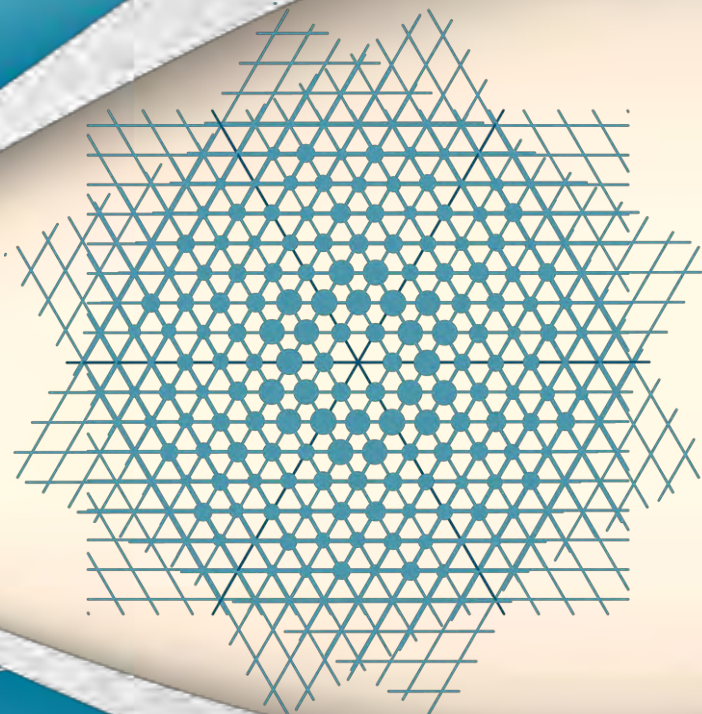


CCP4 Fukuoka 2012

Twinning

Andrea Thorn



OVERVIEW

Introduction:

- Definitions, origins of twinning

Merohedral twins:

- Recognition, statistical analysis: H plot, Yeates-Padilla plot
- Example
- Refinement and R values
- Reticular merohedry

Pseudo-merohedral twins:

- Recognition and treatment

Non-merohedral twins:

- Recognition
- Cell search and integration
- Examples

Not a twin!

Summary and literature



GEORG-AUGUST-UNIVERSITÄT
GÖTTINGEN

Is this a twin?



Image property of Rob Lavinsky,
iRocks.com – CC-BY-SA-3.0

Definition of twinning

Twin: *Two or more crystals of the same species are joined together in different orientation*

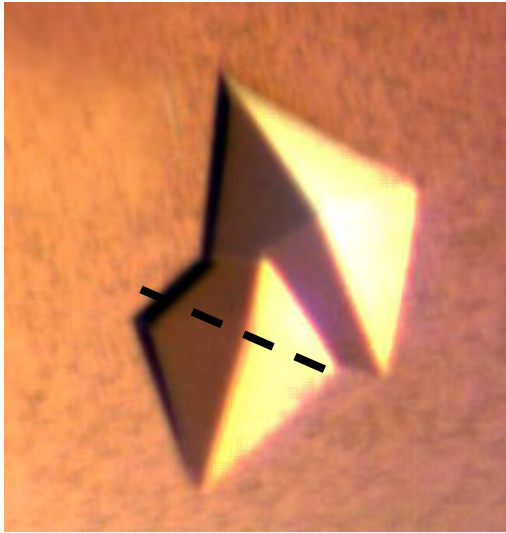
*α is the **twin fraction**:*

$$I_{1+2} = \alpha I_1 + (1-\alpha)I_2$$

*The **twin law** (twin operator) is the operator between the cojoined crystals – an additional symmetry operation.*

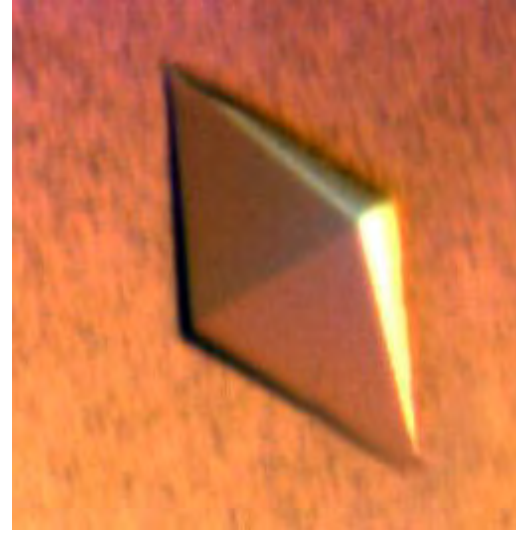
Definition of twinning

Macroscopic twin



Disecting a part might
give a single crystal!

Microscopic twin



Crystal looks fine, but
is twinned.

Definition of twinning

Identifying the twinning type:

**Non-merohedral
twinning**

Merohedral twinning

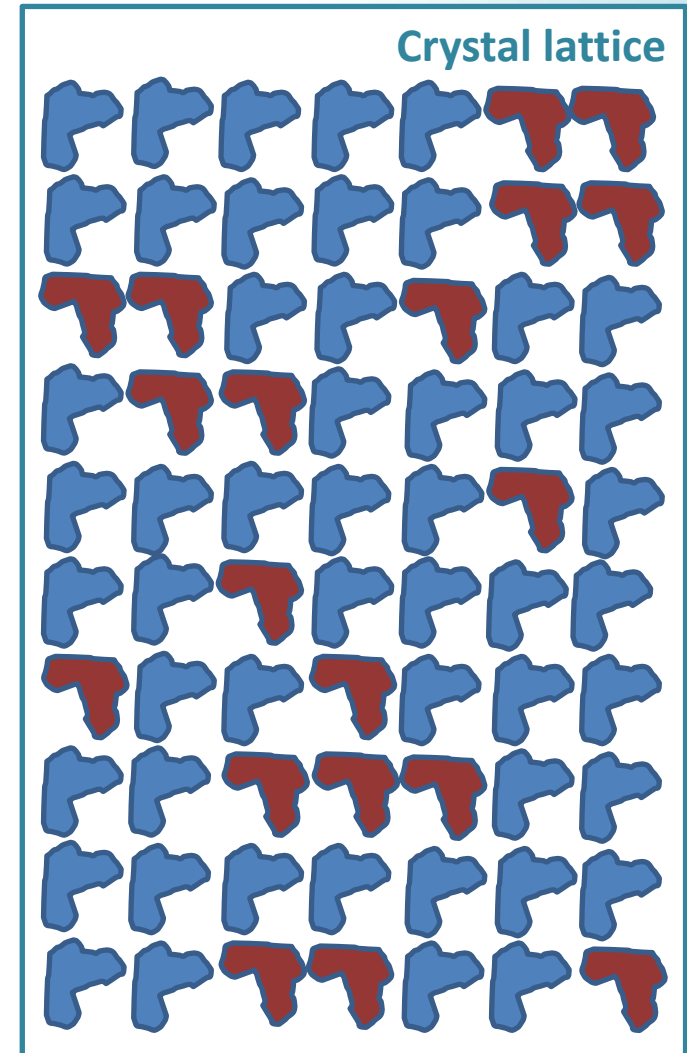
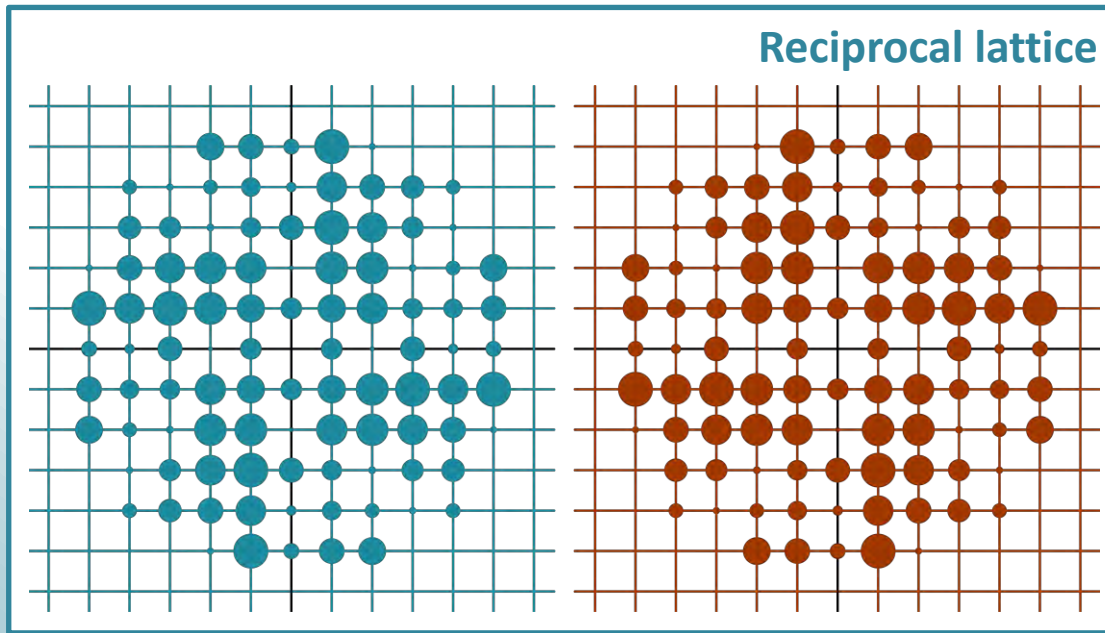
**Pseudo-merohedral
twinning**

Finding the twin law and the twin fraction α

Treat data accordingly

Merohedral twinning

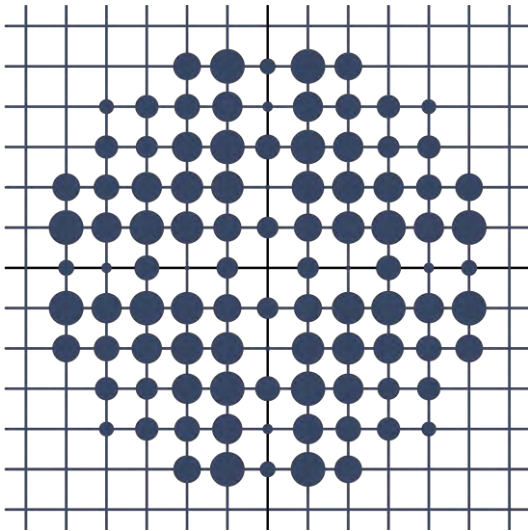
Twin law: Symmetry operator of the crystal system, but not the crystal's point group



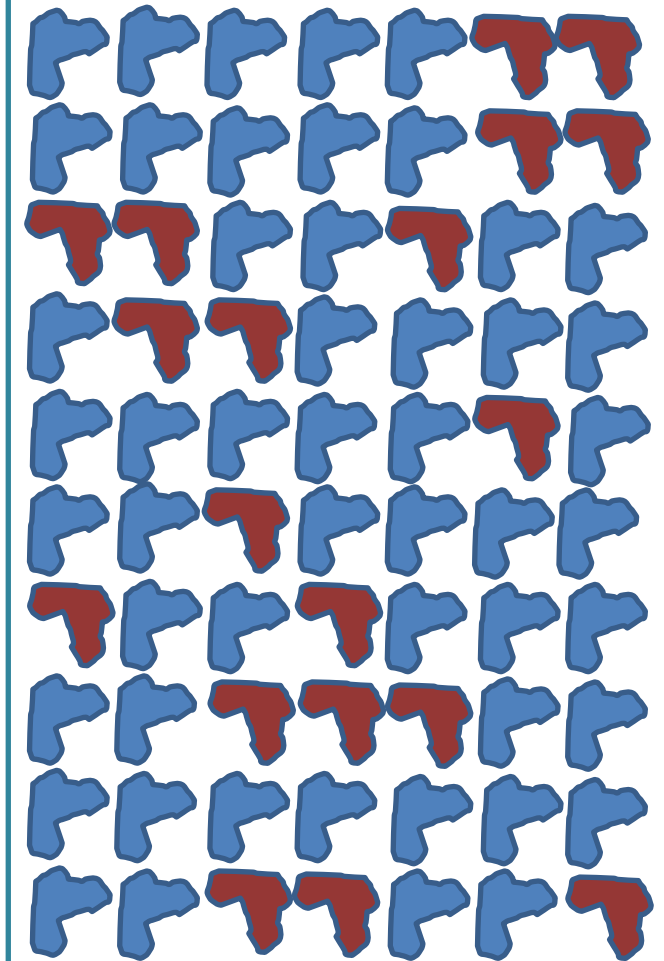
Merohedral twinning

Twin law: Symmetry operator of the crystal system, but not the crystal's point group

Reciprocal lattice

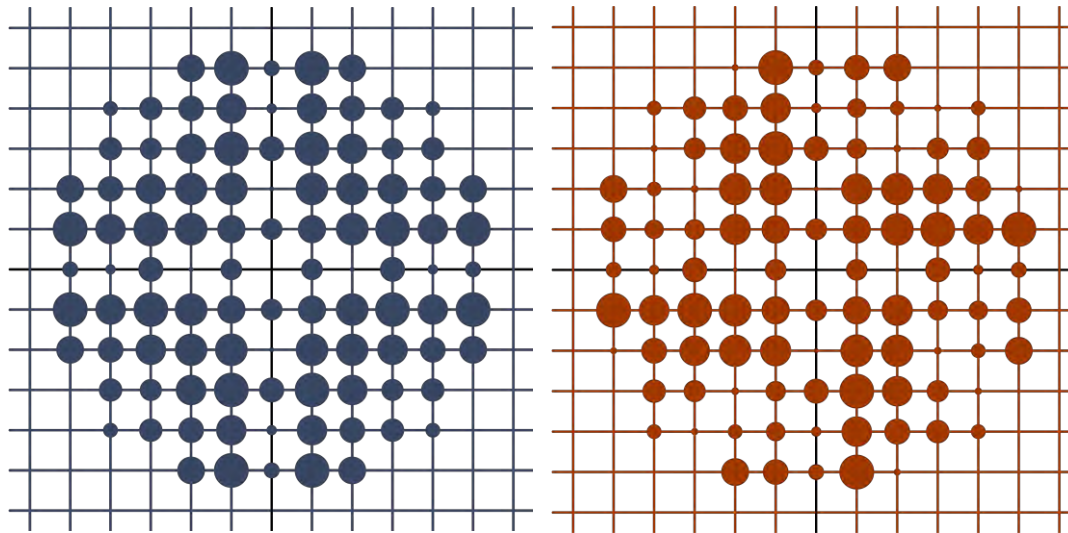


Crystal lattice



Merohedral twinning

Depending on the twin fraction α : The **intensity distribution** has been changed by the twinning. Also, symmetry looks higher!



Merohedral twinning

How to recognize?

- Lower symmetry point group of the trigonal, hexagonal, tetragonal or cubic system
- **Symmetry looks possibly higher than it really is**
- **Changed intensity distribution**
- R_{int} for the higher symmetry
- Typical space group
- No structure solution

Merohedral twinning

Space groups

- Only trigonal, hexagonal and tetragonal space groups
- There is only a limited number of potential twin laws.
- Typical examples:

Is:	Looks like:
$P4_1$	$P4_122$ or $P4_12_12$
$P3_1$	$P3_112$ or $P3_121$ or $P6_4$ or $P6_2$

- Merohedral twinning occurs more frequently than commonly recognized!

Merohedral twinning

Is it really a merohedral twin?

How big is the twin fraction α ?

Two intensities I_1 and I_2 are related by twin law:

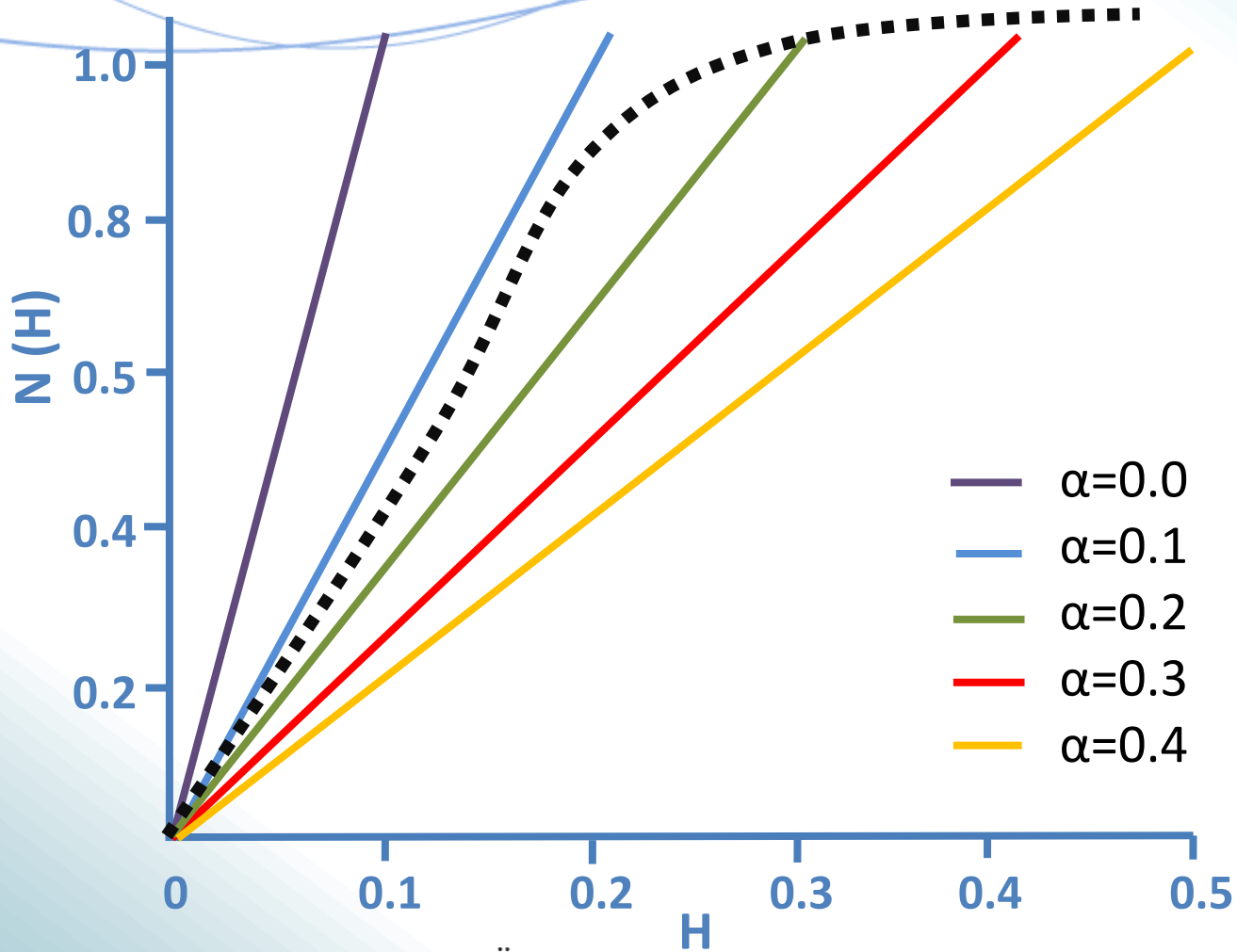
$$H = \frac{|I_A - I_B|}{I_A + I_B}$$

(For acentric reflections:)

**Cumulative probability
distribution**

$$N(H) = \frac{1}{2} \left[1 + \frac{H}{1 - 2\alpha} \right]$$

Merohedral twinning



Merohedral twinning

Drawbacks

Perfect twins are not detectable with this method.

$$H = \frac{|I_A - I_B|}{I_A + I_B}$$

$$I_A = I_B \Rightarrow H = 0$$

We need another test!

Merohedral twinning

Yeates-Padilla Test

The reflections with the intensities I_C and I_D are close to each other in reciprocal space:

$$L = \frac{I_C - I_D}{I_C + I_D}$$

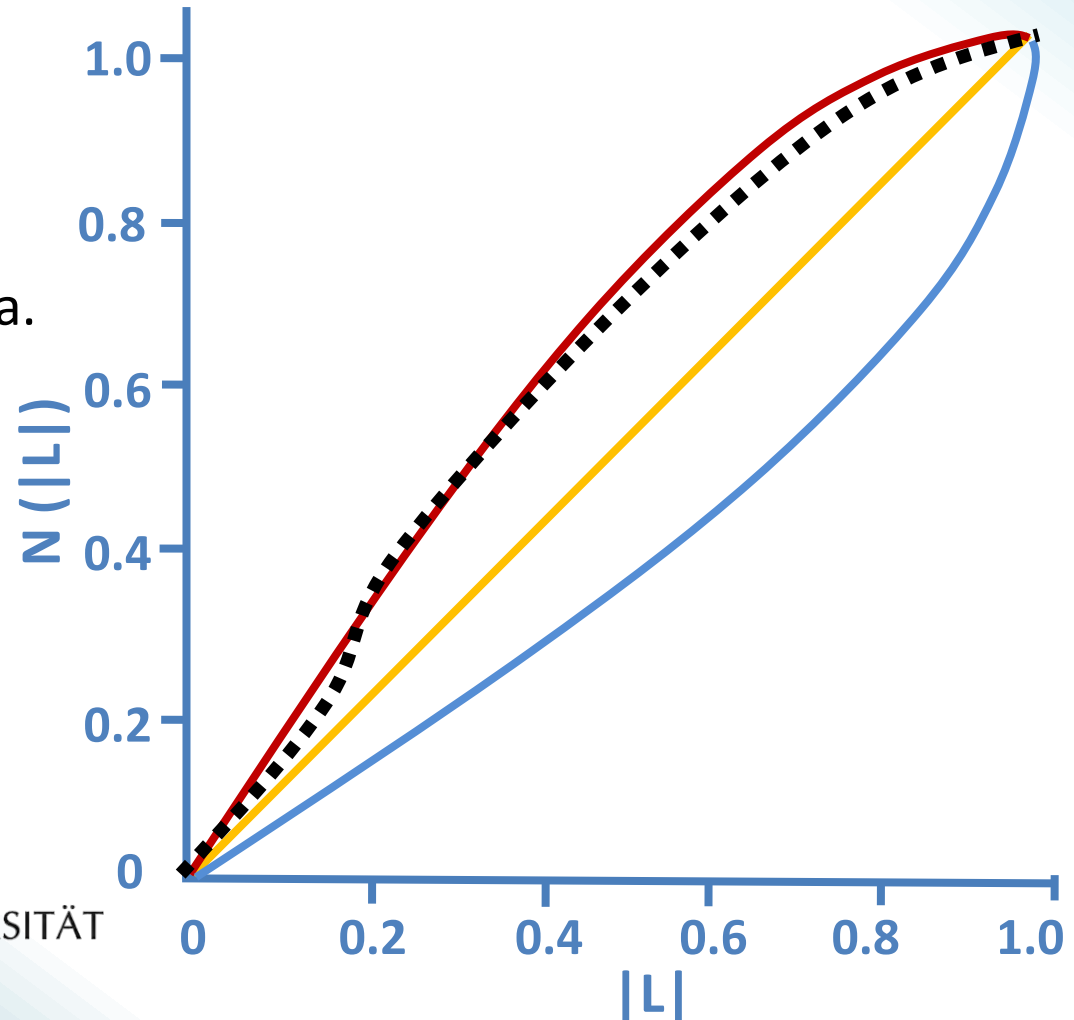
$$N(L) = \frac{1}{2} \left[1 + \frac{L}{1 - 2\alpha} \right]$$

Merohedral twinning

Yeates-Padilla Test

This test shows the expected cumulative distributions for perfect twins and untwinned data. Partial twins will be between the curves.

- acentric reflections, perfect twin
- acentric reflections, untwinned
- Centric, untwinned



Merohedral twinning

- Using the Yeates-Padilla test for the PDB shows that twinning occurs more frequently than commonly recognized!
- Both the **H test** as well as the **Yeates-Padilla plot** can be generated using **CTRUNCATE** or **DETWIN** via CCP4i.
- The $\langle |E^2 - 1| \rangle$ could be too low for twins (below 0.736).

Merohedral twinning

Truncate - Convert Intensities to SFs Initial parameters from /net/home/art/von_regine/baker/CCP

Job title Trying Truncate

Input data are amplitudes

☐ Use old Truncate program

MTZ in baker 115t.mtz Browse View

F F SigF SIGF

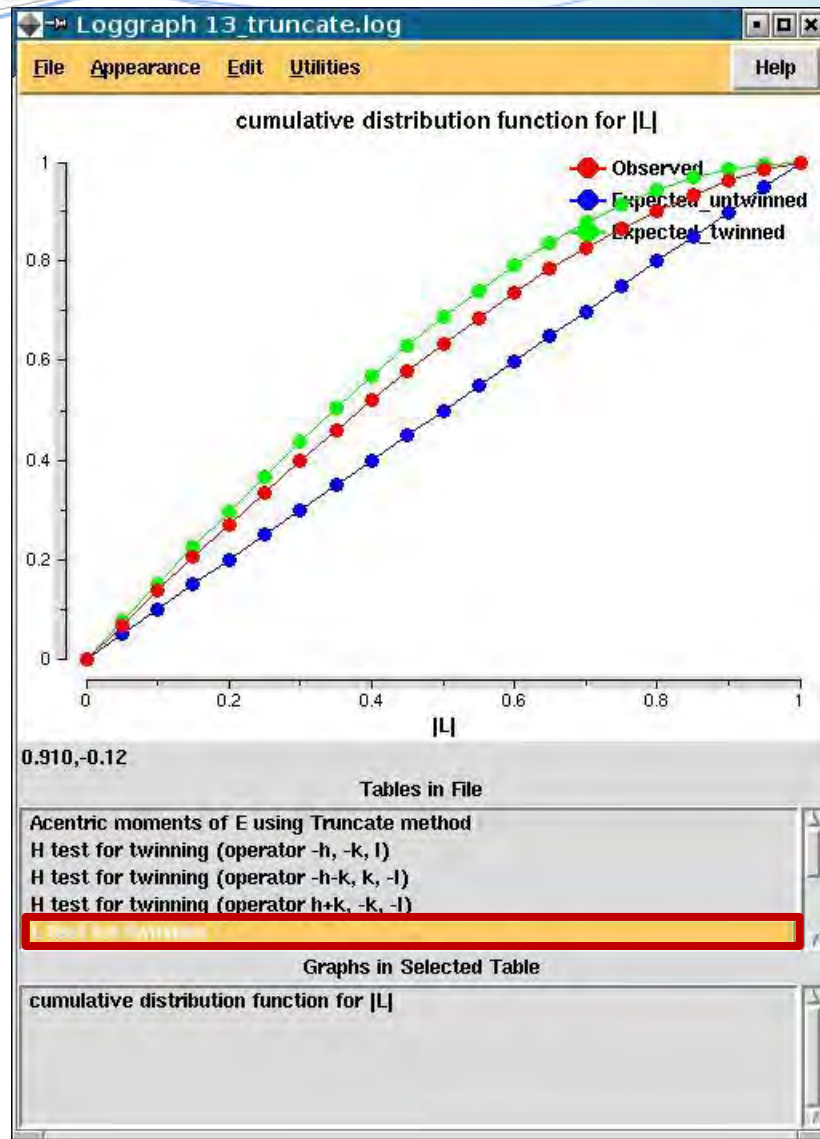
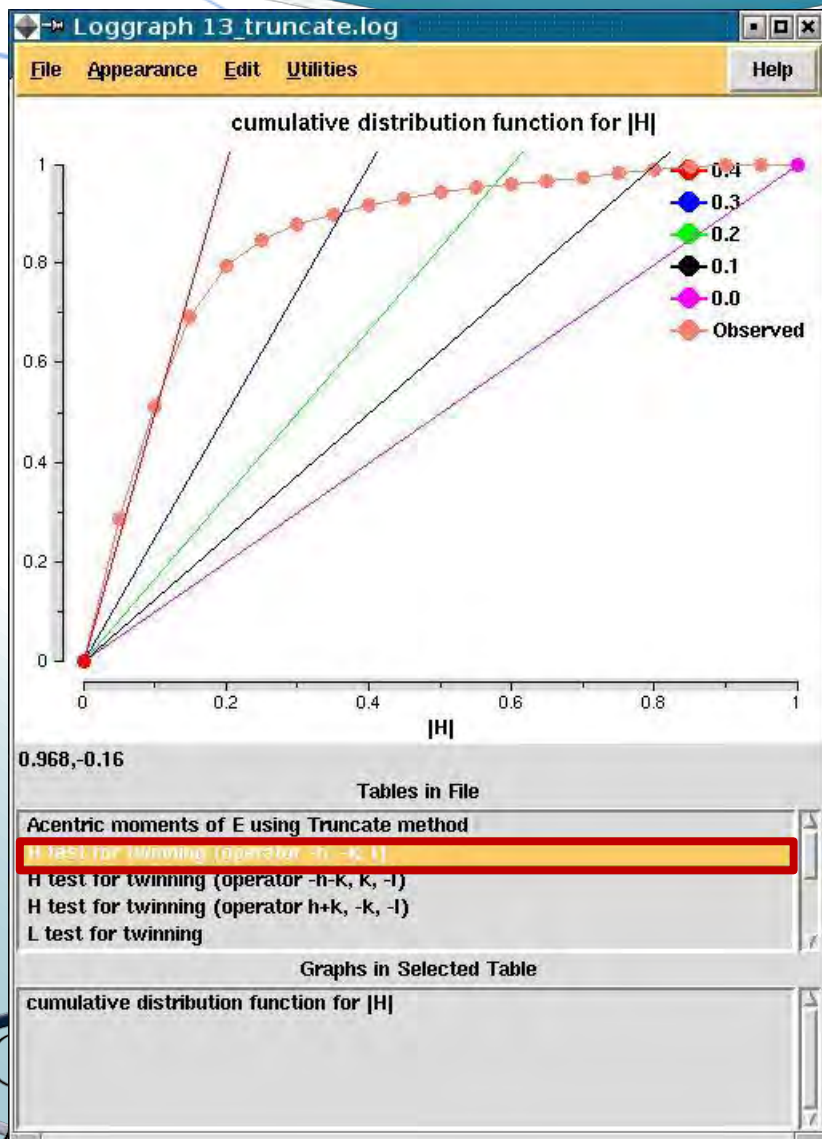
Truncate options

☐ Apply anisotropy correction

☐ Number of residues in asymmetric unit

Run Save or Restore Close

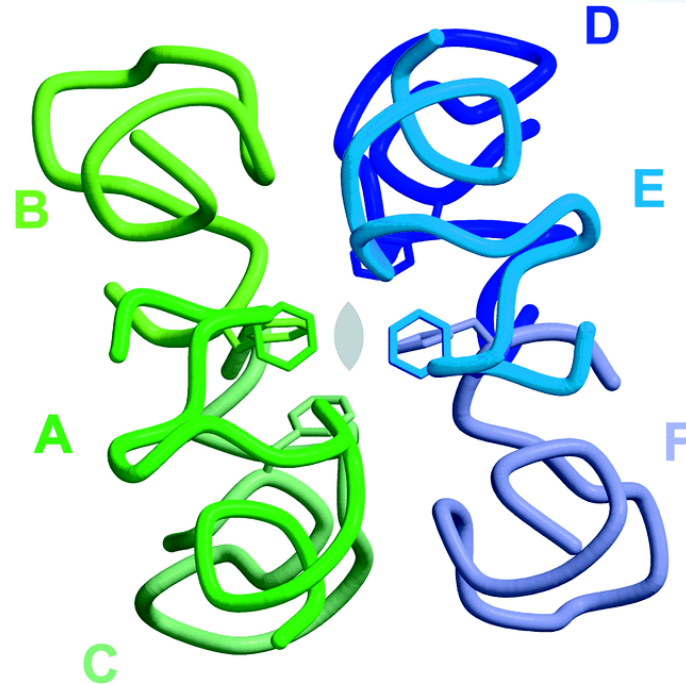
Merohedral twinning



Merohedral twinning

R_{int} in Mersacidin

Resolution (Å)	1.06
$\langle E^2 - 1 \rangle$	0.649
R_{int} (%) $P3_2$	4.9
R_{int} (%) $P3_221$	19.5
R_{int} (%) $P3_212$	44.3



An additional hint could be different R_{int} for higher symmetry space group for different crystals of comparable quality.

* *Acta Cryst* (2000). D56, 705-713

Merohedral twinning

How to integrate?

In lower symmetry group with any integration program!

Structure solution

MR	O good	Sometimes, detwinning the data helps here! SHELXD can use twin laws
MAD, SAD	O ok	
S-SAD	X too sensitive to noise	
SIR, MIR	X several crystals needed	

Merohedral twinning

Refinement

- Several protein refinement programs offer option for the refinement of merohedral twins, for example REFMAC, CNS, phenix.refine and SHELXL. In REFMAC, the twin law is determined automatically.
- Do not use a merohedral twin refinement on data which is not twinned. It will lower the R value possibly, but it is not a valid treatment!

Merohedral twinning

Problematic: R factors

- R_{free} set should include all twin-related reflections.
- R factors may be lower than in single crystals.
(Random R value goes down from 58.5% to 50%!)
- Difference density might have fewer features, as the twinned reflections add noise.

Merohedral twinning

Run Refmac5

Job title

Do using input

☐ Input fixed TLS parameters

PDB in

PDB out

LIB in

Output lib

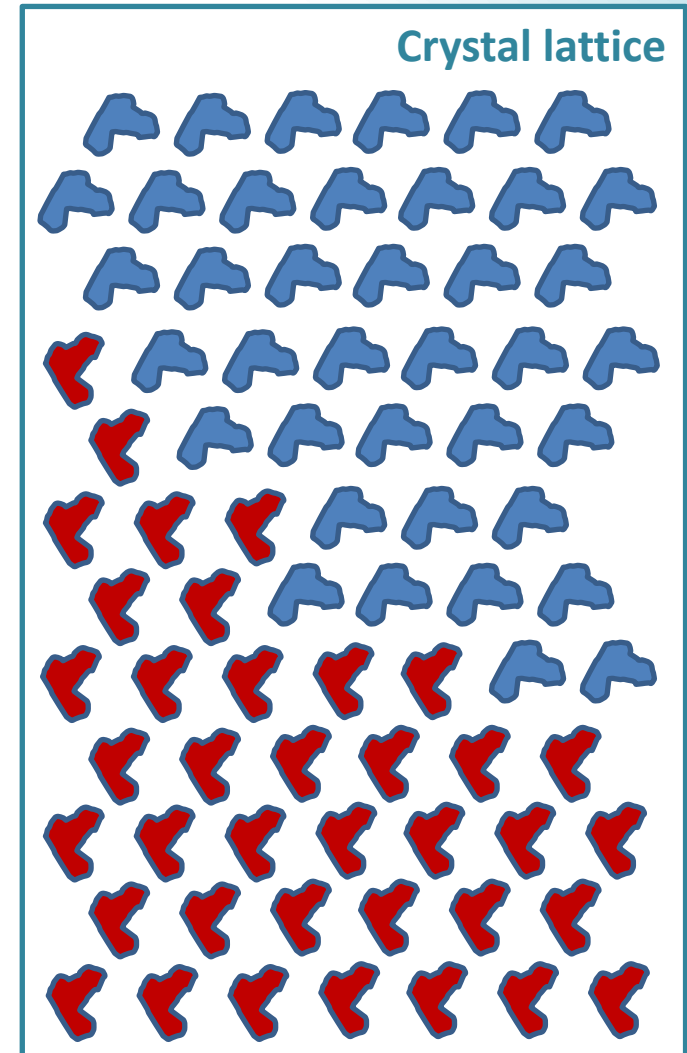
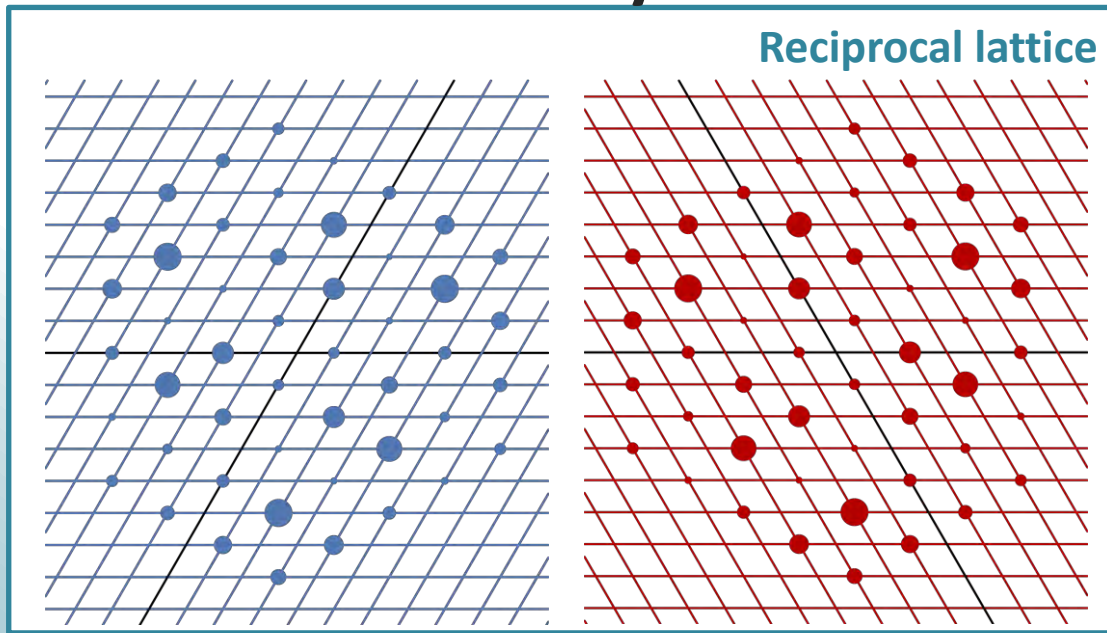
Merohedral twinning

Twin law: Symmetry operator of the crystal system, but not the crystal's point group

- Only in tetragonal, trigonal, hexagonal and cubic space groups possible.
- Exact overlap of reciprocal lattices, but different intensity distribution.
- Usually good results.

Reticular merohedry

**Most common case:
Obverse/reverse twinning in a
rhombohedral crystal**



Reticular merohedry

The typical case

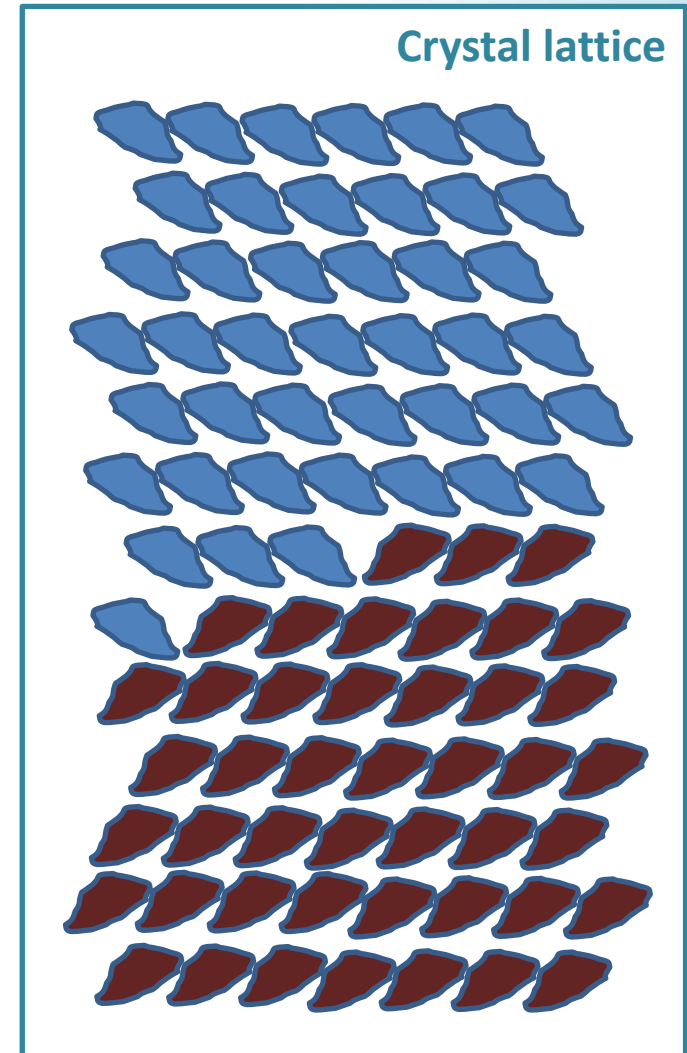
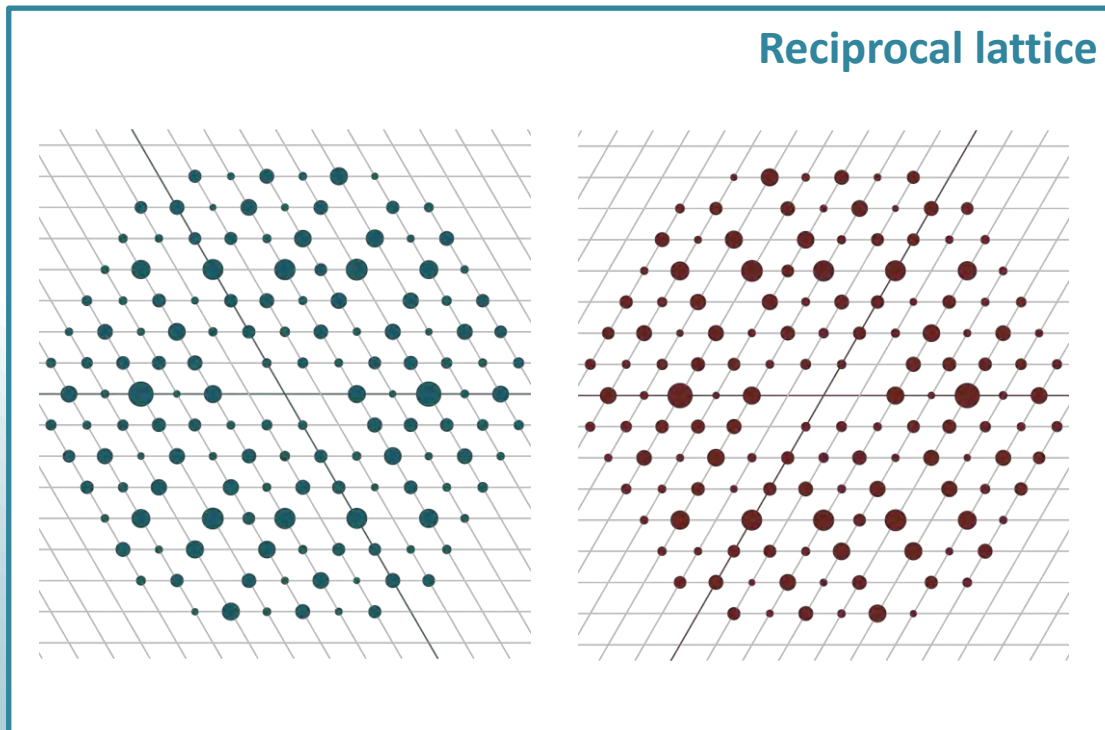
Is:	Looks like:
R32	$P3_121$
R3	$P3_1$

1/3 of all reflections are missing.

The missing reflections form a funny pattern, which is inconsistent with any systematic absence.

Pseudo-merohedral twinning

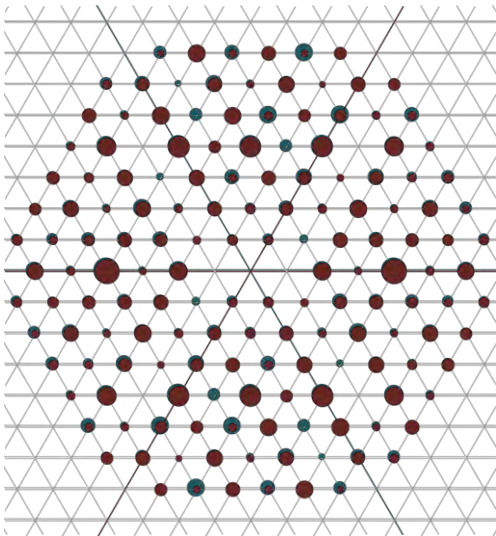
Twin law: Belongs to a higher crystal system than the structure.



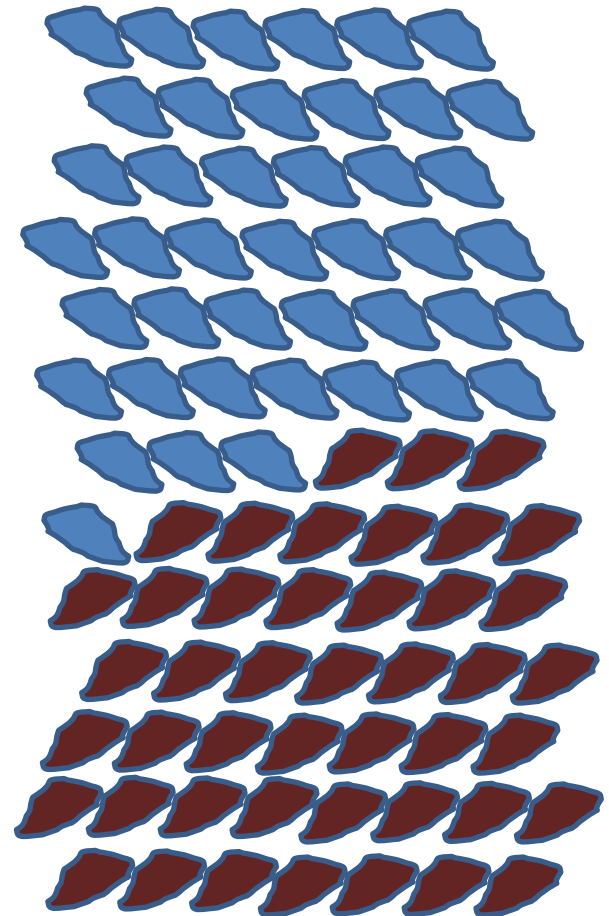
Pseudo-merohedral twinning

Twin law: Belongs to a higher crystal system than the structure.

Reciprocal lattice



Crystal lattice



Pseudo-merohedral twinning

Pseudo-merohedral twinning is only possible if the real unit cell can be transformed into one of a higher crystal system.

Example:

- Monoclinic with $a \approx c$, $\beta \approx 90^\circ$

Is:	Looks like:
$P2_1$	$P222_1$ or $C222_1$

Pseudo-merohedral twinning

How to recognize?

- Like a merohedral twin. The real space group belongs to another crystal system than the observed one.
- The overlap of the lattices might not be perfect for all reflections.
- All hints for merohedral twinning might also work for pseudo-merohedral ones.
- R_{int} behaves like in merohedral twins.

Pseudo-merohedral twinning

How to treat?

- Treatment is very similar to the one for merohedral twins.
- Most programs that can process merohedral data will also process pseudo-merohedral one.
- Be careful to choose the right (lower symmetry) crystal system.

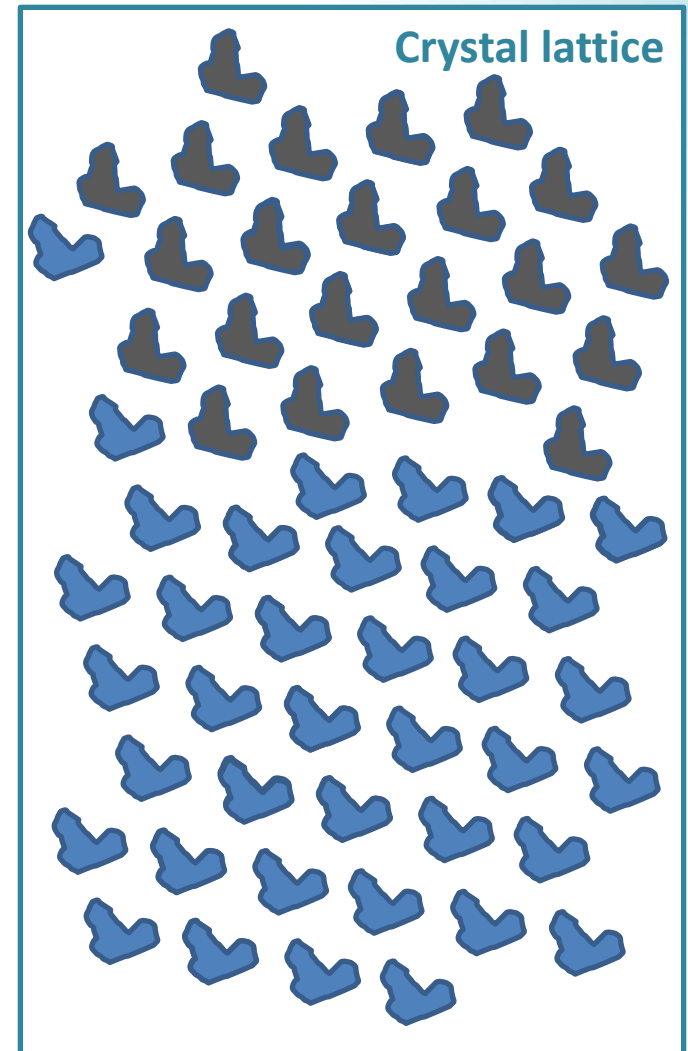
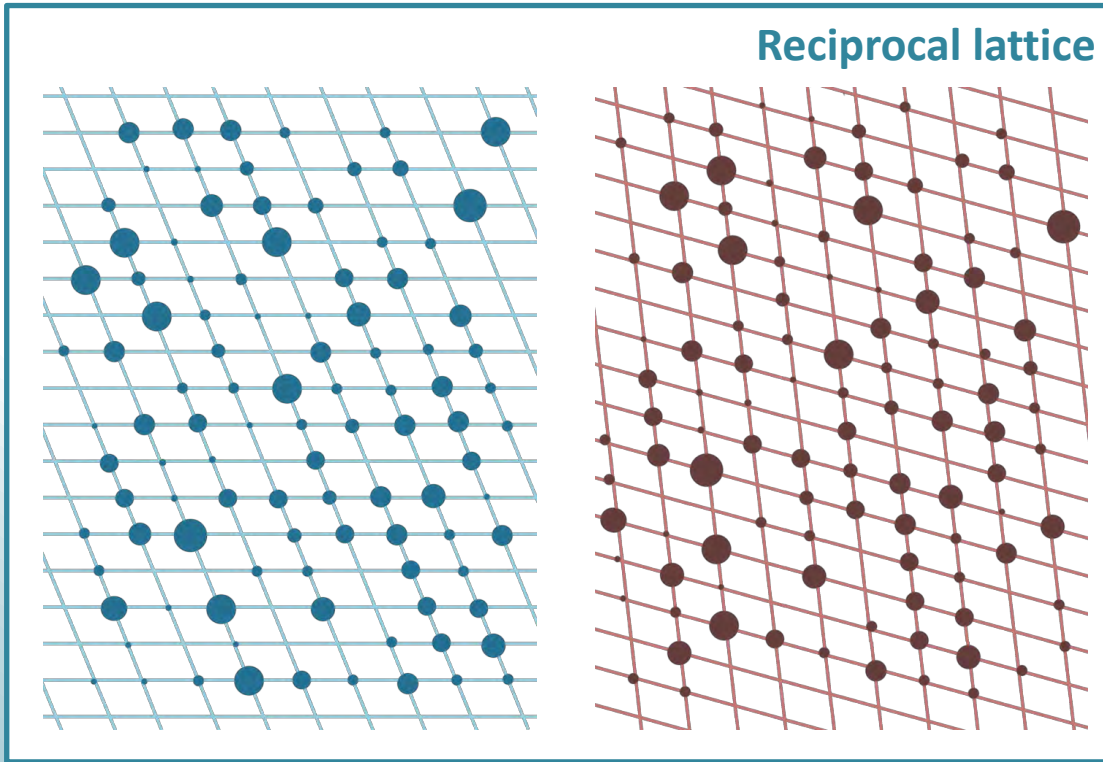
Pseudo-merohedral twinning

Twin law: Belongs to a higher crystal system than the structure.

- Recognition from frames or as for merohedral twins.
- Usually good results.

Non-merohedral twinning

Twin law is relatively arbitrary.



How to recognize?

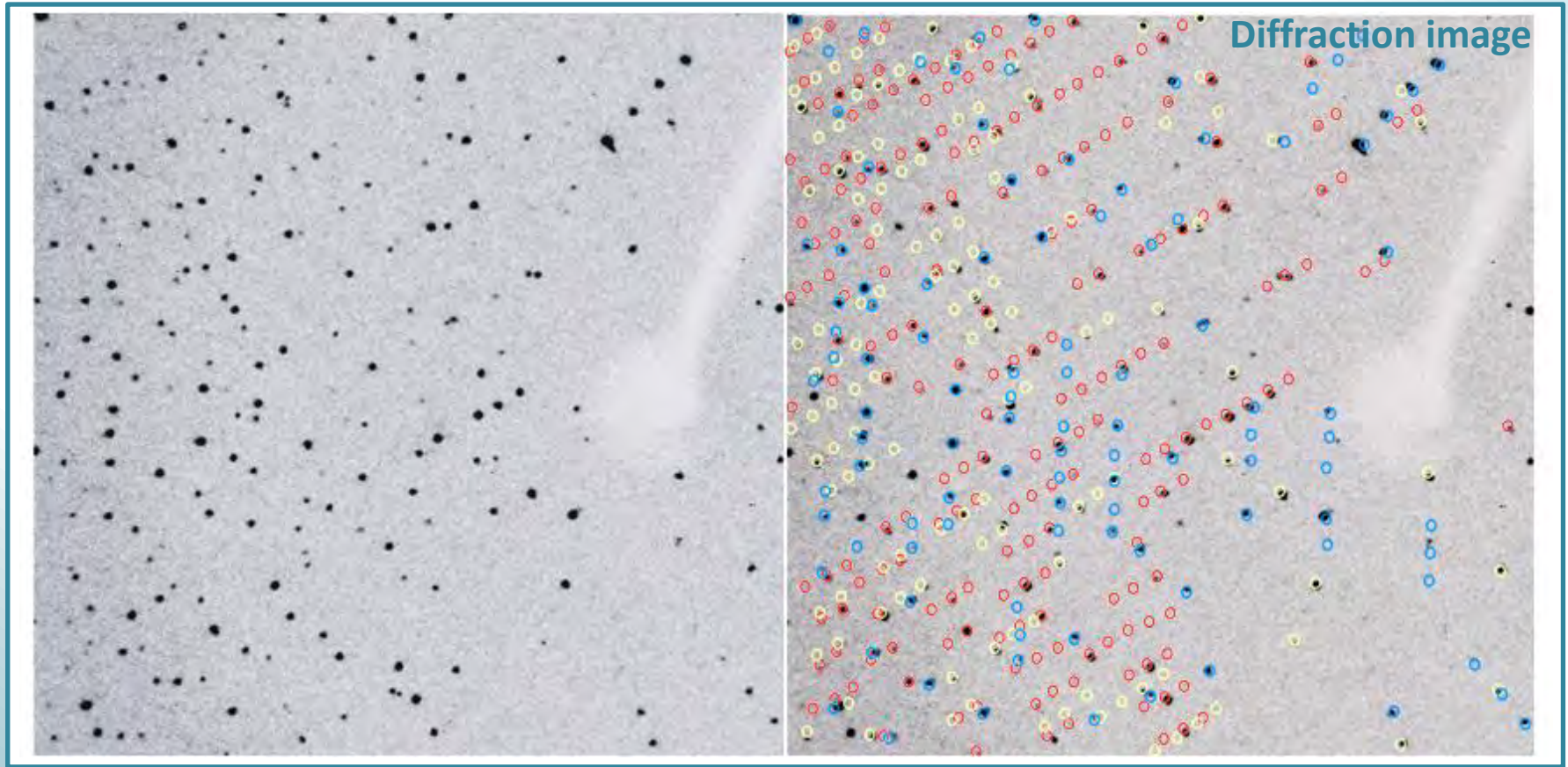
- From detector frames
- By reciprocal lattice viewers: RLATT (*proprietary*) or RLAT4XDS (*available at <http://www.cb-huebschle.de>*)
- No suitable cell for all reflections (many outliers)
- Cell refinement difficult
- An unusual long cell axis
- Some reflections sharp, others split

Example #1: Glucose Isomerase

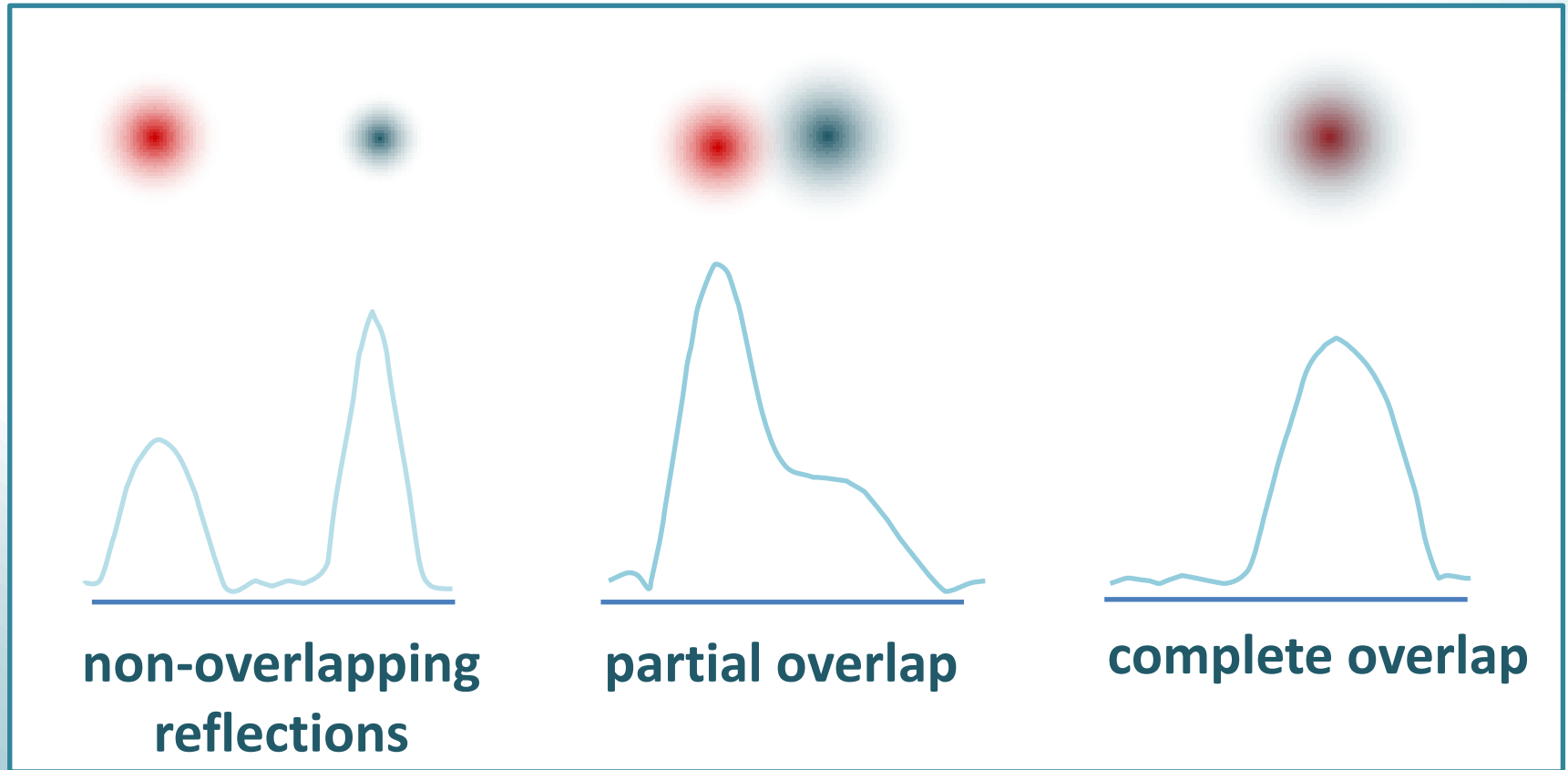


- „Drilling“
- three separate centers
- diffraction pattern consists of
 - single reflections
 - overlapping spots

Example #1: Glucose Isomerase



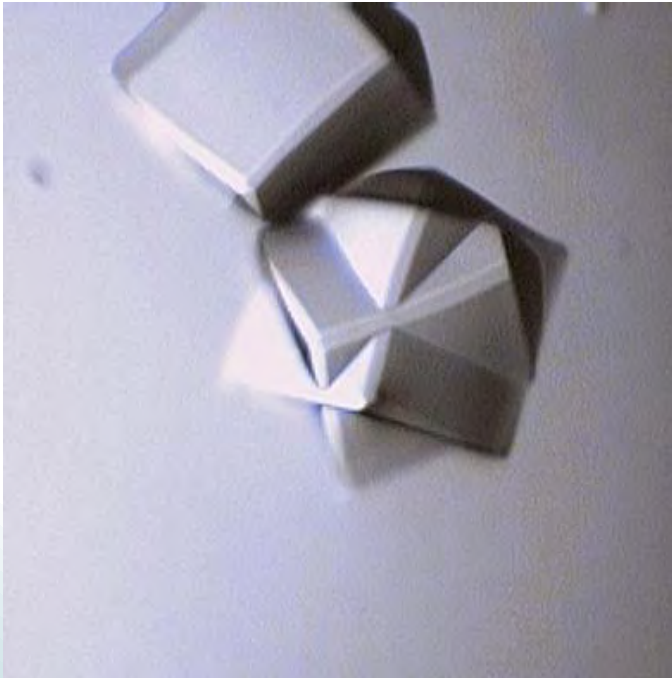
Reflection types



Cell determination

- For indexing, leave out partial overlaps at first.
- Find a cell that fits a reasonable fraction of spots.
- Use the not-yet-indexed reflections to find an alternative orientation of the same cell.
- This can be done with XDS (use omitted reflections) or with CELL_NOW (proprietary)
- If necessary, repeat this step to find more twin domain orientations.

Example #2: Bovine insulin



- Cubic ($I2_13$)
- 51 amino acids
- resolution to 1.60 Å
- interpenetrant twins with approximately the same center

Non-merohedral twinning

CELL_NOW: Determining the twin law and cell

Cell for domain 1: 78.040 77.986 78.024 89.99 89.94 90.01
Figure of merit: 0.560 %(0.1): 51.6 %(0.2): 55.2 %(0.3): 62.6

4072 reflections within 0.250 of an integer index assigned to domain 1

Cell for domain 2: 78.040 77.986 78.024 89.99 89.94 90.01
Figure of merit: 0.910 %(0.1): 91.4 %(0.2): 93.6 %(0.3): 94.5

Rotated from first domain by 89.2 degrees about reciprocal axis
0.928 0.207 1.000 and real axis 0.927 0.208 1.000

Twin law to convert hkl from first to
this domain (SHELXL TWIN matrix):

0.459	-0.625	0.631
0.824	0.036	-0.565
0.330	0.780	0.532

3564 reflections within 0.250 of an integer index assigned to domain 2,
2751 of them exclusively; 184 reflections not yet assigned to a domain

Non-merohedral twinning

How to integrate?



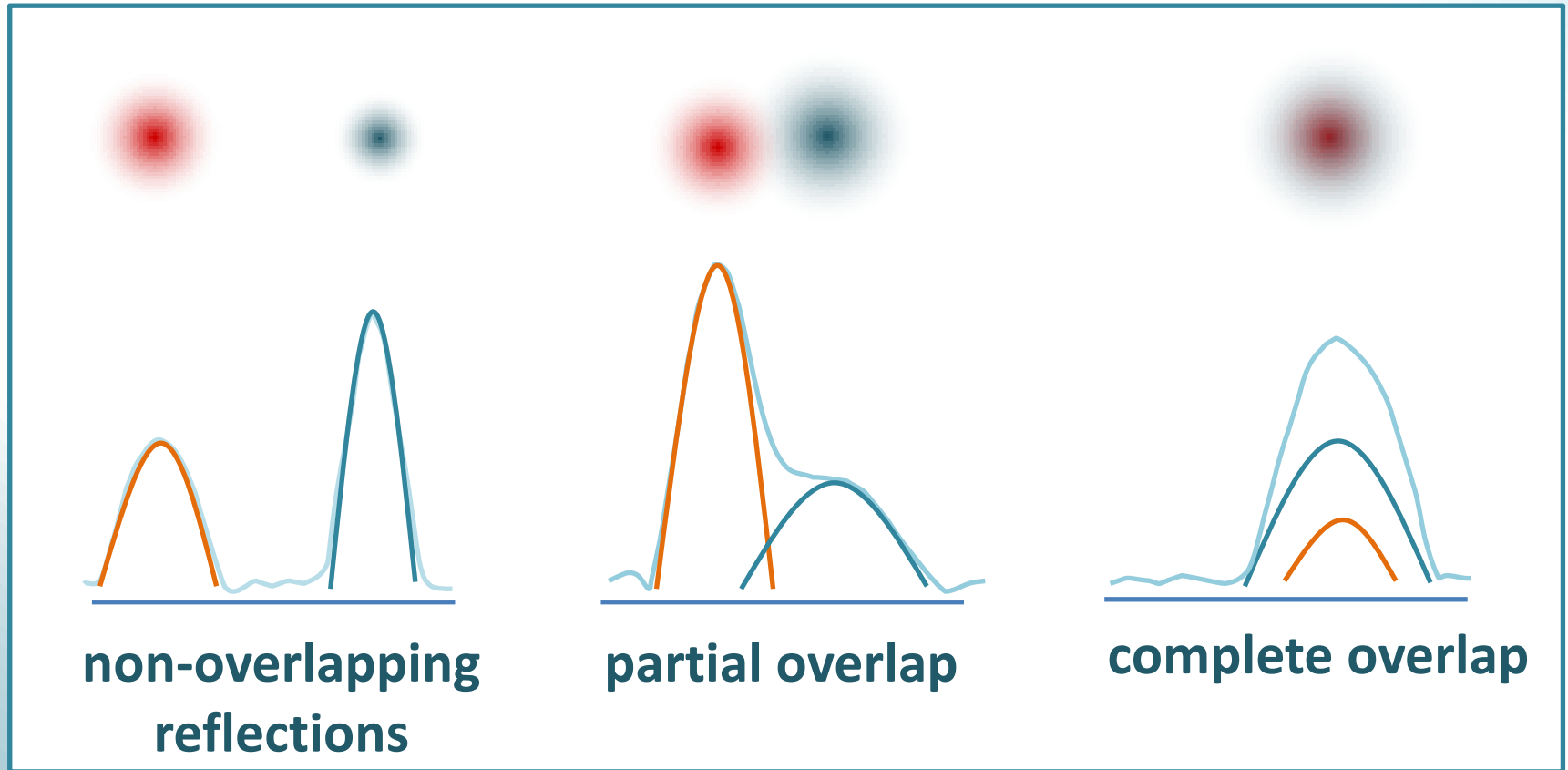
- Few programs can handle non-merohedral twins:
 - SAINT (Bruker)
 - EVALCCD (free)
- Special file format to hold two domains needed (e.g. HKLF5) or intensities have to be merged.

Non-merohedral twinning

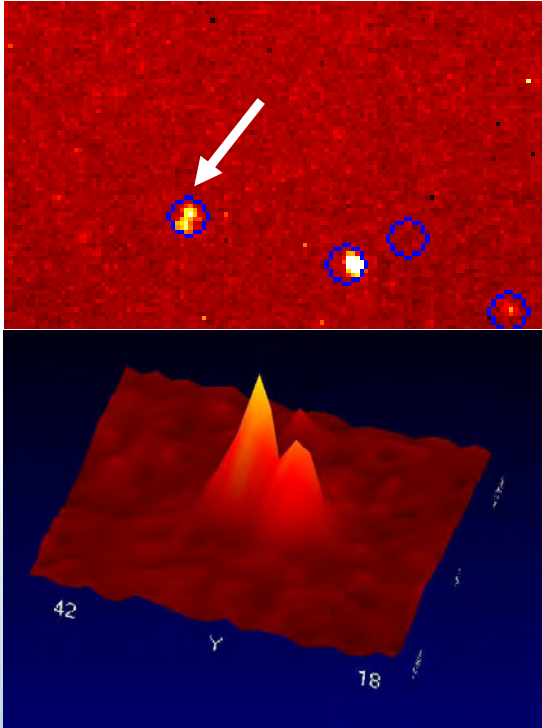
How to integrate?

- Ignoring the twinning: *No special software needed, but poor results (if any).*
- Omission of all overlapped reflections: Possible in *many integration programs, but low completeness.*
- Omission of all partially overlapped reflections
- Integrating both domains by profile fitting the overlapping reflections: *Good, but complicated...*

How to integrate?



How to integrate?

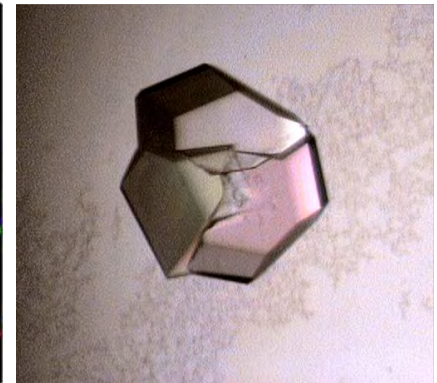
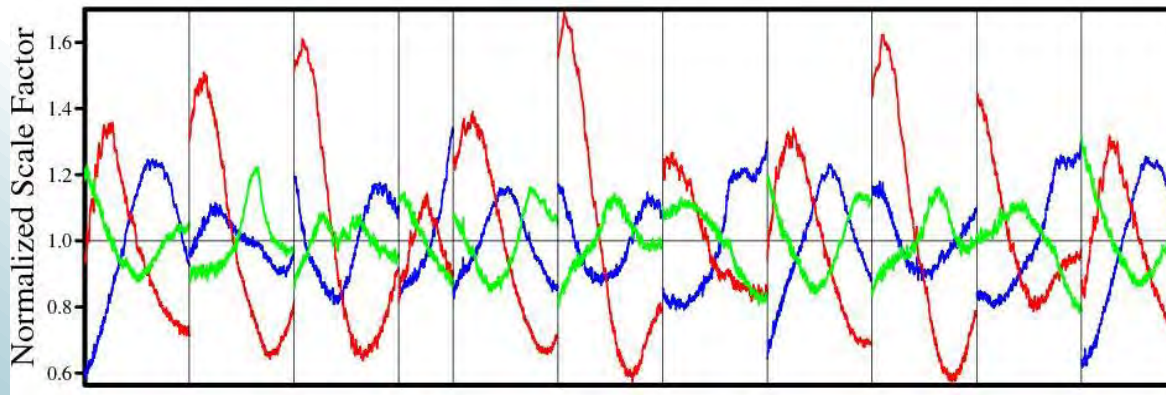
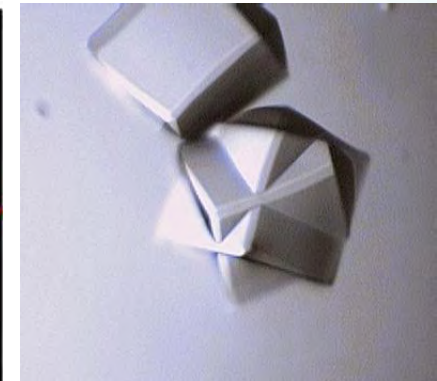
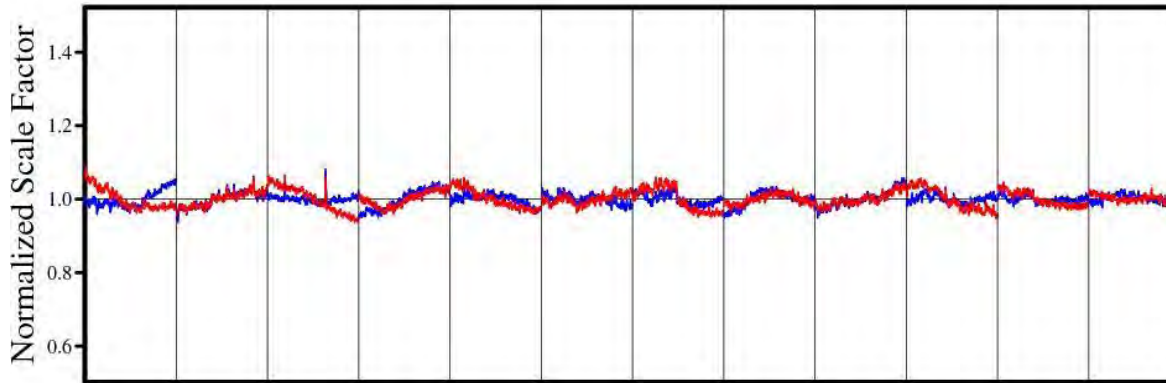


Partially overlapping reflections

- The total intensity of a group of overlapping reflections can be determined precisely!
- Partitioning can be determined by 3D profile fit.
- Special file format to hold two domains needed (e.g. HKLF5) or intensities have to be merged.

Scaling and absorption correction

In TWINABS:



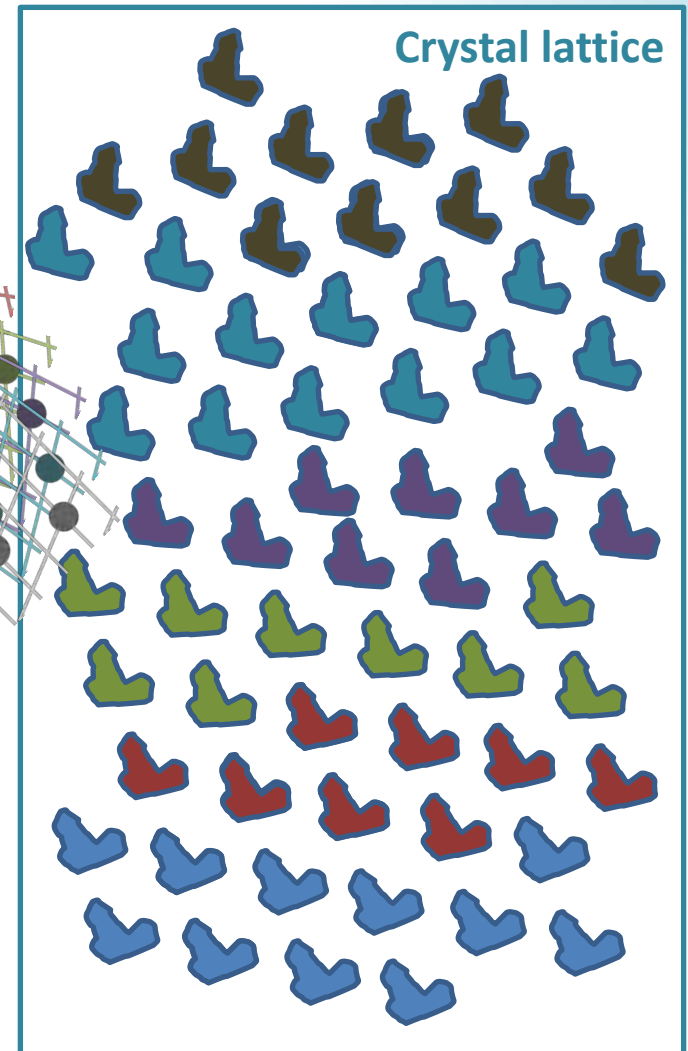
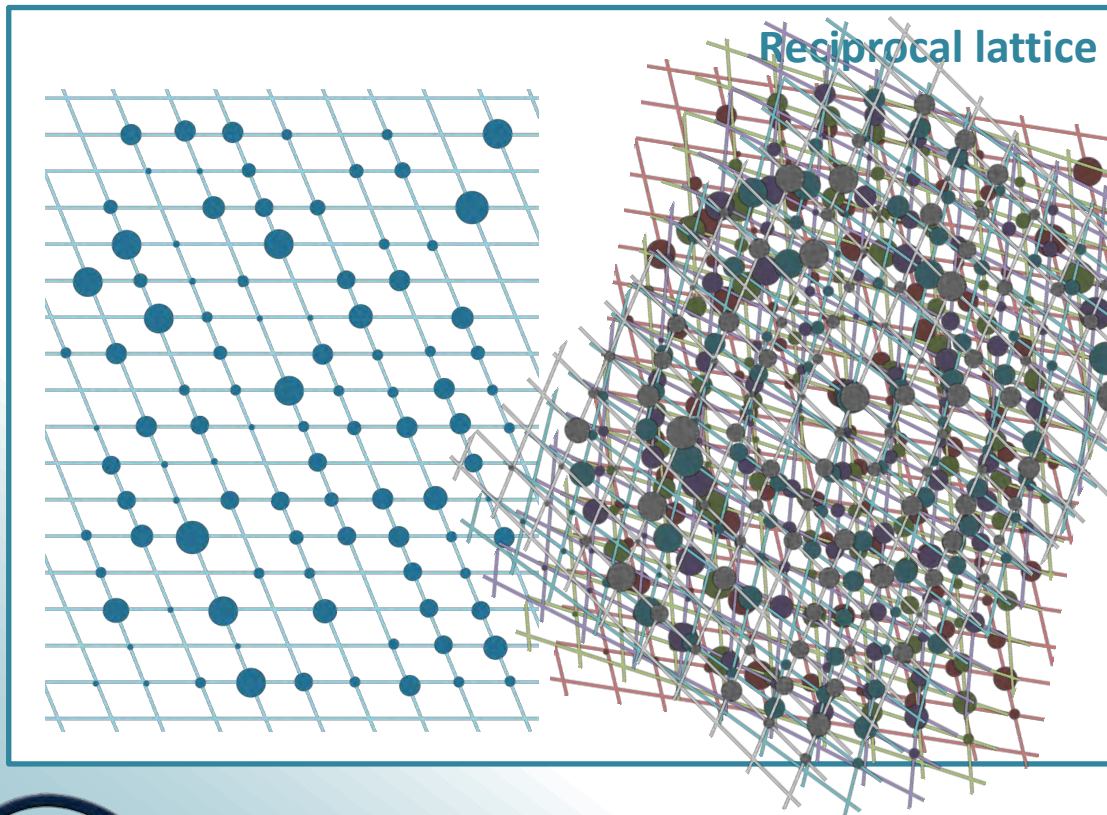
The smallest crystal (red) was furthest from the center.

Non-merohedral twinning

- **Twin law relatively arbitrary, often a two-fold rotation**
- Recognition from frames or reciprocal lattice
- No exact overlap of reciprocal lattices.
- Cell determination, integration, scaling and refinement can be difficult.
- Detwinning is possible, and this data can be used for structure solution

Split crystal

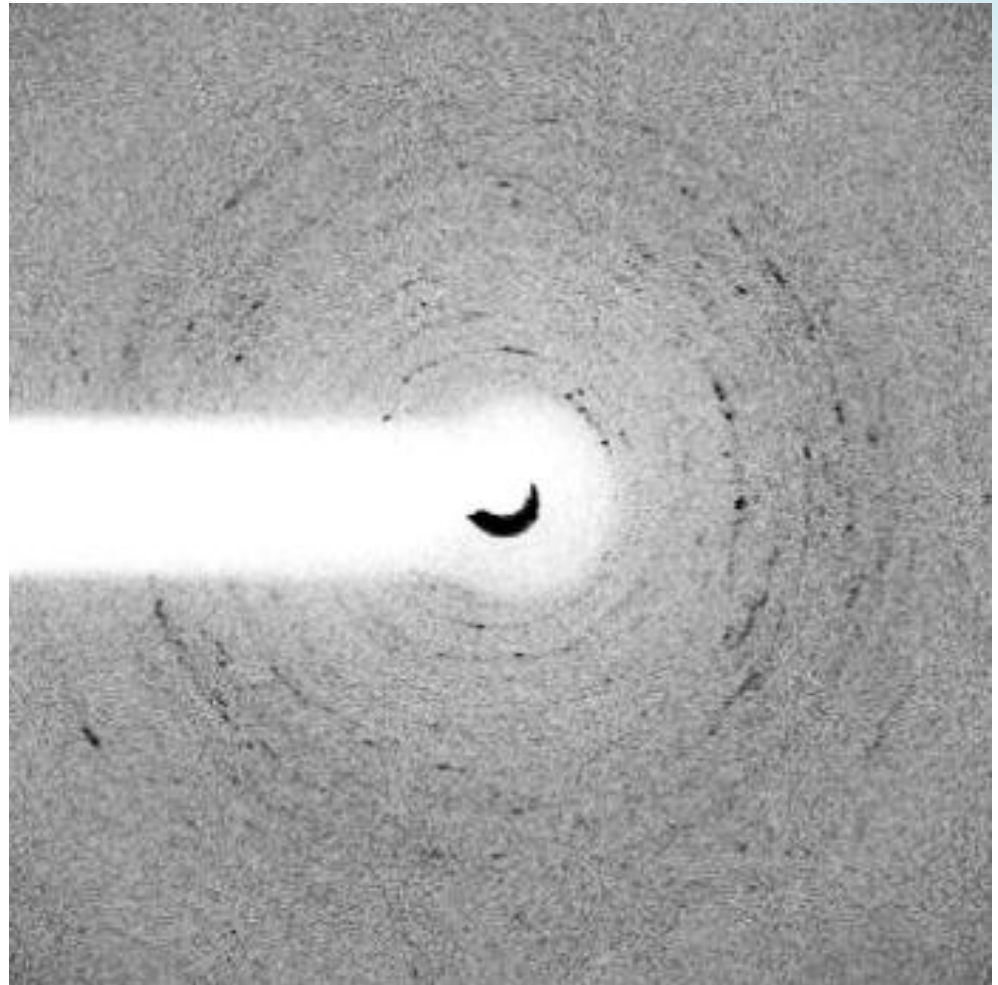
This is NOT a twin!



Split crystal

This is NOT a twin!

- Twin law near to unity
- Indexing gets better with box being bigger
- Bad data quality
- Split crystals give only reasonable data if they have a limited number of domains



Summary

There are several types of twins: Non-merohedral, merohedral and pseudo-merohedral twins.

Twins cannot be detected without prior suspicion.

(Not every data that cannot be solved or properly refined is twinned.)

There are warning signals for twinning in the frames and in the intensity distribution, but not all have to occur.

If the data quality is sufficient, the structure can be elucidated. **Don't throw your only crystal away just because it's twinned!**



Literature

- Bernhard Rupp, **Biomolecular Crystallography: Principles, Practice, and Application to Structural Biology**, 2004
- **Yeates Server:**
<http://nihserver.mbi.ucla.edu/Twinning>
- Simon Parsons, **Introduction to twinning**, Acta Cryst. (2003). D59, 1995-2003
- Zbigniew Dauter, **Twinned crystals and anomalous phasing**, Acta Cryst. (2003). D59, 2004-2016

Macroscopic twins

Can you identify the types of twinning?



GEORG-AUGUST-UNIVERSITÄT
GÖTTINGEN

ACKNOWLEDGEMENTS

I am grateful to **George Sheldrick** and **Regine Herbst-Irmer**, who freely shared with me their knowledge, ideas and material.

The non-merohedral protein data and pictures were taken by **Madhumati Sevvana**.

I also want to thank the **Sheldrick group** for all the support!

