Introduction to single crystal X-ray analysis VIII. Key points for investigation and analysis of twins

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1. Introduction

Among the various analysis techniques, single crystal X-ray structure analysis can be regarded as the most effective analysis technique for acquiring knowledge about the 3-dimensional configuration of molecules. However, in single crystal X-ray structure analysis, there is also a major problem in that measurement cannot be done if the target sample will not form a single crystal. In addition, even if a crystal is obtained, it is often a twin.

Previously, if the crystal was a twin, the usual approach was to examine the crystallization conditions again, and repeat the process a number of times until a single crystal was obtained. However, due to the development of equipment and software in recent years, it has become possible to carry out a certain amount of analysis even if the crystal is twinning. Therefore, this paper discusses the steps for this procedure, from the method of determining twinning to the method of refinement.

2. Classification of twins

Twins are classified into various types based on how they are connected. At the broadest level, they can be classified into so-called twins—crystals connected in accordance with a specific crystallographic orientation—and polycrystals in which crystals are simply joined together (Fig. 1). Twins are divided further into merohedral twin where there is mutual overlap of reciprocal lattice points, and non-merohedral twin in which there is a partial overlap.

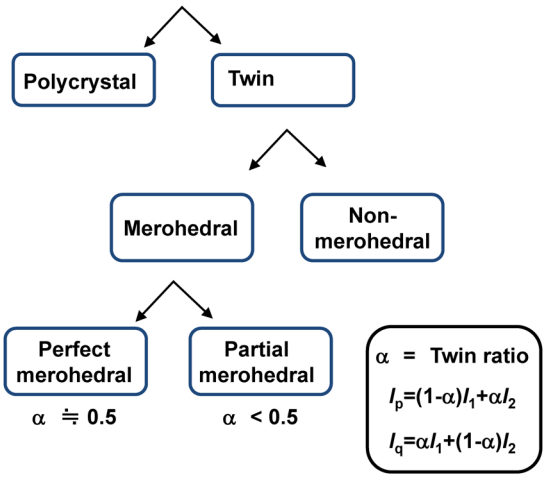

Fig. 1. Classification of twins.

The pattern of observed reflection differs between merohedral twin and non-merohedral twin, and thus there are major differences in the pertinent data processing and analysis. In addition, merohedral twins can be divided further into perfect merohedral twin, whose twin ratio is 1 : 1, and partial merohedral twin which are not perfect.

3. Considering twins based on the reciprocal lattice

This section explains the difference between merohedral twin and non-merohedral twin, from the perspective of the reciprocal lattice[1].

3.1. Case of merohedral twin

Figure 2 shows the reciprocal lattice points of a certain crystal. Looking at the point indicated in red in the diagram at right, when this point is rotated to the right by 90°, it overlaps with the original point. When it is again rotated by 90°, and yet again by 90°, it overlaps again. This shows that the crystal has 4-fold rotational symmetry.

Here it is assumed that there is a crystal with reciprocal lattice points (right side, Fig. 3) in a mirror image relationship with the original reciprocal lattice points (left, Fig. 3).

In the case where these two overlap as twins (Fig. 4), mirror planes for the reciprocal lattice points increase, as indicated by the red solid lines and dashed lines, and it appears as through the original crystal has high symmetry. This is the distinguishing feature of merohedral twin.

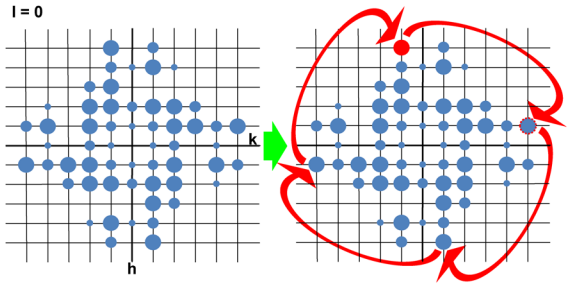

Fig. 2. Reciprocal lattice points of crystal with 4 rotation axes. Lattice point size indicates diffraction intensity.

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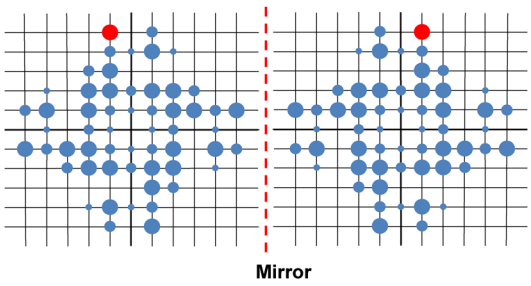

Fig. 3. Mirror image relationship of reciprocal lattice points.

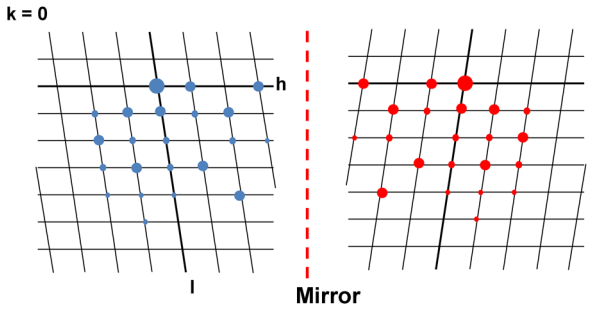

Fig. 5. Reciprocal lattice points of crystals in a mirror image relationship.

3.2. Case of non-merohedral twin

Figure 5 shows the reciprocal lattice points for a certain crystal. The reciprocal lattice points in the right and left diagrams stand in a mutual mirror image relationship.

Figure 6 shows the result when these two are overlapped. There are some overlapping points, but almost all of the points do not overlap, and are each independent. This is the distinguishing feature of non-merohedral twin.

In this way, even if the twin is the same, major differences may arise in the appearance of reciprocal lattice points, i.e., in the diffraction image.

4. Determination of twinning

Now, let us look at what sort of indicators actually appear in crystal observation and data.

4.1. Determination based on crystal observation

To begin, the following summarizes some of the abnormalities which appear in crystal observation:

1) It appears that multiple crystals are overlapped.
2) Stripes appear in the crystal.
3) In plate crystals, thin crystal fragments are stripped off when touched with the point of a needle, etc.
4) Crystal faces are not well defined.
5) When observed with polarized light, uniform quenching does not occur with respect to the crystal face.

Figure 7 shows a crystal photograph of a twin of cytidine. As can be seen, it has stripes like those described in 2) above. In this way, it is possible, to a certain extent, to determine whether a crystal is a twin based on information obtained from crystal observation. Therefore, when selecting crystals, it is best to avoid selecting crystal of this type if possible.

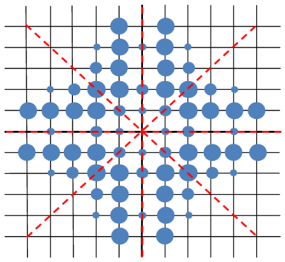

Fig. 4. Reciprocal lattice points when crystals in a mirror image relationship are overlapped. Planes increase, and the crystal has higher symmetry than the original crystal.

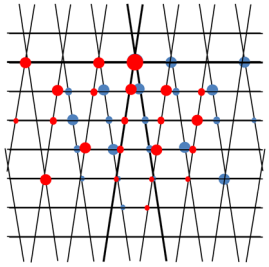

Fig. 6. Reciprocal lattice points when crystals in a mirror image relationship are overlapped. A number of reflections are observed to be split and separated.

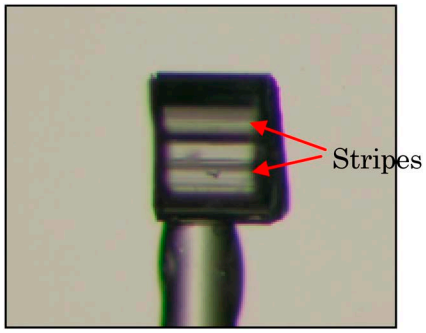

Fig. 7. Cytidine twin.

4.2. Determination based on diffraction data

4.2.1. Case of merohedral twin

Next, the following summarizes the indicators of a merohedral twin in data processing and structure analysis.

Distinguishing features include:

1) The geometrical form of the unit lattice has higher symmetry than the actual Laue symmetry.
2) The Rint value for high Laue symmetry is somewhat higher than the Rint value for lower Laue symmetry.
3) The crystal system tends to occur with tetragonal, trigonal, hexagonal, or cubic space groups.
4) The extinction rule does not apply to any of the space groups.
5) Mean value $|E^2-1| \ll 0.736$ (non-centrosymmetric)
6) There appear to be no problems with measurement

data, but the structure cannot be solved.

7) The structure can be obtained to a certain extent, but the R1 value is high.

As described above, phenomena 1)–4) occur because the apparent symmetry has been set higher than the actual symmetry. Items 5)–7) are described below, in the context of an example of actual analysis.

4.2.1. Case of non-merohedral twin

The following summarizes the indicators of a non-merohedral twin. They include:

1) There is an abnormally long axis.
2) Problems occur in cell determination and refinement.
3) A number of reflections are sharp, but in other reflections, diffraction points are observed to split.
4) The value of K=mean(Fo^2)/mean(Fc^2) is systematically high in weak reflection.
5) In reflections with no match between Fo and Fc, Fo is larger than Fc.
6) Abnormal electron density remains during analysis. That electron density cannot be explained, even if a solvent or disorder are assumed.

These phenomena can be seen not only with twins, but also with crystals containing cracks.

Now, the following will describe how data processing should actually be done for such crystals.

5. Twin measurement and data processing

Looking at actual diffraction images, it is evident that there are peak overlap patterns like those in Fig. 8.

With regard to measurement, in Fig. 7a, there is a complete overlap, and separation is impossible. In cases like those in b and c, it is important to set the smallest possible oscillation angle at measurement, and separate the peaks to the greatest possible extent. In particular, it is necessary, particularly in the case of an imaging plate detector, to decrease the oscillation angle to 5–3° in the case of a Cu beam source, and to 3–1° in the case of a Mo beam source. It is also effective to reduce integration box size so there is as little overlap as possible between integration boxes, as shown in Fig. 7b.

Data processing for each twin pattern can be summarized as in Fig. 9.

6. Analysis of merohedral twin

Analysis of non-merohedral twin has been presented previously in this journal[2], and thus this paper describes only the case of merohedral twins.

In the case of merohedral twin, there is a complete overlap of reflections. Therefore separation is impossible, and it is difficult to see into the structure at the stage of data measurement and processing. Furthermore, data measurement and processing are performed as usual, in the same way as in the single crystal case, and twin law processing is performed during analysis. In addition, with merohedral twins, there is an overlap of crystals in accordance with specific rules, and therefore twin laws are determined which can be applied to each Laue group. For example,

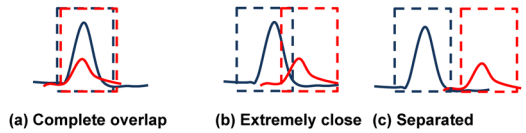

Fig. 8. Extent of peak overlap.

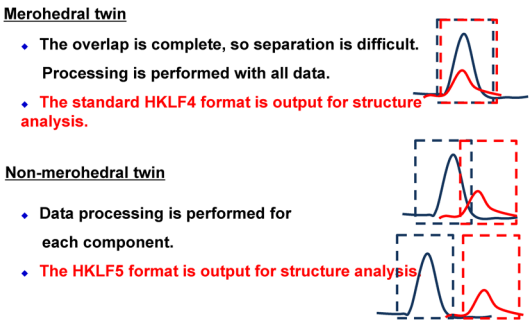

Fig. 9. Processing method for twins.

Table 1. Examples of twin law for merohedral twin.

Actual Laue group	Apparent Laue group	Twin law		
4/m	4/mmm	0 1 0	1 0 0	0 0 −1
−3	−31 m	0 −1 0	−1 0 0	0 0 −1
−3	−3 m1	0 1 0	1 0 0	0 0 −1
−3	6/m	−1 0 0	−1 0 0	0 0 1
−3	6/mmm	0 −1 0	−1 0 0	0 0 −1
		0 1 0	1 0 0	0 0 −1
		−1 0 0	0 −1 0	0 0 1
−3 ml	6/mmm	−1 0 0	0 −1 0	0 0 1
−31 m	6/mmm	0 1 0	1 0 0	0 0 −1
6/m	6/mmm	0 1 0	1 0 0	0 0 −1
m−3	4−3 m	0 1 0	1 0 0	0 0 −1

if the crystal is originally regarded as a single crystal and processing is completed while assuming 6/mmm in the data processing stage, then there is a possibility that the sample is a twin of a crystal with a Laue group such as −3, −3 ml, −31 m or 6/m (Table 1).

Next, the following discusses the procedure up to analysis, taking as an example an actual merohedral twin seen at our company.

For a crystal of the compound in Fig. 10, it was possible to perform processing with the space group $C222_1$. Statistical values in data processing were good, and the value of R_{merge} was also comparatively low (6.19%). However, when analysis was attempted, it was not possible to determine the initial structure (Table 2).

In a case of this type, it is necessary to check for the presence of the signs indicating a merohedral twin described in 4.2.1. The most easily investigated item is the mean value of $|E^2-1|$, i.e., $\langle|E^2-1|\rangle$. E is the normalized structure factor used in the direct method.

Table 2. Ordinary analysis results.

Octhorombic:	$C222_1$
a:	5.30045(12)
b:	12.9001(3)
c:	28.2847(7)
alpha:	90.000
beta:	90.000
gamma:	90.000
volume:	1934.01(8)
Rmerge:	6.19

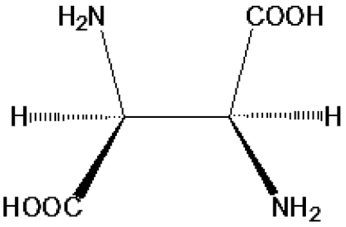

Fig. 10. Structure of 2,3-diamino-succinic acid.
(Courtesy of Dr. Shohei Tashiro, School of Science, University of Tokyo).

sir2004.out (If SHELXS is used: SHELXS.out)

Average values

Average		Numeric		Graphic	
	all data	acentric	centric	hypercentric	a. c. h.
mod(E)	0.927	0.886	0.798	0.718	*
E**2	1.000	1.000	1.000	1.000	
E**3	1.216	1.329	1.596	1.916	*
E**4	1.634	2.000	3.000	4.500	*
E**5	2.398	3.323	6.383	12.260	*
E**6	3.796	6.000	15.000	37.500	*
mod(E**2-1)	0.591	0.736	0.968	1.145	*
(E**2-1)**2	0.634	1.000	2.000	3.500	*
(E**2-1)**3	0.893	2.000	8.000	26.000	*

Fig. 11. Sir2004 output file.

The value of $\langle|E2-1|\rangle$ is listed in the output file of the direct method program (Fig. 11). The theoretical value of $\langle|E^2-1|\rangle$ for non-centrosymmetric structures is 0.736, and the theoretical value for centrosymmetric structures is 0.968. Therefore, this is used to determine whether or not there is a center of symmetry.

The value of $\langle|E^2-1|\rangle$ is also a basis for determining the presence of a merohedral twin. The fact that the $\langle|E^2-1|\rangle$ value is significantly less than the theoretical value can be regarded as indicating that the degree of reflection strength/weakness is low, i.e., that reflections overlap due to the merohedral twin, resulting in averaging of reflection intensity, and thus reduced variation in intensity. Conversely, if this value is small, it suggests the possibility of a merohedral twin.

The value of $\langle|E^2-1|\rangle$ can be confirmed from the

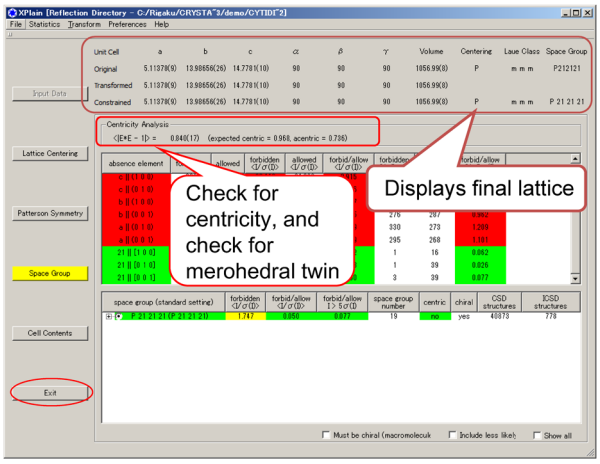

Fig. 12. XPlain screen.

Space group: $P2_1$

Twin law: -1 0 0 0 -1 0 1 0 1

BASF 0.5

Final result:

R1=0.2106 → R1=0.0558

wR=0.5204 → wR=0.1655

BASF=0.510(3)

Flack Parameter: -0.03(4)

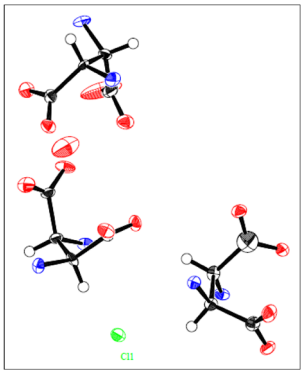

Fig. 13. Analysis results when twin law is incorporated.

output file for the direct method, as indicated above, but in the space group determination which is the previous stage of phase determination, it is possible to recognize the possibility of a merohedral twin at an earlier stage if the space group determination program "XPlain" is used. This program included with CrystalStructure 4.0 and higher.

With XPlain, it is possible to execute each step, from lattice transformation and determination of the presence of a center of symmetry, to determination of the space group, while visually reviewing the situation. At the final stage of space group selection, the value of $\langle|E^2-1|\rangle$ is calculated and displayed (Fig. 12).

As a result of carrying out the usual analysis, the values were R1=21% and wR=52% for the crystal shown in Fig. 10. The value of $\langle|E^2-1|\rangle$ is significantly lower than the theoretical value, and thus the possibility of a merohedral twin was suspected, and the possible twin laws were examined. As a result, when refinement was carried out incorporating twin law −1 0 0 0 −1 0 1 0 1, R1 was improved from 21.06% to 5.58%, and wR was improved from 52.04% to 16.55%. In the end, analysis results were sufficient to enable determination of the absolute structure using the Flack parameter (Fig. 13).

This shows how, in the case of a merohedral twin, the quality of analysis is dramatically changed simply by performing twin law processing.

7. Conclusion

As in the case of the merohedral twin presented here, there are many cases where a determination cannot be made until analysis reaches a certain stage. It is not easy to see through every case, but if the distinguishing features of twins presented here are committed to memory, it should be possible for an analyst to hit upon the possibility of a twin when he realizes, "What? Something is strange here."

As has been presented, there are cases where sufficiently good results can be obtained, even with twins, but there is no change in the fact that obtaining a single crystal, by for example examining crystallization conditions or using another crystal, will facilitate subsequent measurement and analysis.

References

(1) P. Müller, R. Herbst-Irmer, A. L. Spek, T. R. Schneider and M. R. Sawaya: *Crystal Structure Refinement—A Crystallographer's Guide to SHELXL—*, Oxford University Press, (2006).

(2) H. Sato and A. Yamano: *Rigaku Journal (English version)*, **27** (2011), No. 2, 22−23.