PAULING, L. (1930). *Phys. Rev.* **36**, 430–443.
RUHEMANN, M. (1932). *Z. Phys.* **76**, 368–375.
SCHUCH, A. F. & MILLS, R. L. (1970). *J. Chem. Phys.* **52**, 6000–6008.
ST. LOUIS, R. V. & SCHNEPP, O. (1969). *J. Chem. Phys.* **50**, 5177–5183.
STREIB, W. E., JORDAN, T. H. & LIPSCOMB, W. N. (1962). *J. Chem. Phys.* **37**, 2962–2965.
SZABO, A. & OSTLUND, N. S. (1982). *Modern Quantum Chemistry: Introduction to Advanced Electronic Structure Theory*, ch. 6. New York: Macmillan.
VEGARD, L. (1929). *Z. Phys.* **58**, 497–501.
VENABLES, J. A. (1970). *Philos. Mag.* **21**, 147–166.
VENABLES, J. A. & ENGLISH, C. A. (1974). *Acta Cryst.* **B30**, 929–935.
WACHTEL, E. J. (1972). *J. Chem. Phys.* **57**, 5620–5621.

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Evaluation of Reflection Intensities for the Components of Multiple Laue Diffraction Spots. II. Using the Wavelength-Normalization Curve

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Abstract

In a Laue diffraction pattern, 10–20% of the spots result from the exact superposition of two or more reflections that are 'harmonics', *e.g.* hkl, $2h,2k,2l$ *etc.* The use of only the 80–90% of the reflections measurable as singles may not always be sufficient and evaluation of the intensities of the components of the multiple spots is therefore important. A procedure for this deconvolution is given, based on the varying nature of the wavelength-normalization curve. A feasibility trial has been carried out using a single Laue diffraction image of tetragonal hen egg white lysozyme (HEWL) recorded on an image plate. This allowed the intensities of 103 reflections to be evaluated from multiple spots. For these reflections, their agreement with monochromatic diffractometer data gave an R factor of 0.157 for 96 common reflections. An earlier paper described another procedure based on direct methods, which addressed the same problem.

1. Introduction

With the advent of synchrotron radiation, there has been renewed interest in the use of Laue diffraction as a method for obtaining diffraction intensities (Campbell *et al.*, 1987; Helliwell *et al*, 1989; Smith Temple & Moffat, 1987; Smith Temple, 1989; Bartunik, Bartsch & Huang, 1992). Usually, 80–90% of the spots in a single Laue diffraction pattern correspond to single reflections, each with its values of hkl and associated d (plane spacing) and λ (Cruickshank, Helliwell & Moffat, 1987). These are described as singles. The remaining 10–20% of the spots are doubles, triples or higher multiples. If a crystal contains a plane of spacing d, then the spacings $d/2$, $d/3$, or in general d/j, may also occur, where j is any positive integer, Bragg's law is simultaneously satisfied by the sets of values (d, λ), $(d/2, \lambda/2)$, ..., $(d/j, \lambda/j)$, ... and the diffraction spots are exactly superposed. For these, measurement of the spot intensity does not therefore give the component reflection intensities directly. The reflections that cannot be straightforwardly measured as singles are not randomly distributed in reciprocal space; a high proportion of them are low-order reflections, axial reflections or reflections in special planes ($hk0$, hhl *etc.*). The absence of these reflections can be a serious drawback if the data are to be used for structure solution, for example using direct methods. Also, in, for example, larger-unit-cell protein crystals, the absence of low-order reflections has been shown to give electron-density maps that have poor connectivity and are particularly difficult to interpret (Duke, Hadfield, Walters, Wakatsuki, Bryan & Johnson, 1992). For reasons such as these, there has been renewed interest in methods to deconvolute reflection intensities from spots that are multiples. Helliwell *et al.* (1989) have described one procedure for this deconvolution that uses the intensities of spots on successive films in a film pack and the variation of film absorption with λ. With the increasing use of image plates or electronic detectors, it is desirable to develop methods that do not depend on recording the data on such multifilm packs. Hao, Campbell, Harding & Helliwell (1993) have described a use of direct methods to carry out such a deconvolution. This paper describes a method that makes use of the nature of the wavelength-

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normalization curve when multiple reflections or their symmetry equivalents are stimulated at different sets of wavelengths.

2. Method

When estimating reflection intensities from the integrated spot measurements, a wavelength-dependent normalization function needs to be applied to take account of factors such as the variation with wavelength of the incident intensity, sample absorption, detector response and integrated power. This variation with wavelength is an added complication of using the Laue method as the wavelength-normalization curve needs to be determined before the reflection intensities can be determined. However, advantage can be taken of this variation with wavelength and it has been recognised for some time (*e.g.* Helliwell, 1992; K. Moffat, University of Chicago, personal communication) that it provides, in principle at least, information that may be used in the deconvolution of intensities from multiple spots. This can be made use of when a reflection multiple has been measured with the crystal in more than one orientation (*i.e.* at different wavelengths) or when a symmetry-equivalent multiple is present again at different wavelengths from the first multiple. To deconvolute a double, two or more such orientations are needed, three or more for a triple and so on.

The principle is illustrated for a reflection double with its fundamental and first harmonic. Fig. 1 shows the double (or symmetry equivalent) in two different orientations using the Ewald-sphere representation in reciprocal space. In the first orientation, the two components of the double arise from the wavelengths λ_{1a} and λ_{2a} where $\lambda_{2a} = 2\lambda_{1a}$. Similarly, in the second orientation, the two components of the double arise from the the wavelengths λ_{1b} and λ_{2b}. Fig. 2(*a*) shows a wavelength-normalization curve with the four wavelengths marked. Wavelength-normalization factors $g(\lambda)$ can be determined for each of the four wavelengths. If I_1 and I_2 are the normalized reflection intensity components of the double and $I_{\text{obs},a}$ and $I_{\text{obs},b}$ the integrated intensities for the spot in the two orientations, then we can set up and solve the following equations:

$$g(\lambda_{1a})I_1 + g(\lambda_{2a})I_2 = I_{\text{obs},a}$$
$$g(\lambda_{1b})I_1 + g(\lambda_{2b})I_2 = I_{\text{obs},b}.$$

There may of course be more than two orientations present. In general, for an n-multiple spot at m orientations, we have

$$\sum_{i=1}^{n} g(\lambda_{ij})I_i = I_{\text{obs},j} \quad (j = 1, 2, \ldots, m). \qquad (1)$$

This is a set of m linear simultaneous equations in the n unknown intensity components I_i. If $m < n$, no solution can be obtained. When $m = n$, the equations can be solved directly and when $m > n$, a least-squares solution can be found by solving the $1/\sigma^2$ (σ is the standard deviation of the measured spot intensity I_{obs}) weighted 'normal' equations

$$G^T W G \mathbf{I} = G^T W \mathbf{I}_{\text{obs}}, \qquad (2)$$

where G contains $[g(\lambda_{ij})]$, $\mathbf{I} = (I_1, I_2, \ldots I_n)$, $\mathbf{I}_{\text{obs}} = (I_{\text{obs},1}, I_{\text{obs},2}, \ldots, I_{obs,m})$ and W is a weighting matrix, $W = \text{diag}\,(1/\sigma_1^2, 1/\sigma_2^2, \ldots, 1/\sigma_m^2)$. T denotes matrix transpose.

The wavelength-normalization curve will normally be determined using the singles data.

3. A test of the procedure

The potential success of the method will depend on a number of factors, including the following: good

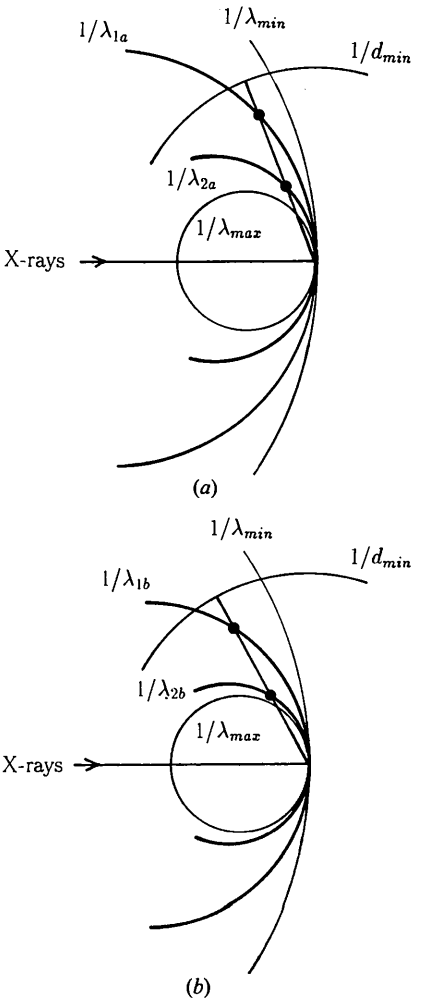

Fig. 1. Example of the diffraction geometry for a Laue double in two different orientations (*a*) and (*b*). For each case, the Ewald spheres are shown for the two wavelength components of the double λ_{1a}, λ_{2a} in the first orientation and λ_{1b}, λ_{2b} in the second orientation. The spheres marking the soft limits λ_{min}, λ_{max} and d_{min} are also shown. Note: although the wavelength-normalization curve and the four wavelengths used in this example are taken from the test data set described in the paper, the illustration in this figure, for clarity, corresponds to a smaller unit cell with higher-resolution data.

integrated spot intensities; a well determined normalization curve; a sufficient number of occurrences of each multiple in different orientations; sufficiently different scaling factors for the various wavelength components to determine sufficiently accurate intenstities and to avoid problems such as collinear sets of equations.

A test was carried out using a crystal of tetragonal hen egg white lysozyme (HEWL) (space group $P4_32_12$, $a = 79.19$, $c = 38.02$ Å). A single Laue diffraction image was recorded, by Dr S. McSweeney, using synchrotron radiation and the MAR image-plate system on station 9.5 of the Daresbury SRS.

When predicting the spot positions prior to integration, the soft limits used were $\lambda_{min} = 0.4$, $\lambda_{max} = 2.2$ and $d_{min} = 2.8$ Å.

The intensity data were processed and normalized curves determined using the programs of the Daresbury Laboratory *Laue Software Suite* (Campbell *et al.*, 1987; Helliwell *et al.*, 1989). The wavelength-normalization curve was determined from symmetry-equivalent data initially for the wavelength range 0.5 to 1.8 Å using the program *LAUENORM*. Reflections with $I < 3.0\sigma(I)$ were rejected. This gave a *LAUENORM* internal *R* factor on intensity, RFACT1, of 0.058 for 1656 reflections (number of unmerged data). To check the quality of the data, a normalization curve was also calculated (program *LAUESCALE*) by scaling the data to a reference set of diffractometer data kindly made available by Dr J. Dewan (Young, Dewan, Nave & Tilton, 1993). An internal *R* factor on intensity, RFACT1, of 0.063 for the 1656 reflections was obtained and the scaling *R* factor on intensity between the Laue singles data and the reference data, RSCALE, was 0.067 for 925 merged unique Laue reflections, which were in common with the reference diffractometer data.

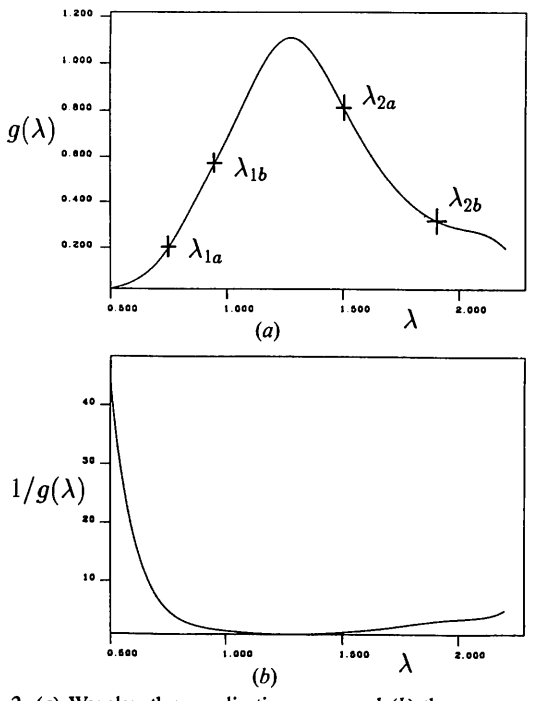

Fig. 2. (*a*) Wavelength-normalization curve and (*b*) the corresponding scaling curve derived from the lysozyme test data set. (*a*) also shows the points on the wavelength-normalization curve for the four wavelengths involved in the example used to illustrate the deconvolution method.

The *LAUENORM* program was modified to allow for the deconvolution of harmonics data as described in this paper. The wavelength-normalization curve for use with the deconvolution method should cover as much of the incident λ range as possible and so, for this test, the wavelength-normalization curve was recalculated with a slightly expanded range from 0.5 to 2.2 Å. This gave an internal *R* factor RFACT1 for the singles of 0.079 for 1707 reflections. After the data were merged, 958 unique singles were output by the program.

For the test crystal, the wavelength-normalization curve from *LAUENORM* and the corresponding scaling curve, $1.0/g(\lambda)$, are shown in Fig. 2. The scaling curve rises very steeply at the low-wavelength end as the reflection intensities get weaker. Large scaling factors tend to be inaccurate and may cause large errors in the deconvolution process. There is therefore an option to exclude equations that contain scale factors above a given threshold and, in the present test, the threshold was set at 25.0.

In a number of cases, the solution of the equations used for deconvoluting a multiple gave rise to a negative value for one of the intensities. In such cases, the components with negative intensities were removed from the equations and the equations were re-solved. The program allows for the inclusion of weights based on the standard deviations of the measured spot intensities as derived by the integration program *INTLAUE*. However, in the present case, unit weights were used as these seemed to give slightly better results, *i.e.* in (2) *W* becomes a unit diagonal matrix.

As a result of the deconvolution process, 103 unique reflections were obtained. Of these, 78 came from 39 doubles with both components giving positive intensities, six from two triples with the three components giving positive intensities and 19 from doubles after eliminating a negative intensity. The program *RSTATS* from the *CCP*4 (1979) program suite was used to scale these to the reference diffractometer data set (determining a scale and temperature factor) and to calculate an *R* factor between the deconvoluted multiples data and these reference data. The *R* factor (on *F*) obtained was 0.157 for the 96 reflections in common. [For the singles processed using the same normalization curve from *LAUENORM*, the *R* factor (on *F*) from the *RSTATS* program was 0.084 for 939 unique reflections in common with the diffractometer data.]

For the 84 reflections from multiples that gave only positive components, the *R* factor was 0.173 for 78 reflections in common with the reference data; for the 19

Table 1. *Analysis of the deconvoluted reflections as a function of* $4\sin^2\theta/\lambda^2$

R factors (on F) are against the common reflections from the reference diffractometer data set.

Range	$4\sin\theta^2/\lambda^2$ (Å^2)	d (Å)	Number	R
1	0.025	6.32	17	0.151
2	0.050	4.47	32	0.181
3	0.075	3.65	11	0.062
4	0.100	3.16	14	0.153
5	0.125	2.83	21	0.194
6	0.150	2.58	1	0.038
All			96	0.157

Table 2. *Analysis of the deconvoluted reflections as a function of F of the reference diffractometer data set* ($\langle F_{reference}\rangle$); $\langle F_{multiples}\rangle$ *refers to the deconvoluted data*

R factors (on F) are for the common reflections in the two data sets.

Range	$\langle F_{\rm reference}\rangle$	Number	R	$\langle F_{\rm multiples}\rangle$
1	1355.9	15	0.337	1404.1
2	3102.1	35	0.228	3109.5
3	5069.0	35	0.123	4750.3
4	6585.3	7	0.106	6635.5
5	9301.0	4	0.081	9542.9
All	4058.6	96	0.157	3966.4

reflections from doubles, after the negative component was eliminated, the R factor was 0.107 for 18 common reflections.

Analyses of the multiples data against the reference data are shown as a function of $4\sin^2\theta/\lambda^2$ (Table 1) and as a function of the reference $F_{\rm obs}$ value (Table 2). These statistics are from the *RSTATS* program. There is no obvious trend with resolution but there is improved data with increasing $F_{\rm obs}$ as would be expected.

In some cases, it is possible that a component of a multiple reflection is also present as a single. Normally, such singles data would be included in the deconvolution process; for the present test, this was not done and there were in fact only five such common reflections.

4. Concluding remarks

The results of the test show that the method can produce reasonable intensities at least for doubles and possibly for some higher multiples. The tetragonal lysozyme in the test case has a high symmetry and therefore it was possible to obtain some useful data from even a single image. Normally, images corresponding to several orientations are measured with more orientations the lower the symmetry. We intend to investigate the types of strategy required, *e.g.* number of images required, best distribution of such images *etc.*, for various symmetries and cell sizes but this will require the development of additional software to do this effectively. We would also like to collect more test data when we have determined a potentially effective strategy for using multiple orientations.

In some cases, *e.g.* with small unit cells and high-resolution data (Cruickshank, Helliwell & Moffat, 1987), the recorded singles may arise from a more restricted wavelength range than the multiples. In such cases, it may be necessary to attempt to extend the wavelength range of the determined normalization curve by using any redundancy of information within the multiples data that is additional to that required for the deconvolution itself.

The success of the method described depends on having a useful variation of normalization factor with wavelength. In some cases, it might be necessary or advantageous to modify the wavelength-normalization curve by making use of a filter in the beam. It would also be possible to use an unfiltered and one or more filtered beams to produce different wavelength-normalization curves and hence provide additional information for deconvoluting harmonics; this would in principle be similar to the original unscrambling method of Helliwell *et al.* (1989), although different experimentally. Again, we would like to simulate such situations to determine what might be worth trying experimentally.

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References

BARTUNIK, H. D., BARTSCH, H. H. & HUANG, Q. (1992). *Acta Cryst.* A**48**, 180-188.

CAMPBELL, J. W., CLIFTON, I. J., ELDER, M., MACHIN, P. A., ZURED, S., HELLIWELL, J. R., HABASH, J., HAJDU, J. & HARDING, M. M. (1987). *Biophysics and Synchrotron Radiation*, edited by A. BIANCONI & A. CONGIU CASTELLANO, pp. 53-60. Heidelberg: Springer Verlag.

*CCP*4 (1979). *The SERC (UK) Collaborative Computing Project* No. 4. *A Suite of Programs for Protein Crystallography*. Warrington: SERC Daresbury Laboratory.

CRUICKSHANK, D. W. J., HELLIWELL, J. R. & MOFFAT, K. (1987). *Acta Cryst.* A**43**, 656-674.

DUKE, E. M. H., HADFIELD, A., WALTERS, S., WAKATSUKI, S., BRYAN, R. K. & JOHNSON, L. N. (1992). *Philos. Trans. R. Soc. London Ser. A*, **340**, 245-261.

HAO, Q., CAMPBELL, J. W., HARDING, M. M. & HELLIWELL, J. R. (1993). *Acta Cryst.* A**49**, 528-531.

HELLIWELL, J. R. (1992). *Macromolecular Crystallography with Synchrotron Radiation*. Cambridge Univ. Press.

HELLIWELL, J. R., HABASH, J., CRUICKSHANK, D. W. J., HARDING, M. M., GREENHOUGH, T. J., CAMPBELL, J. W., CLIFTON, I. J., ELDER, M., MACHIN, P. A., PAPIZ, M. Z. & ZUREK, S. (1989). *J. Appl. Cryst.* **22**, 483-497.

SMITH TEMPLE, B. R. (1989). PhD thesis, Cornell Univ., Ithaca, New York, USA.

SMITH TEMPLE, B. & MOFFAT, K. (1987). *Computational Aspects of Protein Crystal Data Analysis*, edited by J. R. HELLIWELL, P. A. MACHIN & M. Z. PAPIZ, pp. 84-89. *Proceedings of a Daresbury Study Weekend.* Warrington: SERC Daresbury Laboratory.

YOUNG, A. C. M., DEWAN, J. C., NAVE, C. & TILTON, R. F. (1993). *J. Appl. Cryst.* **26**, 309-319.

SHORT COMMUNICATIONS

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Si/SiO_2 interface-depth determination in glancing-incidence X-ray diffraction experiments.

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Abstract

The Si/SiO_2 interface of a single crystal has been investigated by the double-crystal inclination method, the surface peak being measured. The distorted layer depth is shown to be of the order of 1 nm and the amorphous film depth of the order of 6 nm.

In the first experiments on glancing-incidence diffraction by Marra, Eisenberger & Cho (1979), it was shown that the Laue diffraction-surface sensitivity is considerably enhanced. However, the geometry of these experiments suggests a rather high collimation with respect to both the glancing angle Φ_o and the deviation angle from the Bragg condition $\Delta\theta$. The $\Delta\theta$-collimation difficulties are overcome in the scheme suggested by Afanas'ev & Melkonyan (1983) and by Imamov, Golovin, Stepanov & Afanas'ev (1983), which is based on the relationship between the incident angle Φ_o and the exit angle Φ_h:

$$\Phi_h^2 = (\Phi_o + 2\varphi \sin\theta_B)^2 + \alpha, \quad \alpha = 2\Delta\theta \sin 2\theta_B, \qquad (1)$$

where φ is the misorientation angle between the diffraction planes and the surface normal and θ_B is the Bragg angle.

However, there is a real advantage in dealing with the diffraction intensity as a function of Φ_o at fixed α, owing to the rotation around the inverse lattice vector **H** [the so-called inclination method, proposed by Somenkov, Schilstein, Belova & Utemisov (1978)].

Total reflection is always accompanied by an enhancement factor,

$$|2\Phi_o/\Phi_o + (\Phi_o + \chi_o)^{1/2}|^2, \qquad (2)$$

where χ_o is the media polarizability. In the case of X-ray diffraction, the resulting signal is also enhanced by the factor

$$|2\Phi_h/\Phi_h + (\Phi_h^2 + \chi_o)^{1/2}|^2. \qquad (3)$$

Considering the diffraction intensity as a three-dimensional surface over the (α, Φ_o) plane, one can find the following ridges for rather large values of $|\alpha|$:

$$\begin{aligned} &\Phi_o = \Phi_c \text{ at } \alpha \gg \Phi_c^2, \quad \text{from (2);} \\ &\Phi_h = \Phi_c \text{ at } -\alpha \gg \Phi_c^2, \quad \text{from (3);} \\ \text{or} \quad &\Phi_{o\alpha-} = -2\varphi \sin\theta_B - (-\alpha + \Phi_c^2)^{1/2} > 0 \quad \text{and} \\ &\Phi_{o\alpha+} = -2\varphi \sin\theta_B + (-\alpha + \Phi_c^2)^{1/2} > 0, \quad \text{from (1);} \end{aligned} \qquad (4)$$

where Φ_c is the critical angle.

The diffraction intensity is negligible far from the curve $\Phi_{o\alpha+}$ for rather large negative α. On the other hand, the incident beam is collimated with respect to α in a rather narrow interval of the 'Darwin table'. So, the resulting intensity curve consists of two peaks, analogous to triple-crystal diffractometry (TCD) rocking curves. The peak at $\Phi_o = \Phi_c$ corresponds to the 'Darwin table tail' and the diffraction maximum at small α, while the peak shifting with decreasing α according to (4) is due to the diffraction intensity 'tail' at large negative α and to the narrow centre

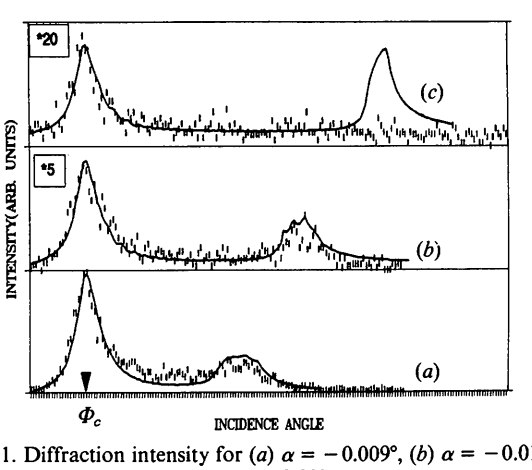

Fig. 1. Diffraction intensity for (*a*) $\alpha = -0.009°$, (*b*) $\alpha = -0.016°$, (*c*) $\alpha = -0.03°$.