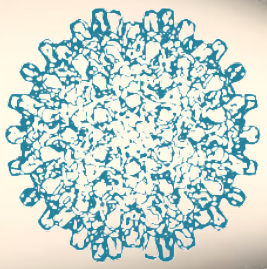
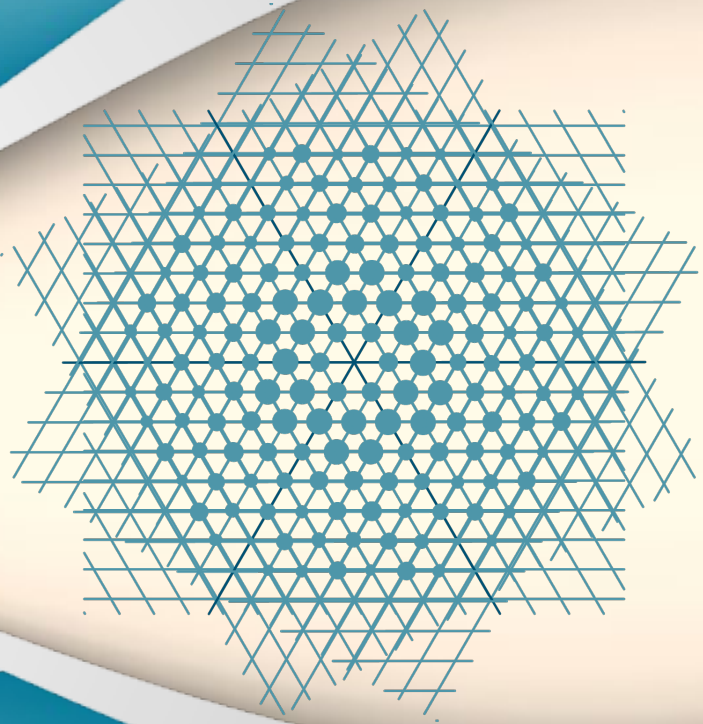
Twinning

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

Is this a twin?

Definition of twinning

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

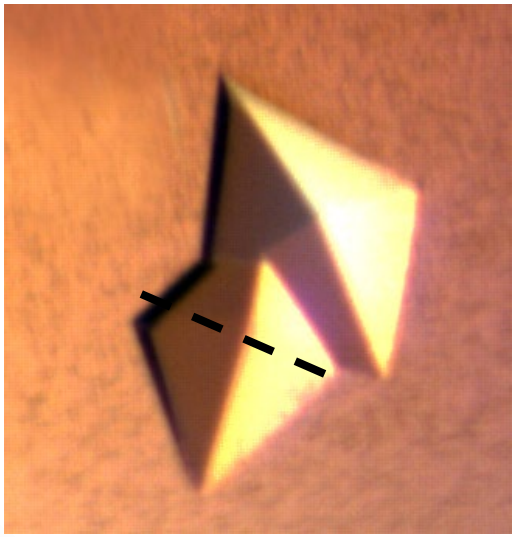
***α** is the **twin fraction**:*

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

*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:

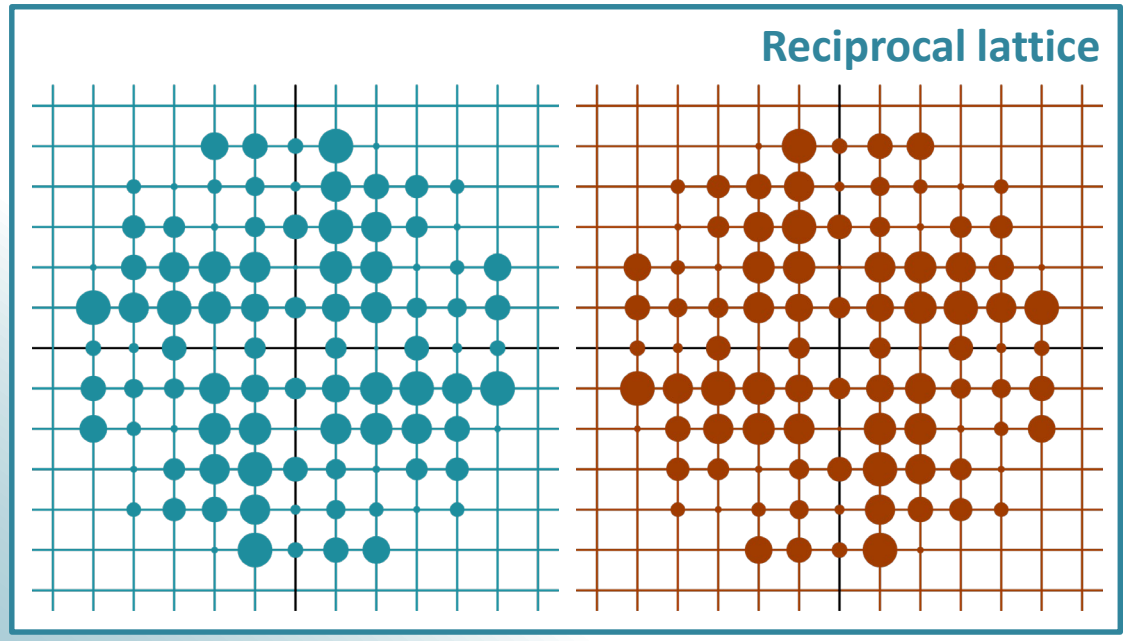
Non-merohedral twinning	Merohedral twinning	Pseudo-merohedral twinning

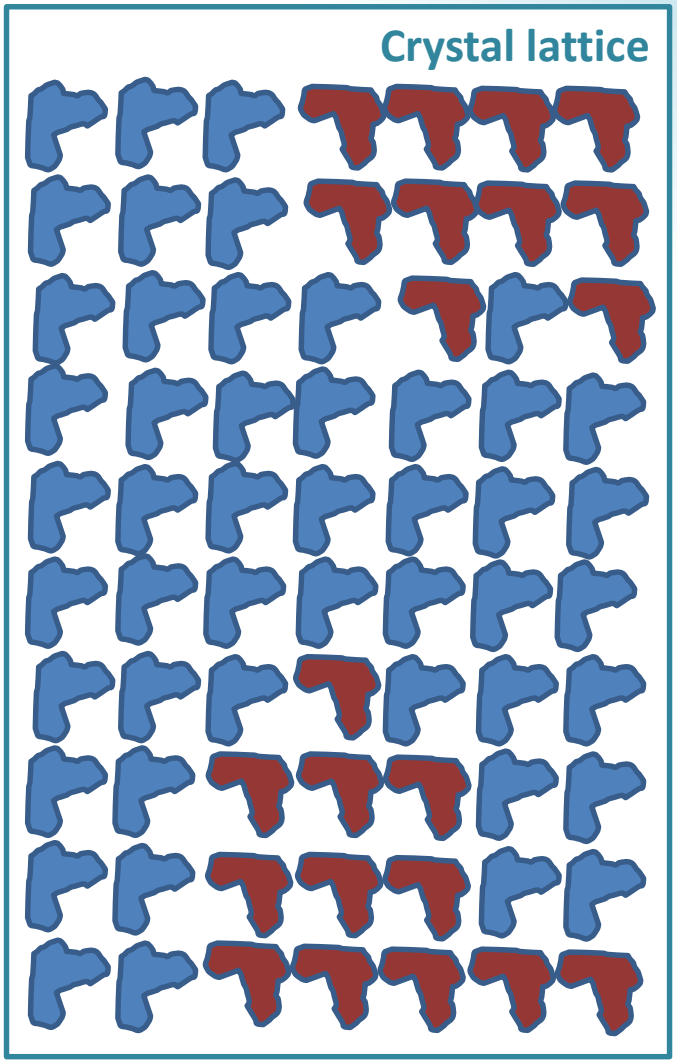
Finding the twin law and the twin fraction α

Treat data accordingly

Merohedral twinning

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

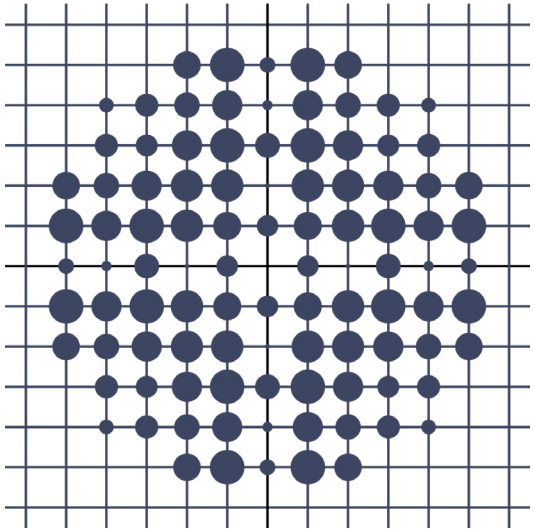




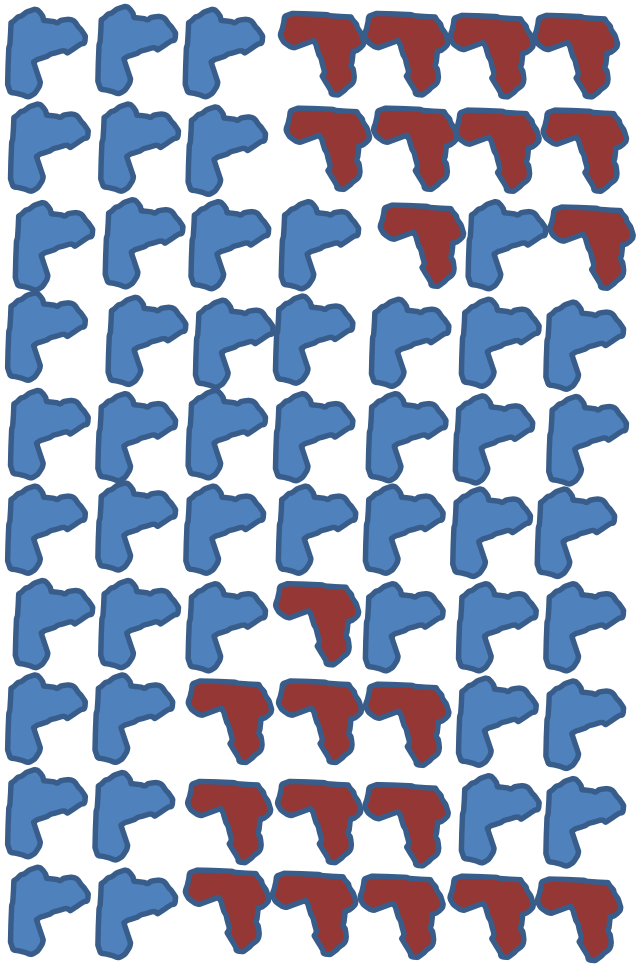
Merohedral twinning

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

Reciprocal lattice

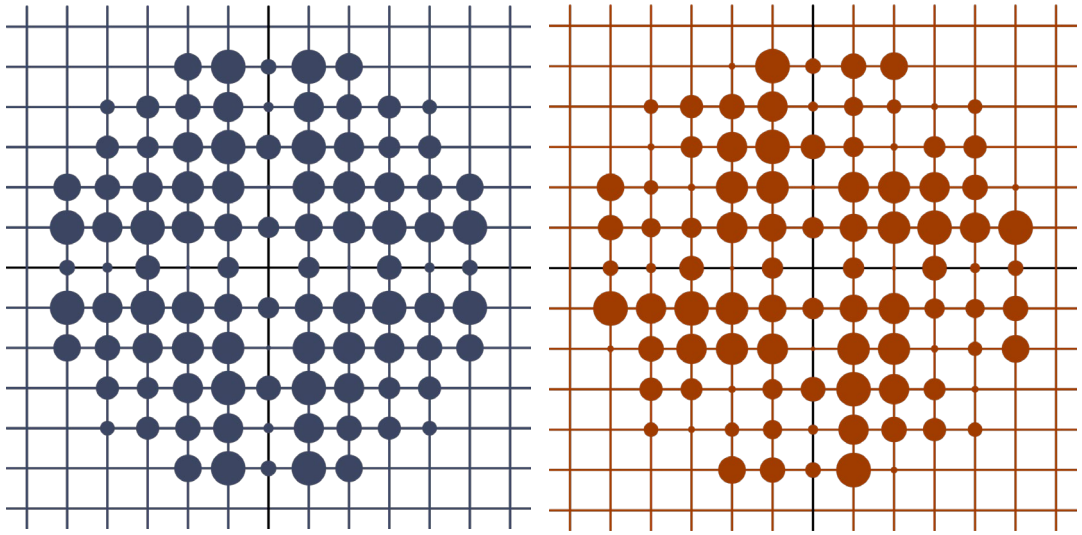


Crystal lattice



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_{merge} for the higher symmetry
- Typical space group
- No structure solution

Merohedral twinning

Space groups

- Only trigonal, hexagonal, cubic 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:

$$H = \frac{|I_1 - I_2|}{I_1 + I_2}$$

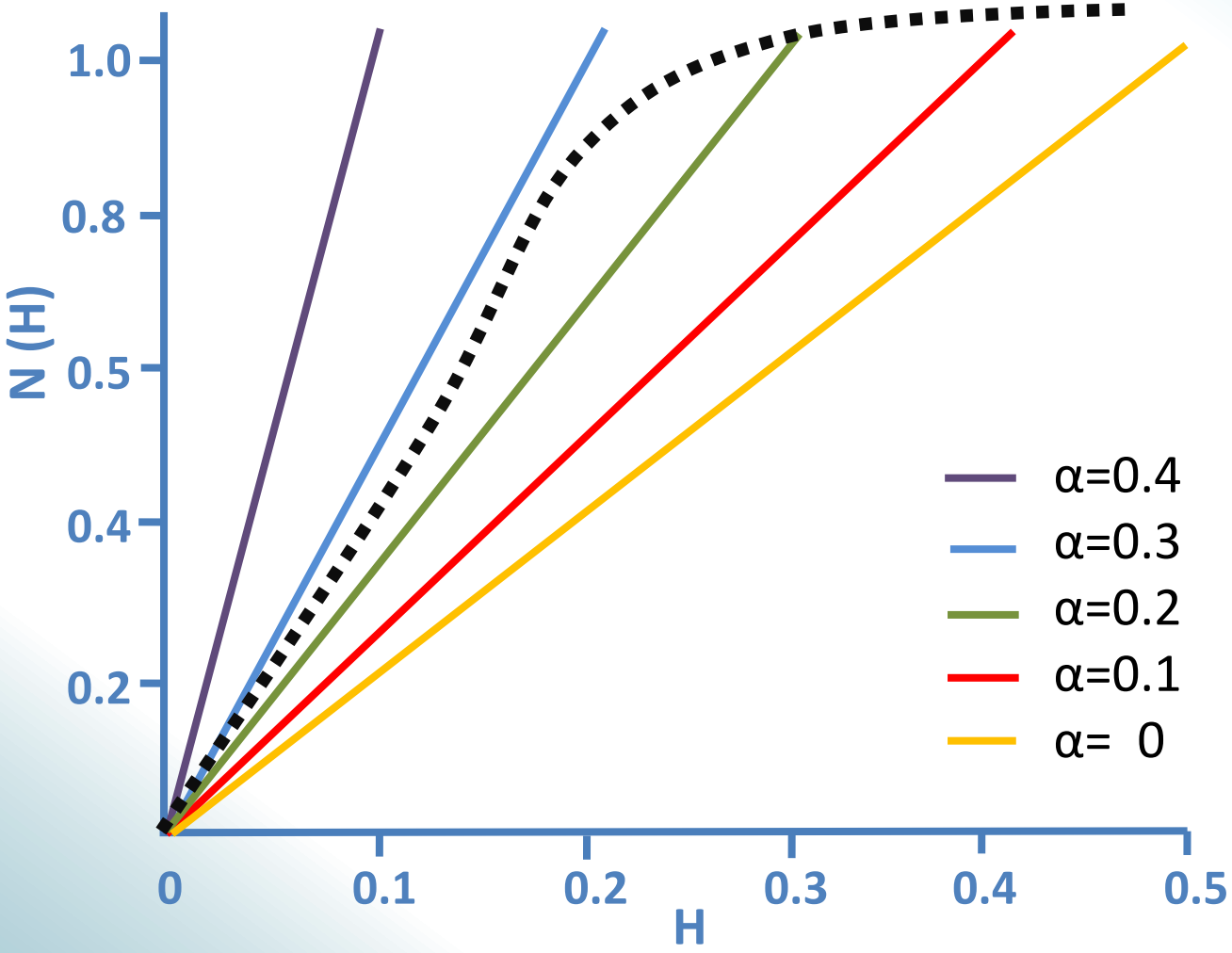
(For acentric reflections:)

Cumulative propability distribution

$$N(H) = \begin{cases} 0 & H<0 \\ \dfrac{H}{1-2\alpha} & 0 \le H \le 1\text{-}2\alpha \\ 1 & H>1\text{-}2\alpha \end{cases}$$

Note: When there is rotation parallel to twinning axis then the distribution will be different

Merohedral twinning



Merohedral twinning

Drawbacks

Perfect twins are not detectable with this method.

$$H = \frac{|I_1 - I_2|}{I_1 + I_2}$$

$$\mathbf{I_1 = I_2} \quad \Rightarrow \quad \mathbf{H = 0}$$

We need another test!

Merohedral twinning

Yeates-Padilla Test

The reflections with the intensities $\mathbf{I_A}$ and $\mathbf{I_B}$ are close to each other in reciprocal space:

$$L = \frac{|I_A - I_B|}{I_A + I_B} \qquad N(L) = \begin{cases} 0 & L<0 \\ \frac{1}{(1-2\alpha)^2}(\alpha^2 + (1-\alpha)^2 - \frac{8\alpha^2(1-\alpha)^2}{1-(1-2\alpha)^2 L^2})L & 0 \le L \le 1 \\ 1 & L>1 \end{cases}$$

If α=0.5:

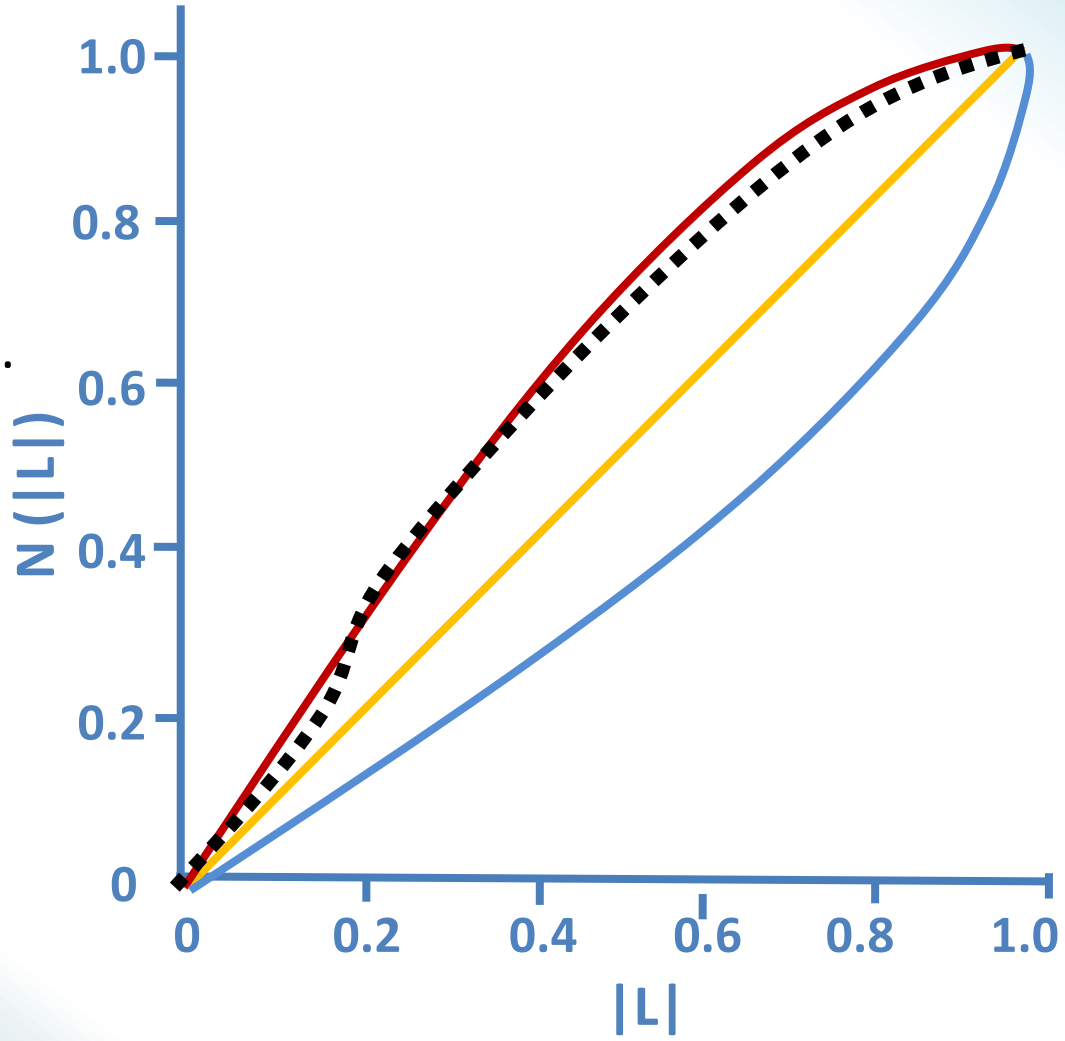
$$N(L) = \begin{cases} 0 & L<0 \\ \frac{3}{2}(L - \frac{L^3}{3}) & 0 \le L \le 1 \\ 1 & L>1 \end{cases}$$

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

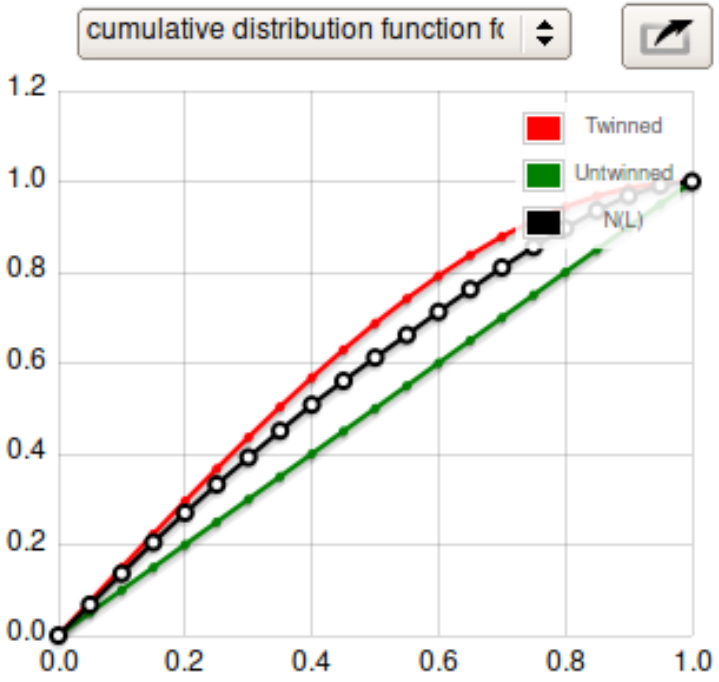
- Using the Yeates-Padilla test for the PDB shows that twinning occurs more frequently then commonly recognized!
- Both the **H test** as well as the **Yeates-Padilla plot** can be generated using **AIMLESS, CTRUNCATE, DETWIN or phenix.xtriage**.
- The $\langle|E^2-1|\rangle$ could be too low for twins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

In Data reduction - AIMLESS output:

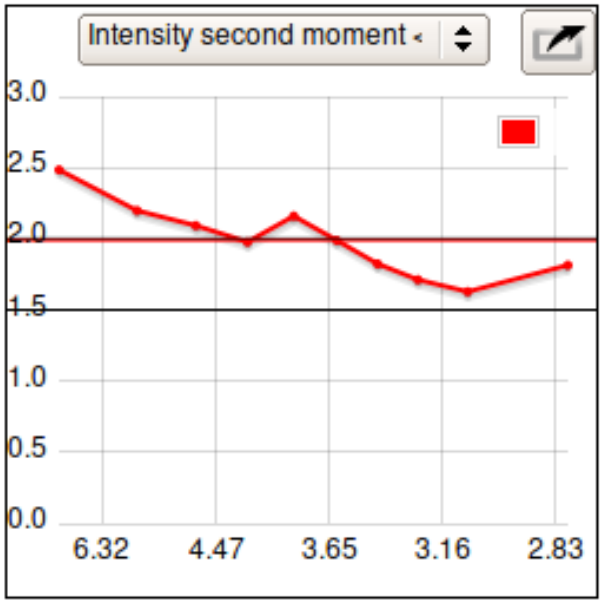
Graphs for detecting twinning etc, more details in Istats section

This dataset is probably twinned

L-test for twinning



Acentric intensity moments



Values for these data, and for ideal data (untwinned or twinned)

Merohedral twinning

How to integrate?

In lower symmetry group with any integration program!

Structure solution

MR	O good
MAD, SAD	O ok
S-SAD	X too sensitive to noise
SIR, MIR	X several crystals needed

Merohedral twinning

Finding the twin law

The correct twin law translates from the (correct) lower into the (wrongly) observed space group.

For merohedral twins, R_{merge} can be used to identify the correct twin law automatically.

Merohedral twinning

Refinement

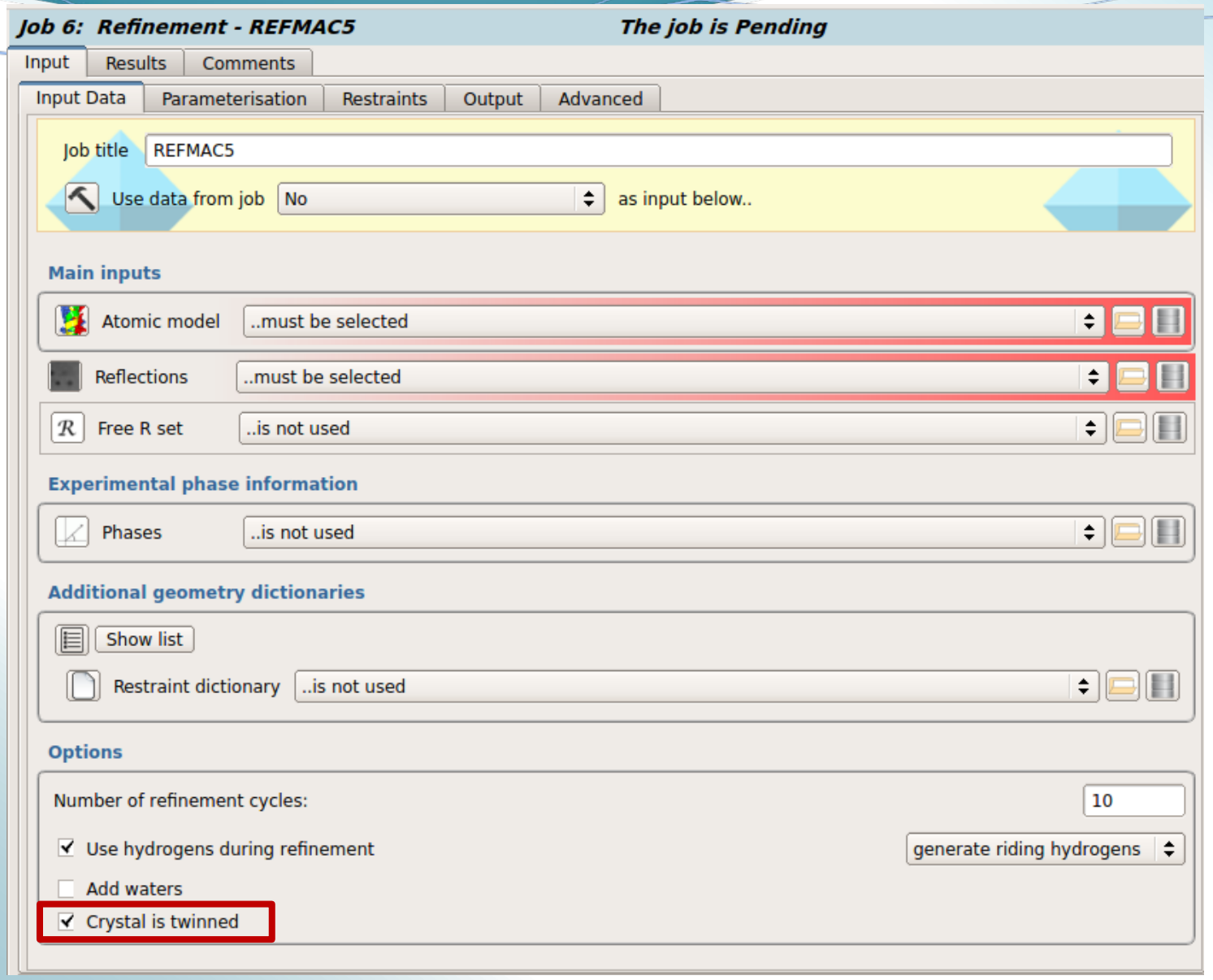
- Protein refinement programs offer option for the refinement of merohedral twins, for example REFMAC, CNS, phenix.refine and SHELXL. In REFMAC, the twin law is determined automatically.
- REFMAC and SHELXL do not de-twin the data but have an adapted target function / model.
- Do not use a merohedral 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.

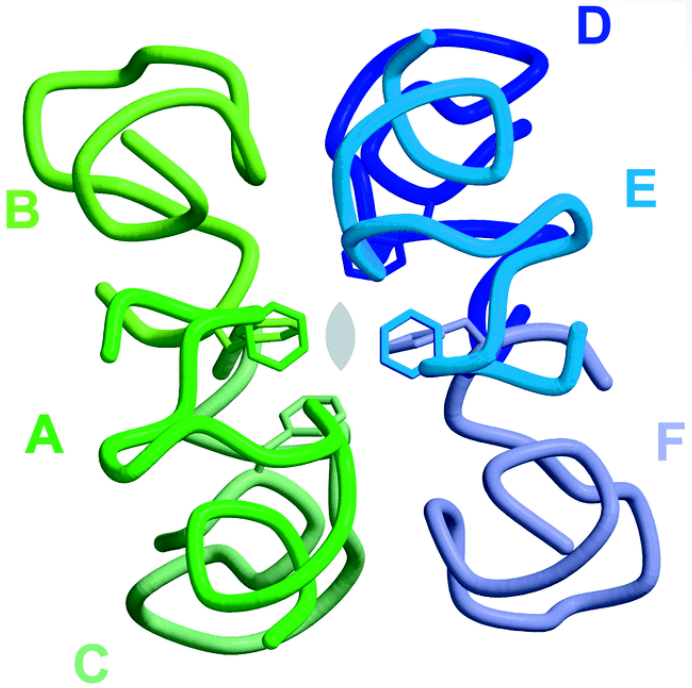
Merohedral twinning



Merohedral twinning

R_{merge} in Mersacidin

Resolution (Å)		1.06
$<\|E^2 - 1\|>$		0.649
R_{merge} (%)	$P3_2$	4.9
R_{merge} (%)	$P3_221$	19.5
R_{merge} (%)	$P3_212$	44.3



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

* *Acta Cryst* (2000). D**56**, 705-713

Merohedral twinning

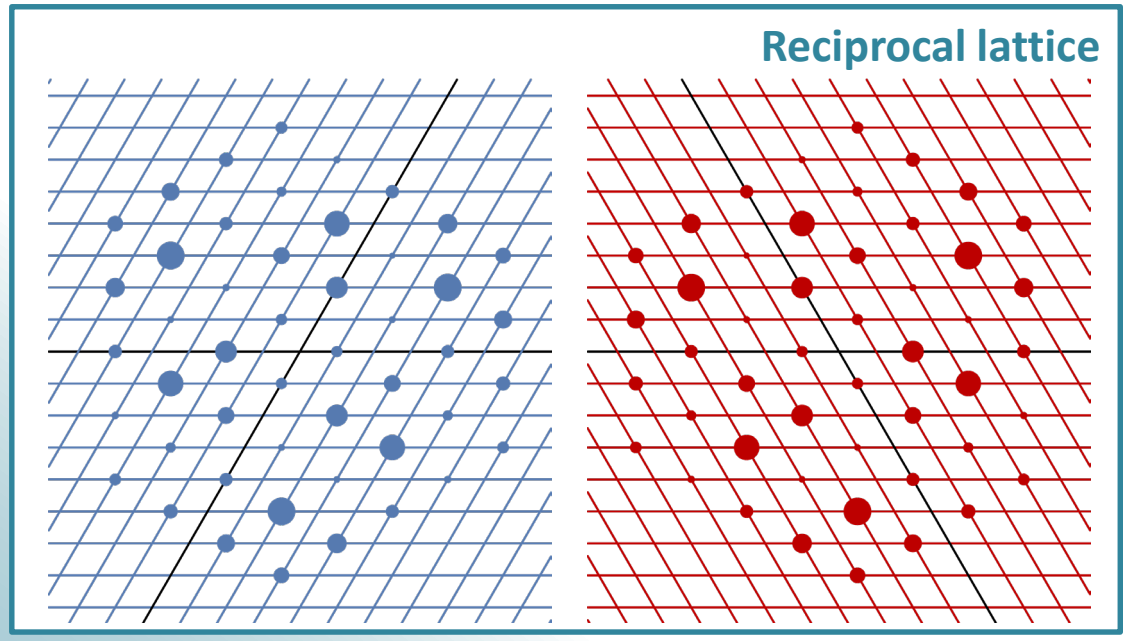
Twin law: Symmetry operator of the crystal system, but not the crystal's point group

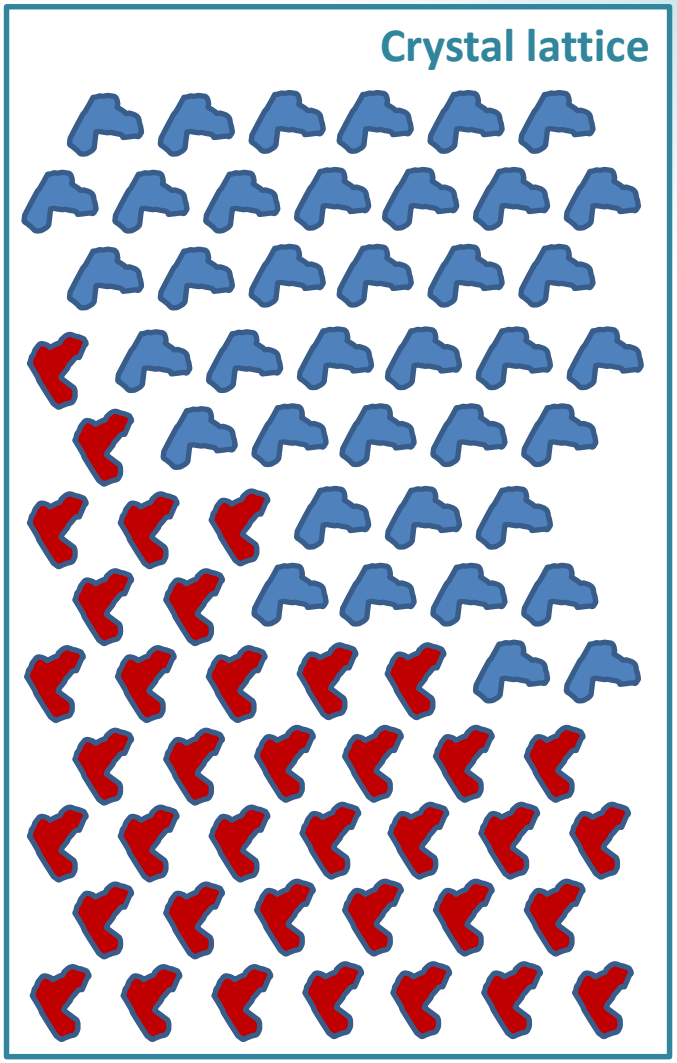
- Only in tetragonal, trigonal, hexagonal and cubic space groups possible.
- Exact overlap of reciprocal lattices, but different intensity distribution.
- Usually good results.

Reticular merohedry

Most common case:

Obverse/reverse twinning in a rhombohedral crystal





Reticular merohedry

The typical case

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

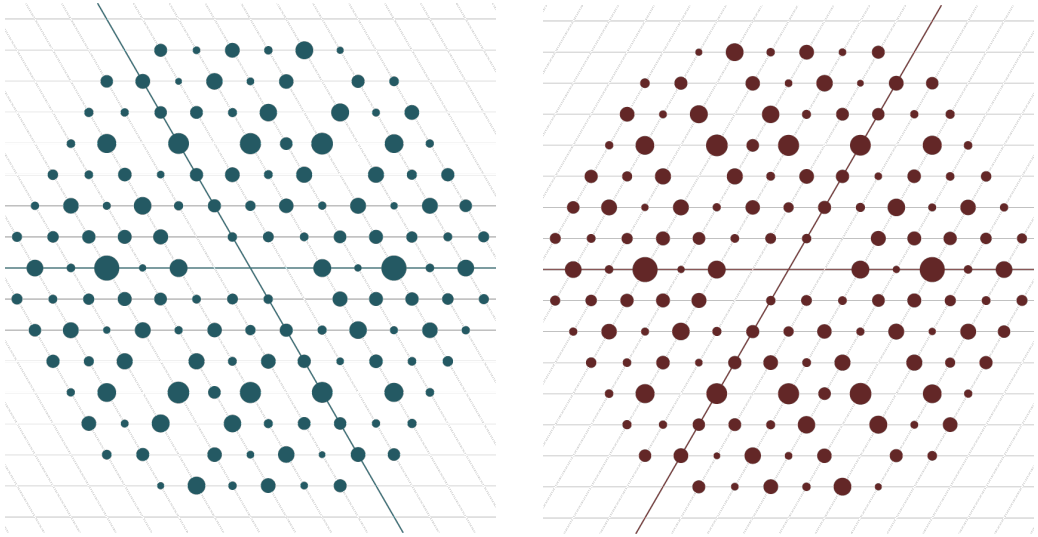
1/3 of all reflections are missing.

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

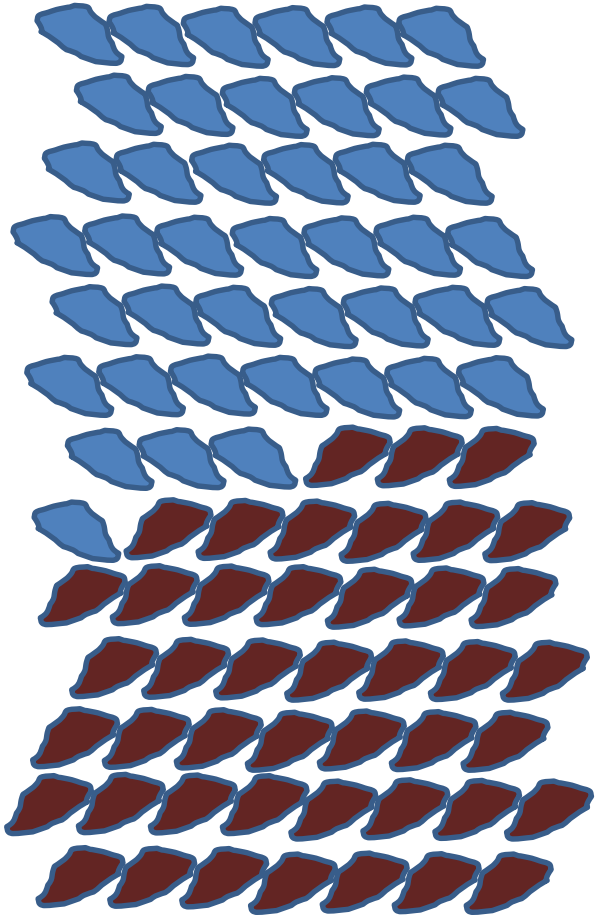
Pseudo-merohedral twinning

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

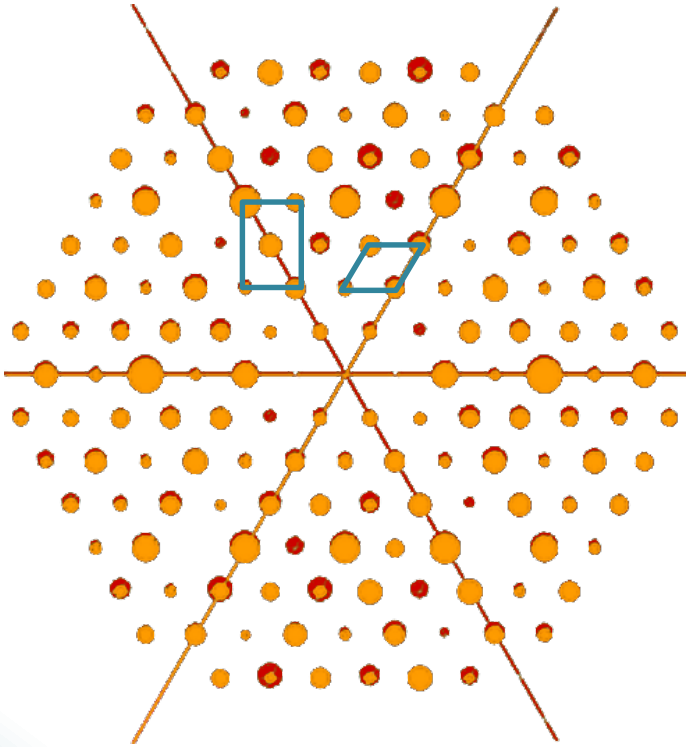
Reciprocal lattice



Crystal lattice



Pseudo-merohedral twinning



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 $a \approx c$, $\beta \approx 90°$

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

Pseudo-merohedral twinning

How to recognize?

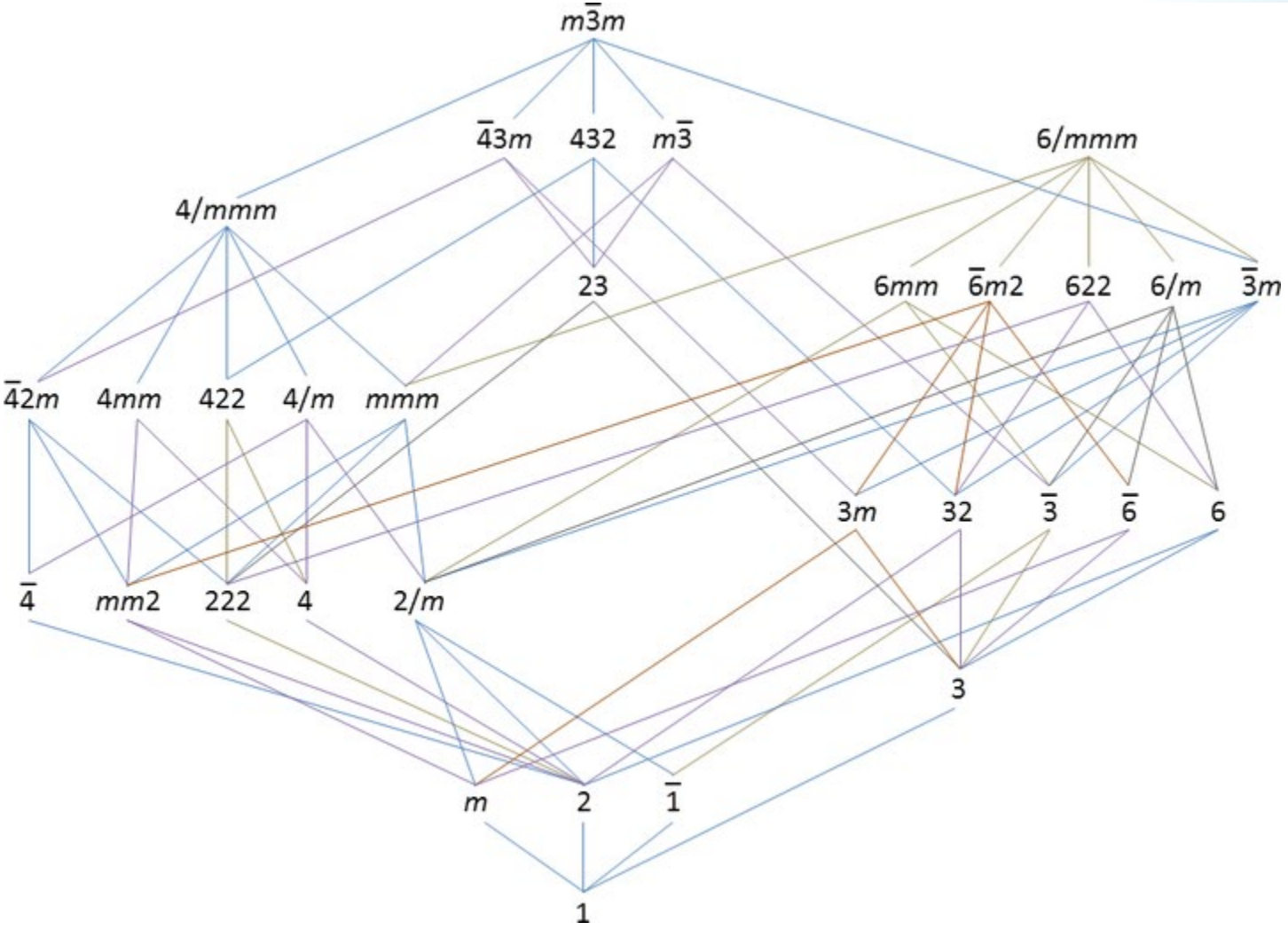
- Like a merohedral twin. The real space group belongs to another crystal system than the observed one.
- The overlap of the lattices might not be perfect for all reflections.
- All hints for merohedral twinning might also work for pseudo-merohedral ones.
- R_{merge} behaves like in merohedral twins.

Pseudo-merohedral twinning

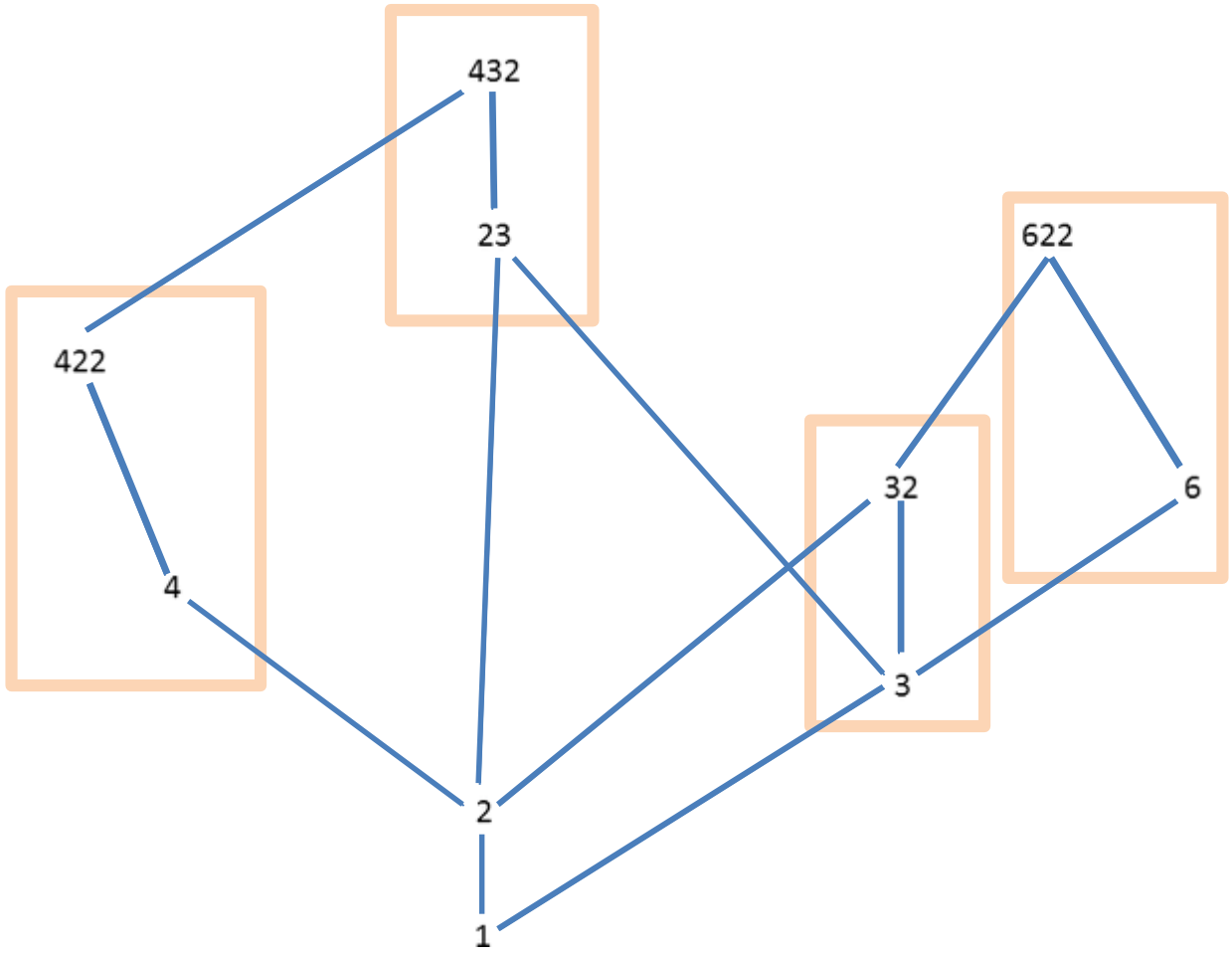
How to treat?

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

Point group relations



Point group relations



Pseudo-merohedral twinning

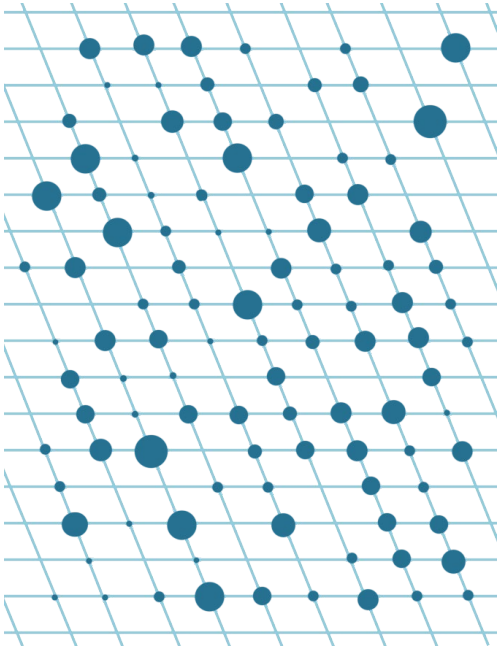
Twin law: Belongs to a higher crystal system than the structure.

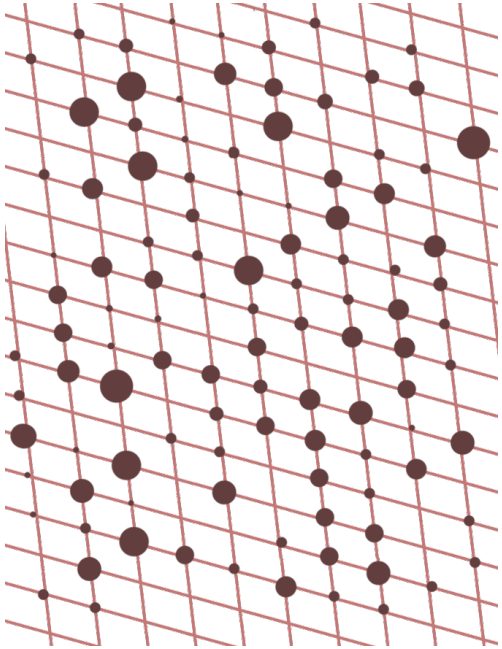
- Recognition from frames or as for merohedral twins.
- Usually good results.

Non-merohedral twinning

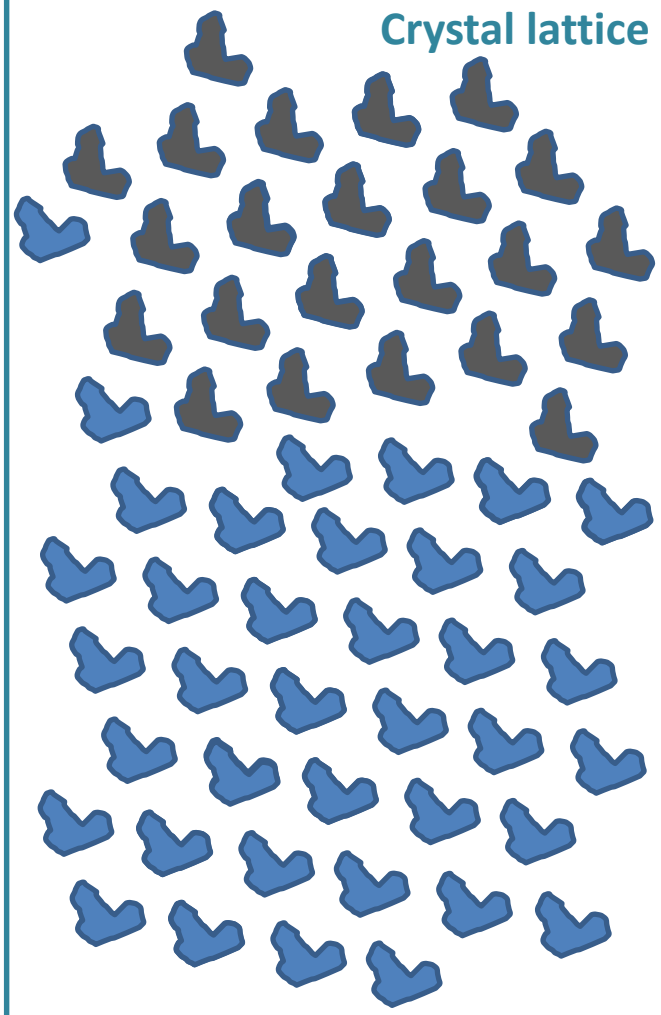
Twin law is relatively arbitrary.

Reciprocal lattice





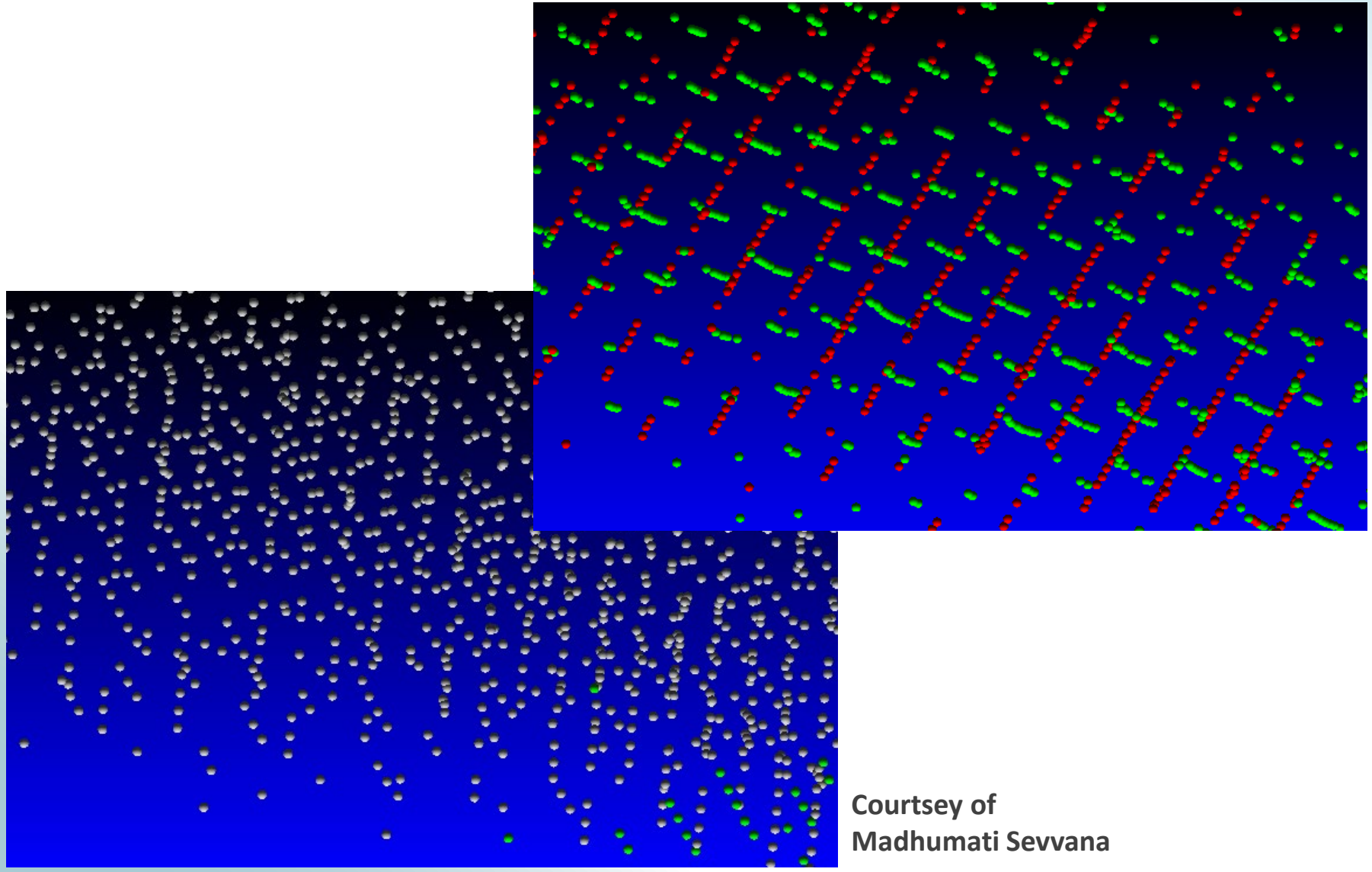
Crystal lattice



How to recognize?

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

How to recognize?



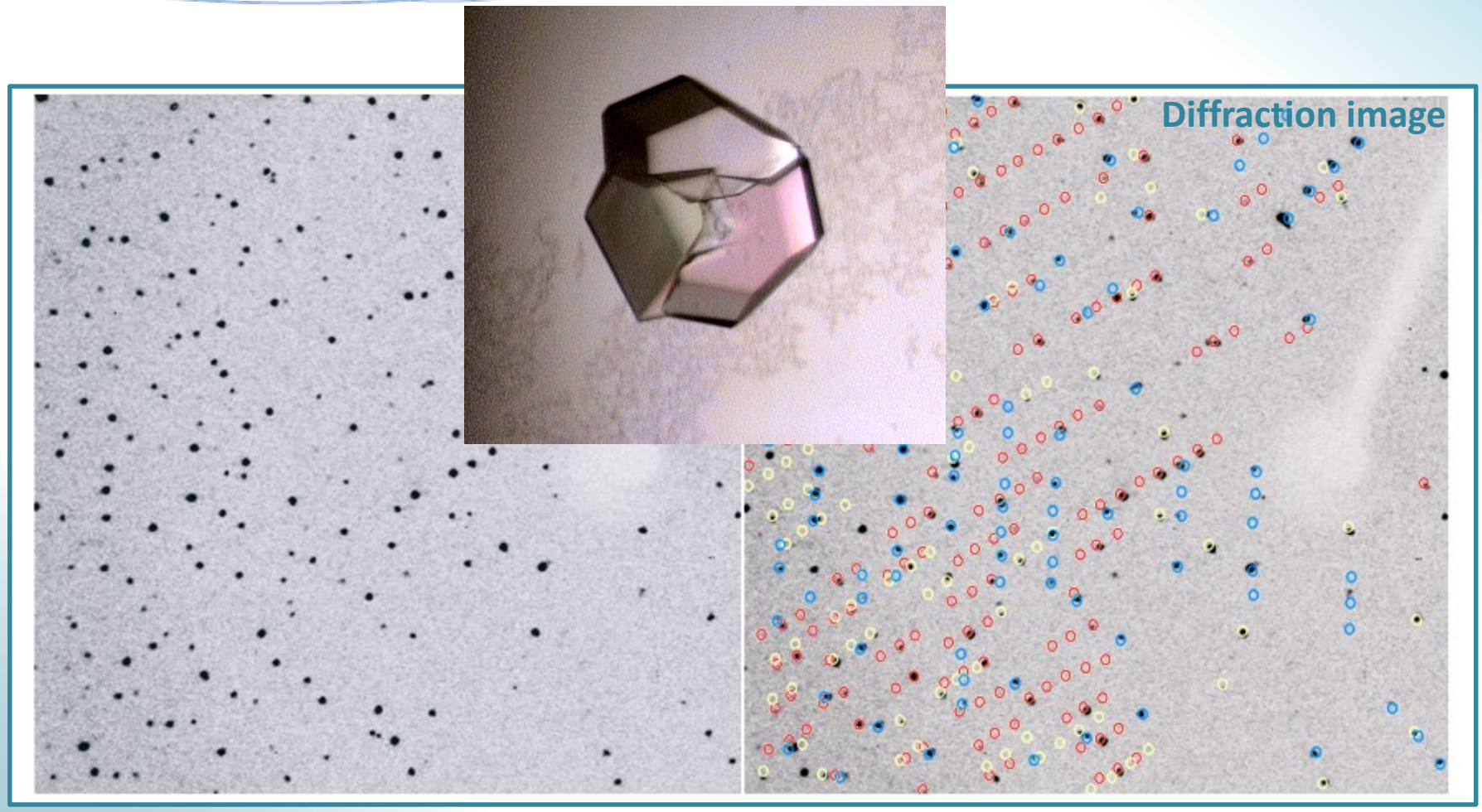
Courtsey of
Madhumati Sevvana

Example #1: Glucose Isomerase

- triplett
- three separate centers
- diffraction pattern consists of
  - single reflections
  - overlapping spots

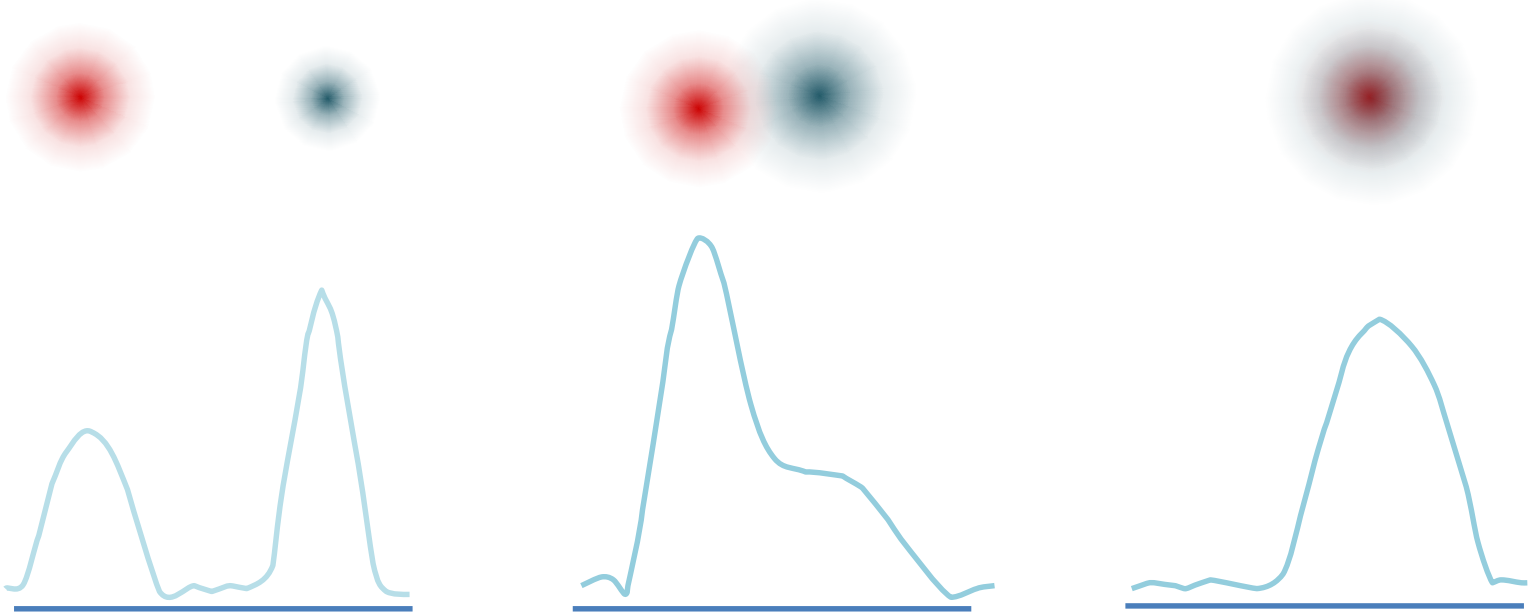
Images courtesy of
Madhumati Sevvana

Example #1: Glucose Isomerase



Images courtesy of
Madhumati Sevvana

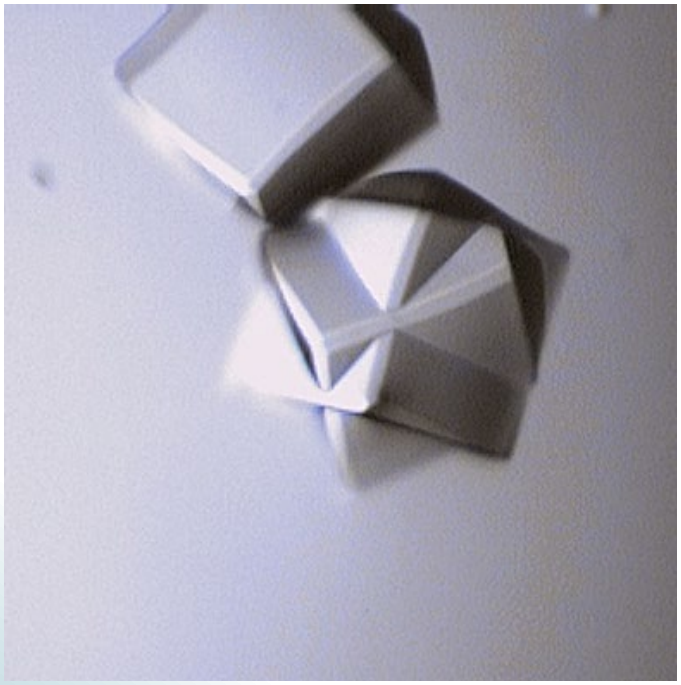
Reflection types



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; repeat as necessary.
- Something like this can be done with
  - XDS (use omitted reflections)
  - DIALS
  - MOSFLM (multi keyword in auto-indexing)
  - CELL_NOW (proprietary)

Example #2: Bovine insulin



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

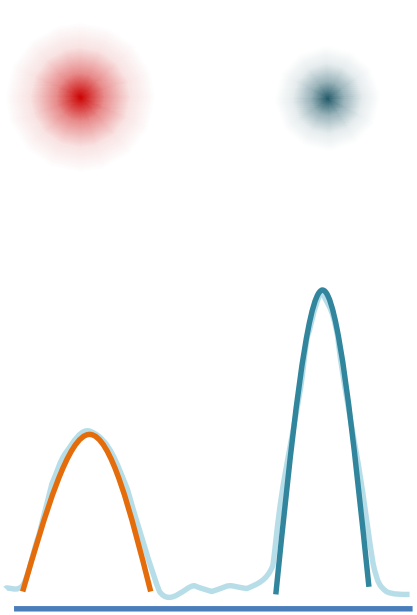
Images courtesy of
Madhumati Sevvana

Non-merohedral twinning

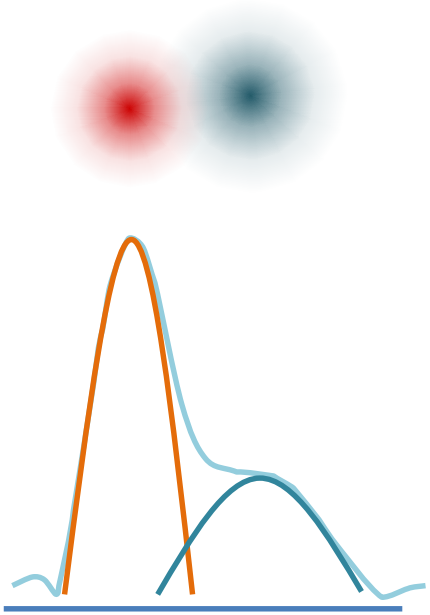
CELL_NOW: Determining the twin law and cell

```
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      0.459   -0.625    0.631
this domain (SHELXL TWIN matrix):          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
```

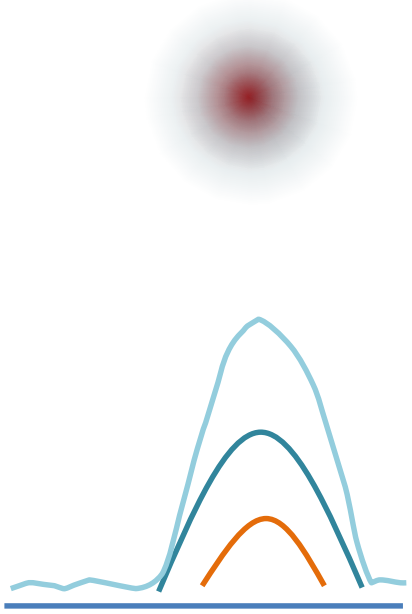
How to integrate?



non-overlapping reflections



partial overlap



complete overlap

Non-merohedral twinning

How to integrate?

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

Non-merohedral twinning

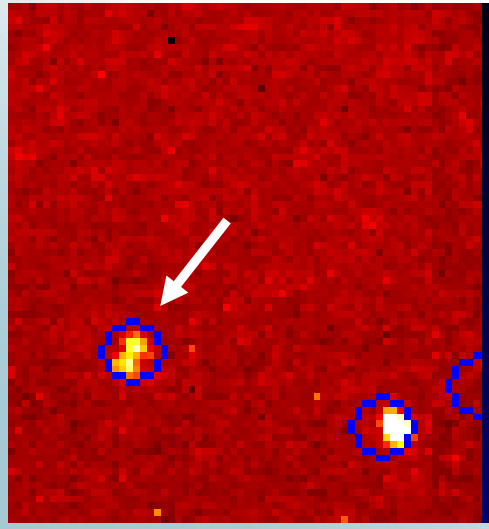
How to integrate?

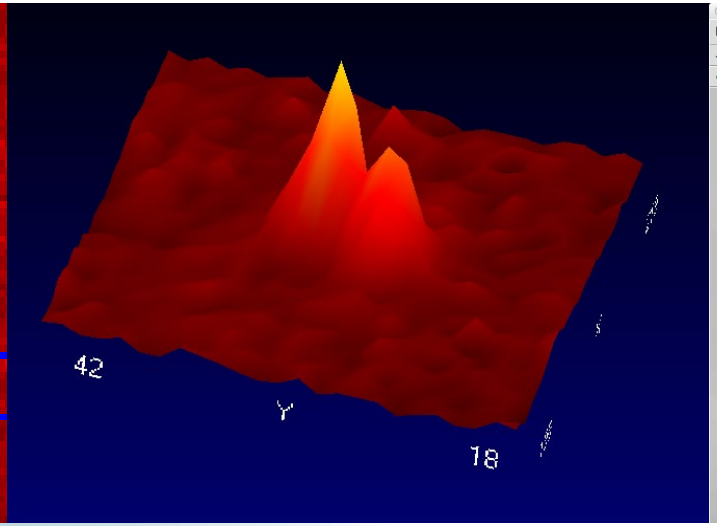
- Ignoring the twinning: P*oor results, if any.*
- Omission of all overlaps: L*ow completeness.*
- Omission of all partial overlaps
- Integrating overlaps without deconvolution
- Integrating both domains by profile fitting the overlapping reflections: *Good, but complicated.*

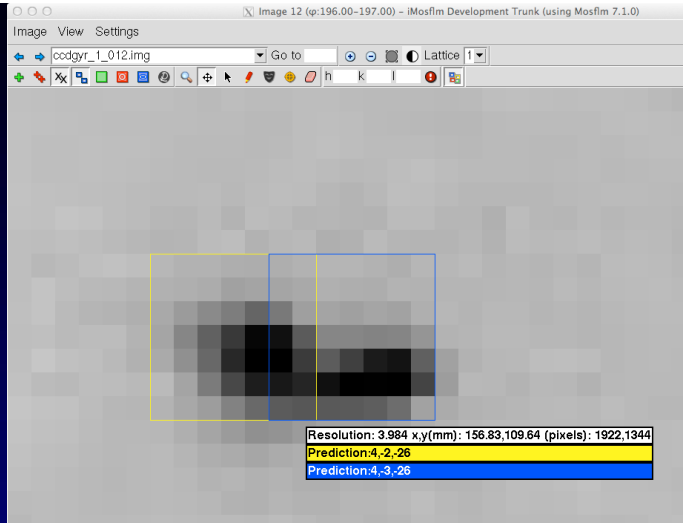
How to integrate?

Partially overlapping reflections

- The total intensity of a group of overlapping reflections can be determined precisely!
- Partitioning by 3D profile fit possible.
- Special file format to hold two domains, multiple files, or intensities have to be merged.

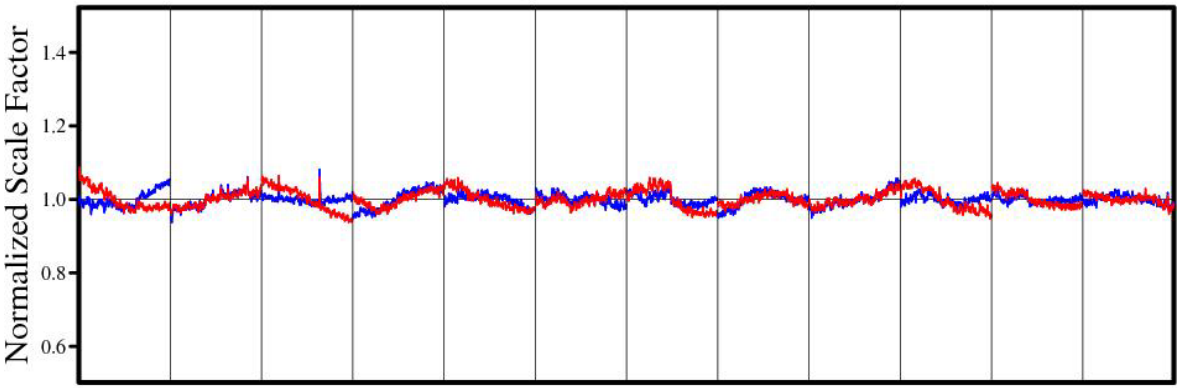


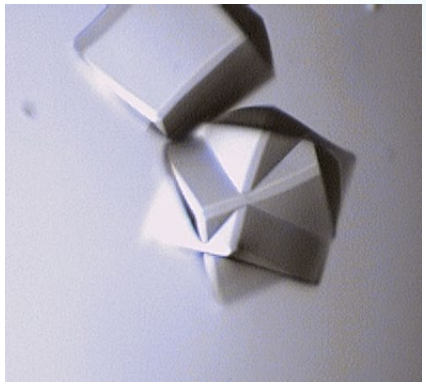


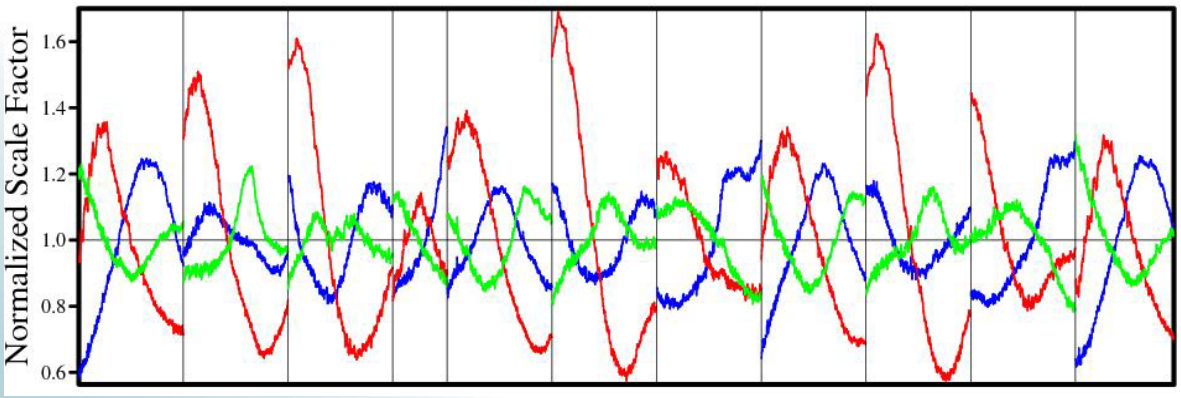


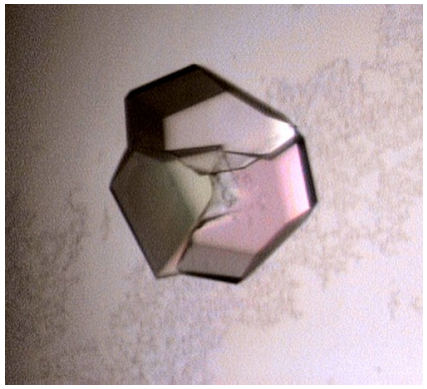
Scaling and absorption correction

In TWINABS:









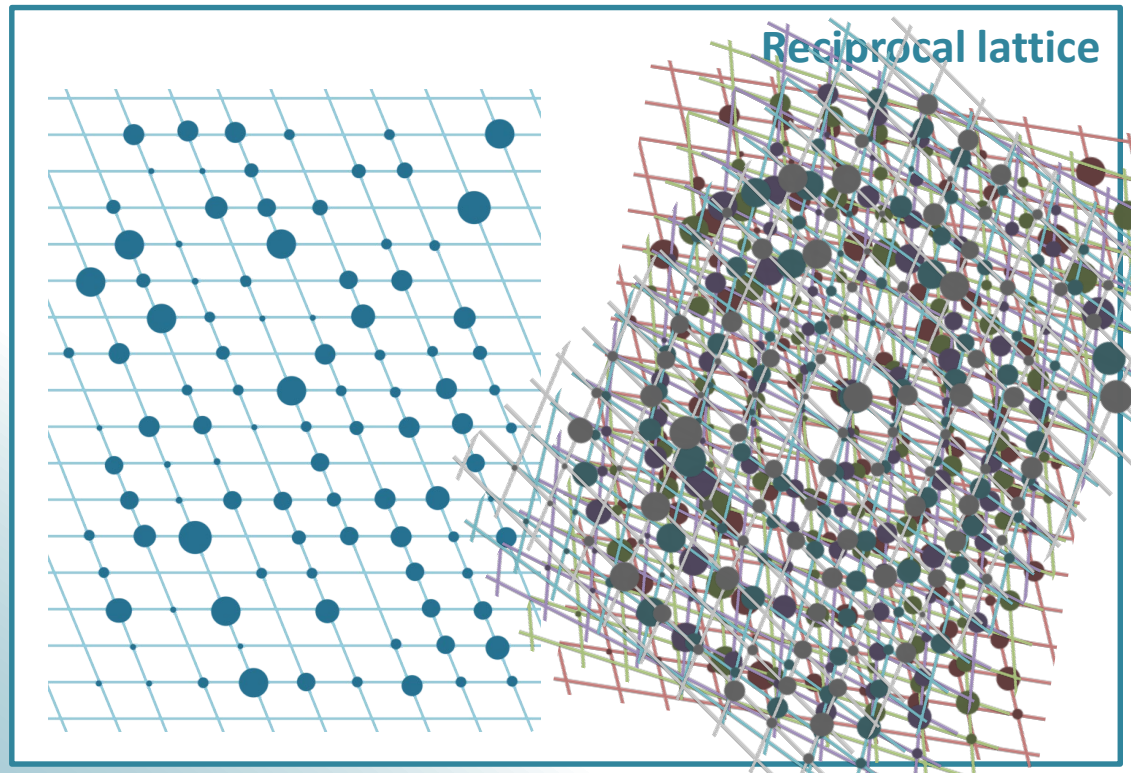
The smallest crystal (red) was furthest from the center.

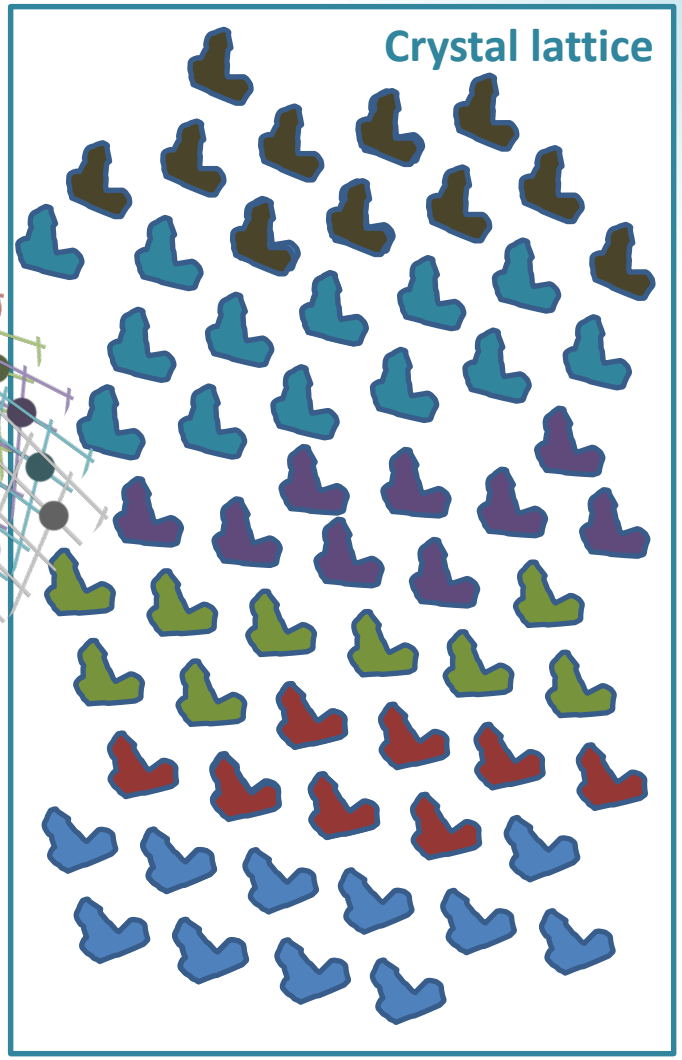
Non-merohedral twinning

- **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
- If feasible: Try irradiating only a part of the crystal, or get another crystal.

Split crystal

This is NOT a twin!

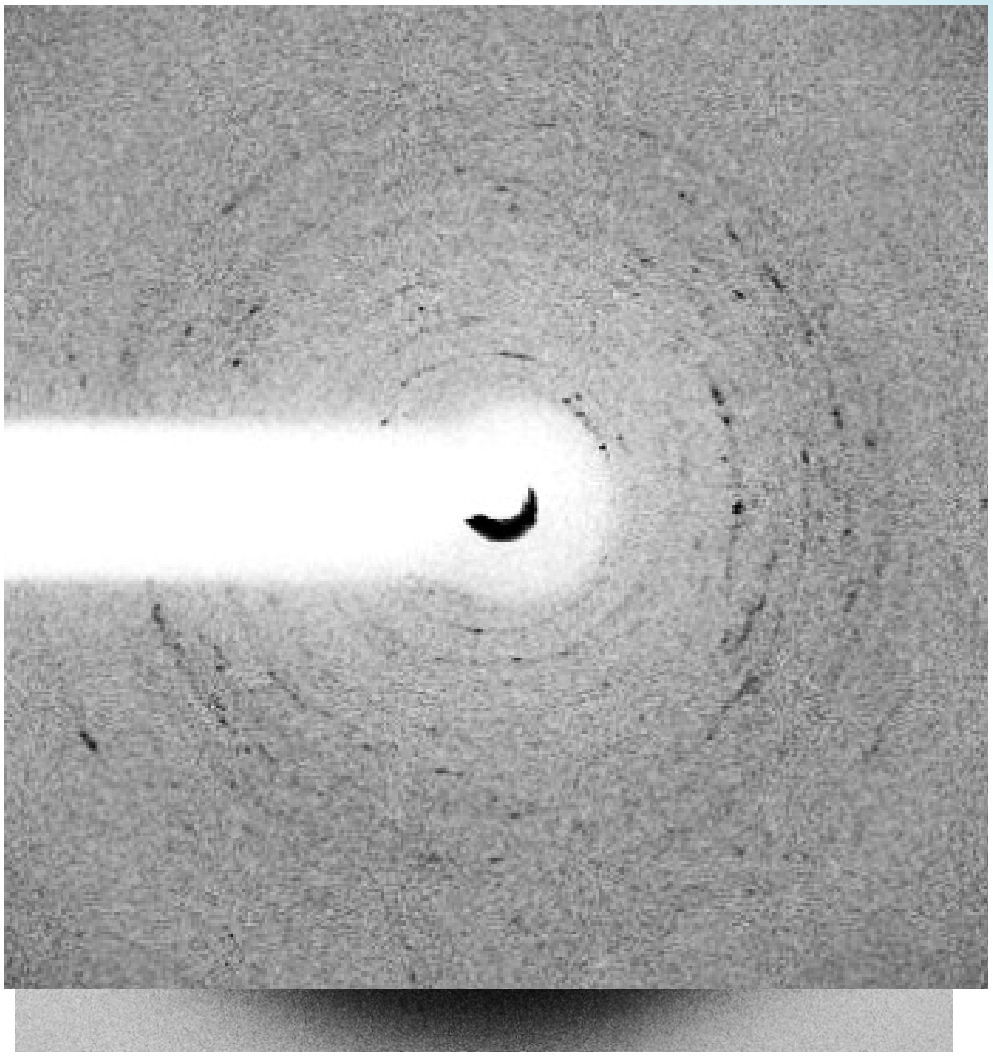




Split crystal

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

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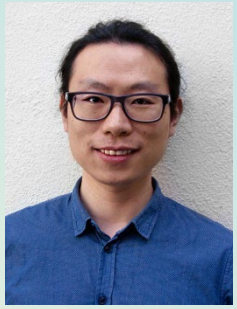

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