

The Mosaic Spread of Very Small Crystals Deduced from Laue Diffraction Patterns

BY S. J. ANDREWS, JANET E. HAILS AND MARJORIE M. HARDING

*Department of Inorganic, Physical and Industrial Chemistry, Liverpool University,
PO Box 147, Liverpool L69 3BX, England*

AND D. W. J. CRUICKSHANK

SERC Daresbury Laboratory, Warrington WA4 4AD, England

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Abstract

Laue diffraction patterns have been recorded with synchrotron radiation for some very small crystals; in most of them the reflections are elongated radially. The Laue geometry is particularly sensitive to mosaic spread, and a relationship between mosaic spread and Laue spot extension is deduced. It appears that many very small crystals have a large mosaic spread ($0.5\text{--}1^\circ$ or more) which is probably the reason why they did not grow bigger.

Introduction

We have been engaged in a project to use the high intensity of the X-rays from the synchrotron radiation source at Daresbury Laboratory to record diffraction data (for structure determination) from crystals which would be too small for study with conventional X-ray sources. Work is proceeding on oscillation photographs taken with monochromatic radiation, using an Arndt-Wonacott oscillation camera and other equipment set up by J. R. Helliwell (Helliwell, Greenhough, Carr, Rule, Thompson & Worgan, 1982), but the procedure is laborious; it is especially so when considered as 'time per reflection intensity measured' for crystals with comparatively small unit cells ($10\text{--}20 \text{ \AA}$).

The possibility of using the white beam (effectively wavelengths in the range $0.3\text{--}2.5 \text{ \AA}$) and recording Laue diffraction patterns seemed to be ideal for work with these very small crystals: the exposure times are very short ($< \frac{1}{2}\text{--}1$ min, even for a $50 \mu\text{m}$ crystal) and a full sphere of reflection data could be recorded in six to ten exposures. Procedures for indexing the patterns and measuring the intensities of all reflections have been developed at Daresbury Laboratory; they are being used for studies of changes in protein crystals and are being checked in two cases by comparison of the Laue-derived intensities with those measured with monochromatic radiation - for the protein pea lectin, and a simple compound

$\text{C}_{10}\text{H}_{15}\text{N}_2\text{PS}_3$ (Elder, Harding, Helliwell & Machin, 1987).

To this end we have recorded Laue diffraction patterns for a variety of very small crystals (of dimensions $50 \mu\text{m}$ or less). To our disappointment the reflections in many cases were substantially streaked or spread (e.g. up to 3 mm in length) and not likely to yield accurate intensity measurements easily by densitometry; examples are shown in Fig. 1 and Table 1. By contrast, in Laue photographs taken under similar experimental conditions for larger crystals (fresh protein crystals, several organic compounds, dimensions $0.2\text{--}0.5$ mm) the reflections are nearly circular and less than 0.2 mm across (when a 0.2 mm collimator is used). Streaked reflections are also observed in photographs of many protein crystals which have suffered radiation damage (e.g. Helliwell & Rule, personal communication; Helliwell, 1985). The 'streaked and spread' reflections indicate the presence of constituent crystallites not perfectly aligned within the crystal, i.e. a mosaic spread; the geometry of the Laue diffraction pattern is particularly sensitive to mosaic spread, and it appears that the reflection profile can be used to make deductions about the mosaic spread and its uniformity.

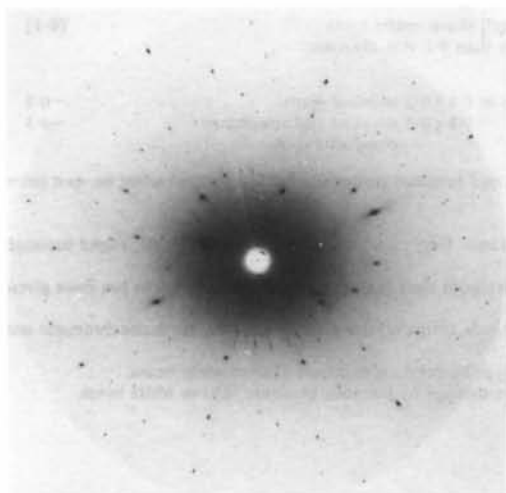
The effect of idealized mosaic spread in the Laue diffraction pattern

In one idealized model, a crystal is made up of many smaller constituent crystallites whose orientations are uniformly distributed within an angular range $\pm \eta/2$. In the reciprocal lattice, this is equivalent to spreading each reciprocal-lattice point over a spherical cap of radius $\eta a^*/2$.

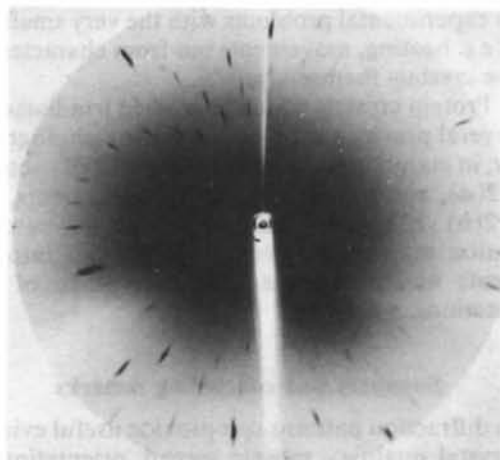
In the plane of the incident beam and a particular reciprocal-lattice point, the angular width of the diffracted beam is 2η , since the angle of incidence $\theta \pm \eta/2$ must equal the angle of reflection $\theta \pm \eta/2$ for each crystallite. On a flat film at distance D from the sample, this angular divergence produces a radial

spot extension of length $2\eta D/\cos^2 2\theta$ (with a wavelength spread implied by Bragg's law for $\theta \pm \eta/2$).

In the perpendicular direction the spherical cap has a height $\pm \eta d^*/2$. To first order in η the reflection condition is still satisfied by an Ewald sphere with the original radius $1/\lambda$, so the angular divergence of the diffracted beam is $\eta d^* \lambda = 2\eta \sin \theta$. The resultant tangential spot extension is $2\eta D \sin \theta / \cos 2\theta$ (as the obliquity factor $\cos 2\theta$ now occurs only once). To first order in η there is no change in wavelength across the spot in the tangential direction.



(a)



(b)

Fig. 1. Laue diffraction photographs with SRS 'white' beam, wavelengths approximately $0.3\text{--}2.5 \text{ \AA}$, collimator 0.2 mm , crystal-film distance 60 mm , maximum $\theta = 21^\circ$. (a) Ammonium oxalate monohydrate, crystal $0.3 \times 0.2 \times 0.1 \text{ mm}$. (b) A disaccharide, crystal $270 \times 25 \times 20 \text{ \mu m}$, X-ray beam perpendicular to needle axis.

Thus for $\eta = 0.5^\circ$ and crystal-film distance 55 mm a radial extension of 1 mm is predicted near the centre of the film, rising to 1.6 mm at $\theta = 20^\circ$ near the edge, and a tangential extension rising from near zero to 0.5 mm over the same range.

For $\eta = 0.1^\circ$ the radial extension would be 0.2 to 0.4 mm and the tangential 0 to 0.1 mm . In a real experiment the overall spot shape will depend on collimator size and beam divergence as well as mosaic spread. For many good crystals mosaic spread is *ca* 0.1° and the reflections are approximately circular and 0.2 mm in diameter; these dimensions appear to be controlled by collimator size (0.2 or 0.3 mm here) and beam divergence. But when mosaic spread rises to $0.3\text{--}0.5^\circ$, the reflections will become radial streaks or ellipses, with dimensions given by the expressions above.

In real crystals the constituent crystallites may not have uniformly distributed orientations or sizes, and there may be anisotropy in the distribution of orientations.

Observations on real crystals and their interpretation

The observations are summarized in Table 1. All photographs were taken on the wiggler beamline of the SRS at Daresbury Laboratory, using components of an Arndt-Wonacott oscillation camera. At $1.8\text{--}2 \text{ GeV}$ the effective wavelength range is *ca* $0.3\text{--}2.5 \text{ \AA}$. Crystals were mounted with araldite, on very short lengths ($< 1 \text{ mm}$) of glass wool, attached in turn to stronger glass rods. A specially designed backstop and screen were used to reduce background scattered intensity and improve the signal-to-noise ratio. The dimensions of reflections were estimated visually using a microscope with eyepiece graticule; since the reflection boundary is not sharp this is inevitably slightly subjective, and dependent on intensity. Reflections were measured in different parts of the film and at different θ values. The observations and their interpretation can be divided into groups:

1. 'Normal'-sized good-quality crystals give small circular spots, as already described, and the mosaic spread is 0.1° or less.

2(a). Some very small crystals show simple radial streaks approximating to the shape in Fig. 2(a). The ratio of streak length to width and their increase with θ are in accordance with the formulae above. These correspond to a fairly ideal mosaic spread of 0.5° and more.

2(b). For other very small crystals the spots appear to be composite, made up of a number of smaller streaks, spread over a larger area of film as in Fig. 2(b). In these cases the crystal consists of a small number of component crystallites, with orientations varying by $1\text{--}2^\circ$ from each other; each of the constituents may have a substantial mosaic spread, like those in 2(a). All the crystals in this group [2(a) and

Table 1. Summary of observations on real crystals

Sample	Crystal size (μm)	Laue spot size (mm) and shape*	Mosaic spread derived from spot extension (radial) ($^\circ$)
1. Normal-sized good crystals ammonium oxalate† i proflavine hemisulfate‡	300 × 200 × 100 500 × 500 × 600 (larger than collimator)	~Circular 0.15–0.20 mm (diffuse haloes round very intense spots)	[0.1]
2. Very small crystals			
(a) ammonium oxalate† ii disaccharide§ i disaccharide§ ii	40 × 40 × 20 150 × 25 × 5 250 × 20 × 20	~1 × 0.1 } Streaks ~1.5 × 0.1 } or ~3.5 × 0.3 } ellipses	~0.4 ~0.6 ~1.5
(b) Rh ₆ (CO) ₁₂ dppm¶ trisaccharide** i	35 × 25 × 10 250 × 25 × 20	~1 × 0.5 } Composite to 0.7 × 0.3 } of smaller 2 × 0.3 } sharper streaks	0.4 to 0.3 ~0.8
3. Very small crystal chipped from larger one of proflavine hemisulfate‡ i ii	80 × 80 × 15 80 × 70 × 25	Small sharp round spots less than 0.1 mm diameter	[0.1]
4. Radiation-damaged protein crystal β -lactoglobulin†† monoclinic human insulin‡‡	> 300 × 300 × 300 > 300 × 300 × 300	Up to 0.8 × 0.2 streaked spots 0.8 × 0.3 streaked and sometimes composite spots	~0.3 ~0.3

* For crystal film distance 55 mm and $2\theta \sim 25^\circ$, a visual estimate (with microscope and eyepiece graticule), and a little dependent on spot intensity.

† Commercial sample, both crystals from same sample.

‡ $(\text{C}_{13}\text{H}_{12}\text{N}_3)^+ \cdot \frac{1}{2}\text{SO}_4^{2-} \cdot \frac{1}{2}\text{H}_2\text{O}$ from Dr S. Neidle.

§ Xylose β 1-2 arabinose, $\text{C}_{10}\text{H}_{18}\text{O}_9$, from Dr W. Mackie, Biophysics Department, Leeds Univ.; both crystals from same sample; i used successfully for monochromatic oscillation photographs.

¶ dppm is the ligand $\text{Ph}_2\text{P}\cdot\text{CH}_2\cdot\text{PPh}_2$, sample from J. A. Clucas and A. K. Smith, Liverpool Univ. Larger crystal from same batch has since given results similar to group 1, and Laue photographs are being used for intensity measurement.

** Xylose β 1-4 xylose β 1-4 xylose, from Dr W. Mackie, Biophysics Department, Leeds Univ.; crystal used successfully for monochromatic oscillation photographs.

†† Sample and photographs of Dr M. Papiz, Daresbury Laboratory, radiation damage by previous exposure (1 s) to white beam.

‡‡ Sample and photographs of Dr C. D. Reynolds, Liverpool Polytechnic, radiation damage by previous exposure (6 s) to white beam.

2(b)] are either very small crystals in batches where some other crystals have grown larger, or very small crystals of compounds which have never yielded larger crystals despite repeated attempts at crystallization. It would appear that growth to a larger crystal is inhibited if the mosaic spread is large, *i.e.* when the constituent crystallites are poorly oriented in relation to each other.

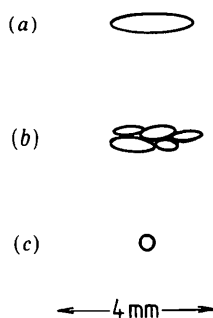


Fig. 2. Diagrammatic illustration of types of spot profile observed, (a) and (b) from small crystals with substantial mosaic spread, and (c) on the same scale from a 'normal' crystal. In all cases the horizontal direction is radial from the centre of the film. Note that the Laue geometry has cylindrical symmetry about the X-ray beam direction.

3. Two very small crystals of proflavine hemisulfate, chipped from a much larger good crystal, gave very small sharp reflections, like those in group 1. This shows that the streaks above do not result from experimental problems with the very small crystals (*e.g.* heating, movement) but from characteristics of the crystals themselves.

4. Protein crystals which have been irradiated (*e.g.* by several previous exposures for Laue photographs) show, in many cases, substantially streaked spots, like Fig. 2(a), and sometimes several components, as in Fig. 2(b). It appears that one of the effects of the radiation may be to break these crystals into constituents and/or increase the divergence of their orientations.

Summary and concluding remarks

Laue diffraction patterns can provide useful evidence on crystal quality – mosaic spread, orientation and size of constituent crystallites. This could be exploited further, particularly for very small crystals.

Many very small crystals have unusually large mosaic spread and this is probably the reason why they would not grow to bigger crystals. When present this large mosaic spread will make it more difficult to use Laue diffraction patterns for intensity measure-

ment (for structure determination), while for some crystals with only moderate mosaic spread such data collection will be practicable.

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One-Wavelength Technique: Estimation of Centrosymmetrical Two-Phase Invariants in Dispersive Structures

BY C. GIACOVAZZO

Centro di Ricerca Interdipartimentale di Cristallografia, Università, Campus Universitario, Via Salvemini, 70124 Bari, Italy

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Abstract

Two-phase structure invariants of type $\Phi = \varphi_{\mathbf{h}} + \varphi_{-\mathbf{h}}$ are estimated in centrosymmetrical space groups, and in non-centrosymmetrical ones when $\varphi_{\mathbf{h}}$ is a symmetry-restricted phase. The distributions are of von Mises type with concentration parameters half those occurring in non-centric distributions.

Notation

N number of atoms in the unit cell
 t number of atoms in the asymmetric unit
 $f = f' + if''$ general expression for the atomic scattering factor
 $F^+ = A^+ + iB^+$ } structure factors of the reflexions
 $F^- = A^- + iB^-$ } \mathbf{h} and $-\mathbf{h}$ respectively
 φ^+, φ^- phases of F^+, F^-
 $C_S \equiv (\mathbf{R}_S, \mathbf{T}_S)$ S th symmetry operator: \mathbf{R}_S is the rotational part, \mathbf{T}_S the translational part
 $\xi_j = \sum_{s=1}^m \cos 2\pi\mathbf{h}(\mathbf{R}_S\mathbf{r}_j + \mathbf{T}_S)$
 $\eta_j = \sum_{s=1}^m \sin 2\pi\mathbf{h}(\mathbf{R}_S\mathbf{r}_j + \mathbf{T}_S)$
 $\varepsilon_{\mathbf{h}}$ Wilson's (1980) statistical weight of the reflection \mathbf{h}
 $\Sigma_+ = \varepsilon \sum_{j=1}^N (f_j'^2 + f_j''^2)$ average value of $|F_{\mathbf{h}}|^2$ at given $|\mathbf{h}|$
 $\Sigma_- = \varepsilon \sum_{j=1}^N (f_j'^2 - f_j''^2)$
 $R = F/\Sigma_+^{1/2}$ normalized modulus of the structure factor
 $D_1(x) = I_1(x)/I_0(x)$ ratio of modified Bessel functions of order 1 and 0
 cs, ncs centrosymmetric, non-centrosymmetric

1. Introduction

Probabilistic approaches have been applied by several authors to crystal structures with dispersive atoms

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(see Srinivasan & Parthasarathy, 1976, and literature there quoted; Kroon, Spek & Krabbendam, 1977; Heinerman, Krabbendam, Kroon & Spek, 1978). Among the most recent results connected with this paper we quote:

(a) estimation of two-phase and three-phase structure invariants (Hauptman, 1982; Giacovazzo, 1983);

(b) estimation of probabilistic coefficients for a Patterson synthesis devoted to finding the positions of anomalous scatterers (Cascarano & Giacovazzo, 1984);

(c) formulas estimating phases of the complete crystal structures when the position of the anomalous scatterers are known (Cascarano & Giacovazzo, 1985).

Probabilistic treatment of the anomalous dispersion effect is more important in ncs space groups: accordingly all the approaches (a), (b), (c) were described in Cascarano & Giacovazzo (1985). It is, however, of non-negligible interest to apply probabilistic methods for the estimation of the structure invariants in cs space groups, or in ncs space groups when the invariants are constituted by symmetry-restricted phases. This paper is devoted to the estimation of two-phase structure invariants in the above conditions. For the sake of brevity we give here only the final formulas: for the mathematical approach the reader is referred to a recent book (Giacovazzo, 1980).

2. The estimation of $\Phi = \varphi_{\mathbf{h}} + \varphi_{-\mathbf{h}}$ in centrosymmetric crystals

In cs space groups $F^+ \equiv F^-$: thus, in order to calculate $\Phi = \varphi_{\mathbf{h}} + \varphi_{-\mathbf{h}}$, the simpler distribution $P(A, B)$ may be calculated instead of $P(A^+, A^-, B^+, B^-)$, as has