or the crystal-to-detector distance must be increased, thus

references