recommended in practice. In fact, unlike representation, the complementary invariant method is too sensitive to changes in the parameters $E_{h\min}$, $E_{k\min}$, *etc.* If the values of these parameters are too high, no quartet may, on occasion, be available for a given $\varphi_{\mathbf{H}}$; if they are too low, too many unreliable quartets may be calculated with waste of computer time.

It is anticipated that the estimation of one-phase structure seminvariants *via* the second representation will play an important role in a new procedure which exploits also the information contained in the two-phase structure seminvariants (Burla, Giacovazzo, Nunzi & Polidori, 1980). It is expected that a similar role may be played by a procedure using the three-phase structure seminvariants as well (Giacovazzo, 1978*b*, Hauptman & Potter, 1979).

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The Influence of Twinning by Merohedry on Intensity Statistics

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Abstract

A simple test based on intensity statistics is presented for the detection of twinning by merohedry. Using relationships derived in the text, the twinning fraction of a crystal may be estimated from the intensity probability distribution. Unlike most methods for the detection of twinning, application of this test does not require knowledge of the twinning operation. Two possible mechanisms for increasing the apparent diffraction symmetry of a crystal, twinning by merohedry and crystal disorder, may be distinguished in certain cases by these procedures.

Crystals twinned by merohedry present special problems in X-ray crystal-structure determinations since the reciprocal lattices of the twins have identical orientations (Buerger, 1960). This class of twinning may occur in space groups of tetragonal or higher symmetry whenever the point symmetry of the crystal is lower than that of the lattice (Catti & Ferraris, 1976). Since the twinning operation exactly superimposes non-

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equivalent reflections from the twin domains, the diffraction pattern provides no obvious indication of the composite nature of the reciprocal lattice. As perfect twinning introduces extra symmetry into the diffraction pattern, even the space group may be misidentified. Consequently, considerable effort can be expended towards solving a twinned structure before recognizing the problem and appropriately modifying the intensities or refinement procedure.

Conventional procedures for the detection of twinning and estimation of the twinning fraction from intensity data require correlation of twin-related reflections (Britton, 1972; Murray-Rust, 1973). To apply these tests, however, the nature of the twinning operation must be known. In the event that the true space group of the twinned crystal is misidentified, the required pairs of reflections may not have even been measured. Furthermore, these methods cannot distinguish a perfectly twinned crystal from an untwinned crystal in a higher-symmetry space group. To overcome these restrictions, we have developed a method for the detection of twinning by merohedry based on intensity statistics, which requires no *a priori* knowledge of the twinning operation.

The observed intensities from a twinned crystal are derived from the untwinned values by the relationships:

$$I_1 = (1-\alpha)J_1 + \alpha J_2,$$

$$I_2 = \alpha J_1 + (1-\alpha)J_2, \qquad (1)$$

where I_1 and I_2 are the observed twinned intensities produced by superimposing the untwinned intensities J_1 and J_2, and α is the volume fraction of the smaller twin mate. If the probability distribution for the untwinned intensities, $P(J)$, is known, the probability distribution of the twinned intensities, $P(I,\alpha)$, for nonzero α, is given by

$$P(I,\alpha) = \frac{\int\limits_0^{I/(1-\alpha)} P(J)\,P\{[I-(1-\alpha)J]/\alpha\}\,\mathrm{d}J}{\int\limits_0^{\infty} P(I,\alpha)\,\mathrm{d}I}. \qquad (2)$$

The implicit assumption that the probability distributions for the twin-related reflections are independent will be examined below.

Using Wilson's (1949) intensity distributions, (2) may be evaluated. For non-centrosymmetric reflections, an analytical solution has been obtained for the probability distribution ${}_1P(z,\alpha)$:

$${}_1P(z,\alpha) = [e^{-z/(1-\alpha)} - e^{-z/\alpha}]/(1-2\alpha), \qquad (3)$$

where z represents the intensity relative to the mean intensity. Values of the probability distribution curve for the normalized structure-factor amplitude $P_2(y,\alpha)$, where $y^2 = z$, may be calculated from $P(z,\alpha)$ using the relationship

$$P_2(y,\alpha) = 2yP(y^2,\alpha) \qquad (4)$$

(Srinivasan & Parthasarathy, 1976).

It has not been possible to derive general analytical expressions for the corresponding centrosymmetric distributions, although numerical solutions can be calculated. Interestingly, however, the distribution formulas simplify to the untwinned non-centrosymmetric distributions in the case of perfect twinning. This behavior illustrates the important point that twinning and disorder may have opposite effects on intensity statistics. Whenever crystal disorder introduces an apparent center of symmetry in certain classes of reflections of a non-centrosymmetric crystal, the intensity distribution of those reflections will be more centric-like in disordered than in ordered crystals. Twinning of the ordered crystals, however, although resulting in a diffraction pattern of higher symmetry, will yield a hypo-non-centrosymmetric intensity distribution. Thus, even though twinning and disorder may have similar effects on the symmetry of the diffraction pattern, in principle, they would be distinguished by the intensity distribution.

The cumulative distribution function $N(z,\alpha)$ which gives the fraction of reflections having relative intensities less than z, for a crystal of twinning fraction α, may be calculated from $P(z,\alpha)$ (Howells, Phillips & Rogers, 1950). For non-centrosymmetric reflections,

$${}_1N(z,\alpha) = \frac{[\alpha(e^{-z/\alpha}-1) - (1-\alpha)\,(e^{-z/1-\alpha}-1)]}{(1-2\alpha)}. \qquad (5)$$

In the case of perfect twinning, the expressions $N(z,0\cdot5)$ reduce to

$${}_1N(z,0\cdot5) = 1-(1+2z)e^{-2z},$$

$${}_{\bar{1}}N(z,0\cdot5) = 1-e^{-z}. \qquad (6)$$

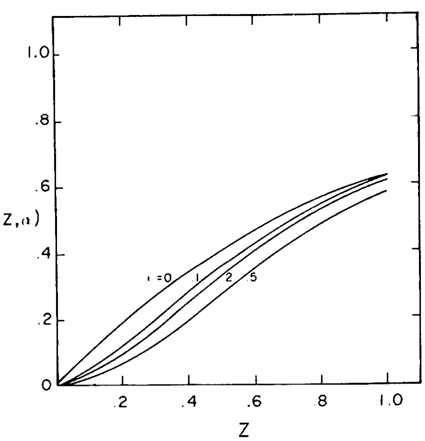

Fig. 1. The cumulative distribution function ${}_1N(z,\alpha)$ for non-centrosymmetric reflections, with $\alpha = 0\cdot0, 0\cdot1, 0\cdot2$ and $0\cdot5$.

Table 1. ${}_1P(z,\alpha)$ *for noncentrosymmetric reflections*

z	α 0·0	0·1	0·2	0·3	0·5
0·1	0·91	0·66	0·46	0·38	0·33
0·2	0·82	0·83	0·68	0·60	0·54
0·3	0·74	0·83	0·77	0·71	0·66
0·4	0·67	0·78	0·79	0·75	0·72
0·5	0·61	0·71	0·76	0·75	0·74
0·6	0·55	0·64	0·70	0·72	0·72
0·7	0·50	0·57	0·64	0·68	0·69
0·8	0·45	0·51	0·58	0·62	0·65
0·9	0·41	0·46	0·52	0·57	0·60
1·0	0·37	0·41	0·47	0·51	0·54

Table 2. ${}_{\bar{1}}P(z,\alpha)$ *for centrosymmetric reflections*

z	α 0·0	0·1	0·2	0·3	0·5
0·1	1·20	1·28	1·07	0·97	0·90
0·2	0·81	1·00	0·92	0·86	0·82
0·3	0·63	0·81	0·80	0·77	0·74
0·4	0·52	0·66	0·69	0·68	0·67
0·5	0·44	0·55	0·60	0·61	0·61
0·6	0·38	0·47	0·53	0·55	0·55
0·7	0·34	0·41	0·47	0·49	0·50
0·8	0·30	0·35	0·41	0·44	0·45
0·9	0·27	0·31	0·36	0·39	0·41
1·0	0·24	0·28	0·32	0·35	0·37

Table 3. ${}_1N(z,\alpha)$ *for noncentrosymmetric reflections*

z	α 0·0	0·1	0·2	0·3	0·5
0·1	0·10	0·04	0·03	0·02	0·02
0·2	0·18	0·12	0·08	0·07	0·06
0·3	0·26	0·20	0·16	0·14	0·12
0·4	0·33	0·28	0·24	0·21	0·19
0·5	0·39	0·36	0·31	0·28	0·26
0·6	0·45	0·42	0·39	0·36	0·34
0·7	0·50	0·48	0·45	0·43	0·41
0·8	0·55	0·54	0·52	0·49	0·48
0·9	0·59	0·59	0·57	0·55	0·54
1·0	0·63	0·63	0·62	0·61	0·59

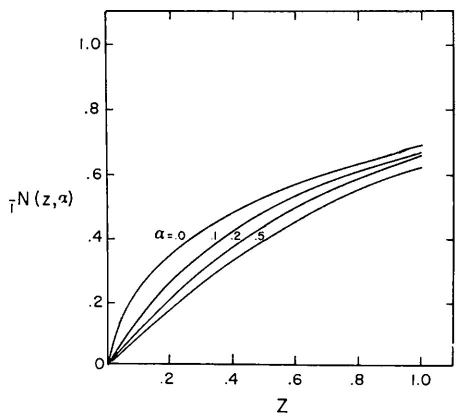

Fig. 2. The cumulative distribution function ${}_{\bar{1}}N(z,\alpha)$ for centrosymmetric reflections, with $\alpha = 0{\cdot}0, 0{\cdot}1, 0{\cdot}2$ and $0{\cdot}5$.

Table 4. ${}_{\bar{1}}N(z,\alpha)$ *for centrosymmetric reflections*

z	α 0·0	0·1	0·2	0·3	0·5
0·1	0·25	0·15	0·12	0·10	0·10
0·2	0·35	0·26	0·22	0·19	0·18
0·3	0·42	0·35	0·30	0·28	0·26
0·4	0·47	0·42	0·38	0·35	0·33
0·5	0·52	0·48	0·44	0·41	0·39
0·6	0·56	0·53	0·50	0·47	0·45
0·7	0·60	0·58	0·55	0·52	0·50
0·8	0·63	0·62	0·59	0·57	0·55
0·9	0·66	0·65	0·63	0·61	0·59
1·0	0·68	0·68	0·66	0·65	0·63

Curves of $N(z,\alpha)$ for non-centrosymmetric and centrosymmetric reflections appear in Figs. 1 and 2, respectively. Tabulated values for the various distribution functions are given in Tables 1–4. Integrals for the centrosymmetric distribution function were evaluated using Gaussian quadrature formulas (Stroud & Secrest, 1966).

For the special case of perfect twinning, these results agree with the distribution functions obtained by Stanley (1972), based on a consideration of the distribution of the mean intensity of pairs of reflections chosen at random from a population. The generality of the present approach has several advantages over Stanley's method, however, for deriving distribution functions: (i) the effect of twinning on any probability distribution for untwinned intensities may be evaluated using (2), whereas Stanley's approach restricts the untwinned probability distributions to certain specific classes; (ii) probability distributions may be obtained for arbitrary twinning fractions. However, the intensity distributions for perfect higher-order twins [for example, twinning by tetartohedry, in which each reflection may have contributions from four non-equivalent reflections (Catti & Ferraris, 1976)] would be more easily calculated using Stanley's method.

The criteria for independence of the probability distributions for twin-related reflections may be determined by considering the correlation of intensities from pairs of structures with the same lattice dimensions and number of atoms (Srinivasan & Parthasarathy, 1976). For the present problem, the two structures correspond to the twin-related domains, for which the coordinate sets are related by application of the twinning operation. The intensity distributions for a given reflection, **h**, for these structures will be uncorrelated if the average $\langle\cos(2\pi\mathbf{h}.\Delta\mathbf{r})\rangle$ over all the atoms vanishes, where $\Delta\mathbf{r}$ is the difference in coordinates between pairs of atoms in the two structures. Since twinning is facilitated in structures with pseudosymmetry, the two coordinate sets may be correlated, although not identical. Consequently, to ensure independence of the intensity distributions, it is essential to use reflections of sufficiently high order so that the non-integer part of $\mathbf{h}.\Delta\mathbf{r}$ is distributed randomly, and the cosine average vanishes. A similar point has been made by Stanley (1972).

The non-centrosymmetric distribution has been used to establish twinning in crystals of the protein complex between carboxypeptidase *A* and the potato carboxypeptidase inhibitor (Rees & Lipscomb, 1980). This complex crystallizes in space group $P3_2$, with two protease-inhibitor molecules in the asymmetric unit. A 180° rotation about the $[11\bar{2}0]$ axis relates the twin domains, which gives rise to pseudo $P3_221$ symmetry in the diffraction pattern. Heavy-atom difference-Patterson maps and rotation and translation functions demonstrated that the non-crystallographic twofold axis relating the two independent complexes is nearly parallel to the $[11\bar{2}0]$ axis, but fails to intersect the *c* axis by 2·3 Å. $N(z)$ plots for two data sets collected from single crystals are illustrated in Figs. 3 and 4. Reflections with $\sin^2\theta/\lambda^2$ between 0·004 and 0·012 Å^{-2} were used to calculate the distribution function, although the curve for crystal 101 was identical when an upper limit for $\sin^2\theta/\lambda^2$ of 0·032 Å^{-2} was used. The $N(z)$ distributions for the *h*0*l* reflections, which are unaffected by twinning, follow the theoretical untwinned distribution closely. The twinning fractions estimated from the $N(z)$ curves for the twinned reflections (18% for crystal 101 and 45% for crystal 150) agree to within several percent with the values calculated using the methods of Murray-Rust (1973) and Britton (1972). Finally, the $N(z)$ curves calculated using all the reflections clearly indicate the presence of twinning. This distribution could be calculated routinely in the preliminary stages of a crystal-structure determination to test qualitatively for the presence of twinning. By examining the intensity distributions for selected zones of reflections, the nature of the twinning operation could subsequently be established.

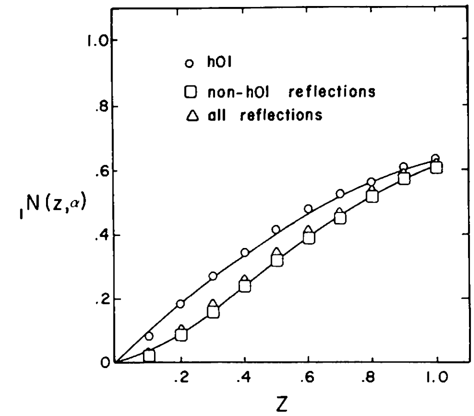

Fig. 3. Experimental points of $N(z,\alpha)$ for crystal 101 of the carboxypeptidase *A*–potato inhibitor complex. The solid lines indicate the theoretical curves for $\alpha = 0{\cdot}0$ and $0{\cdot}2$.

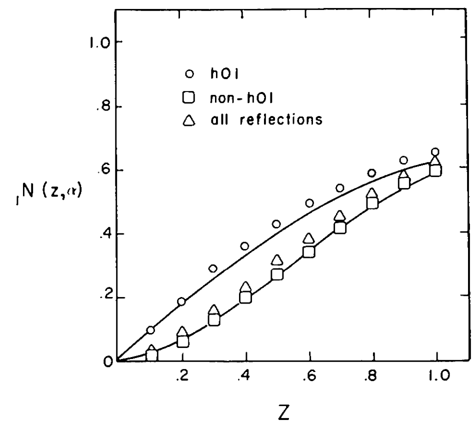

Fig. 4. Experimental points of $N(z,\alpha)$ for crystal 150 of the carboxypeptidase *A*–potato inhibitor complex. The solid lines indicate the theoretical curves for $\alpha = 0{\cdot}0$ and $0{\cdot}5$.

Although the distribution functions presented here are strictly valid only for structures which satisfy the assumptions used in deriving the untwinned intensity probability distributions, non-ideal conditions may be treated by substituting the appropriate distribution functions in (2). Twinning tests based on intensity statistics have the important advantage of not requiring knowledge of the twinning operation, or measurement of both twin-related reflections. Furthermore, such tests may distinguish perfectly twinned crystals from untwinned crystals of higher symmetry, or disordered crystals.

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