

Things you don't want to see in your diffraction data

(Why we do want to see diffraction patterns in 3D)

Andrey Lebedev, CCP4

A questions to ask if structure determination or refinement does not go smooth:

Is there any crystal pathology? (Crystal pathology = no global periodicity)

If yes:

- successful structure solution is less likely
- even if the structure is solved, bad refinement stats are quite likely
 - explain in the manuscript
(e.g. a picture from `dials.reciprocal_lattice_viewer`)
- consider the option of finding better crystallisation conditions

Merohedral twinning cannot be detected by direct examination of diffraction patterns. There are other crystal pathologies that can be.

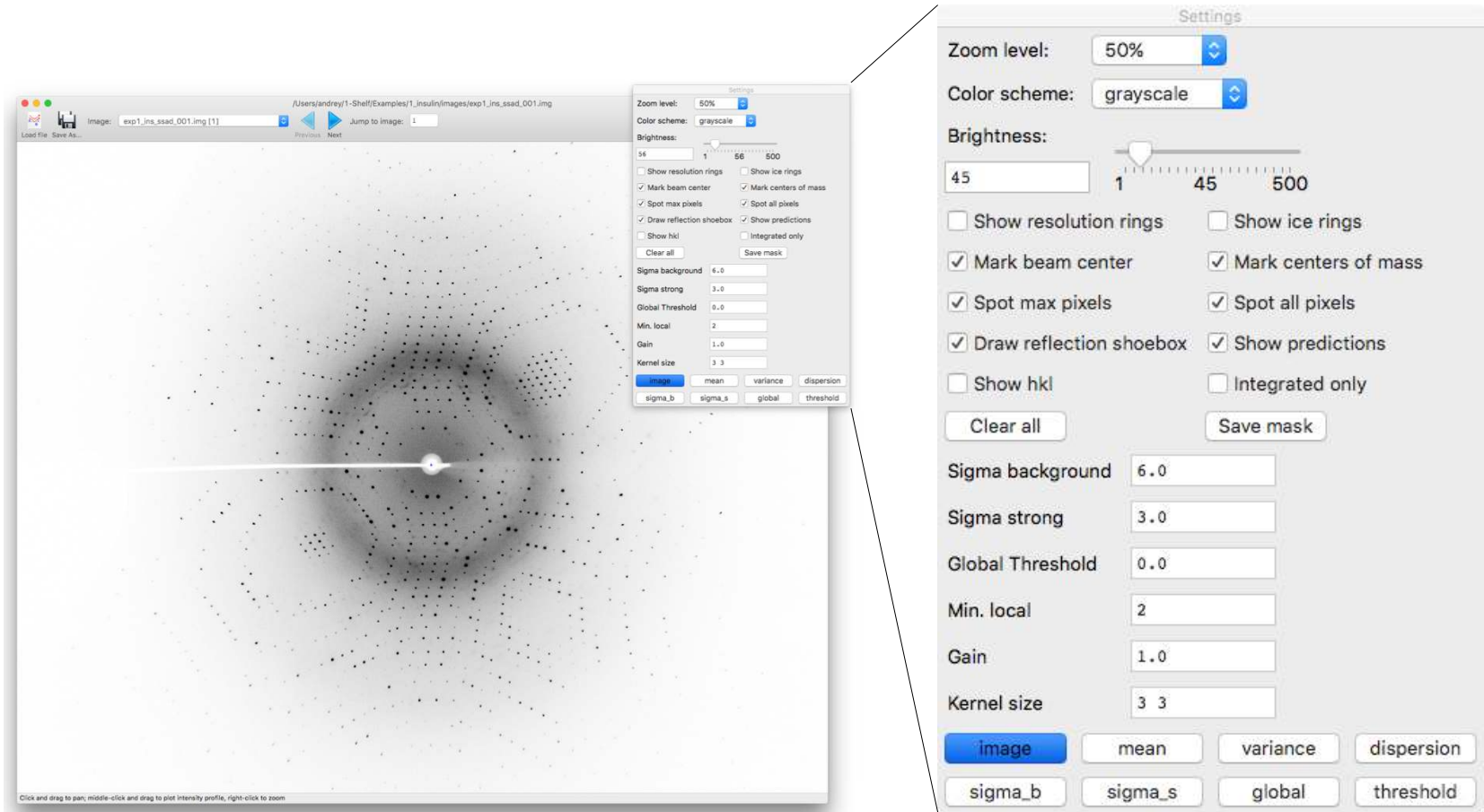
A few examples are presented here.

If my explanation is not clear enough – look at the pictures anyway

- Graphical facilities in DIALS
- Inter-grown crystals
- OD-structures
- Partially disordered OD-structures
- Pseudo-translation

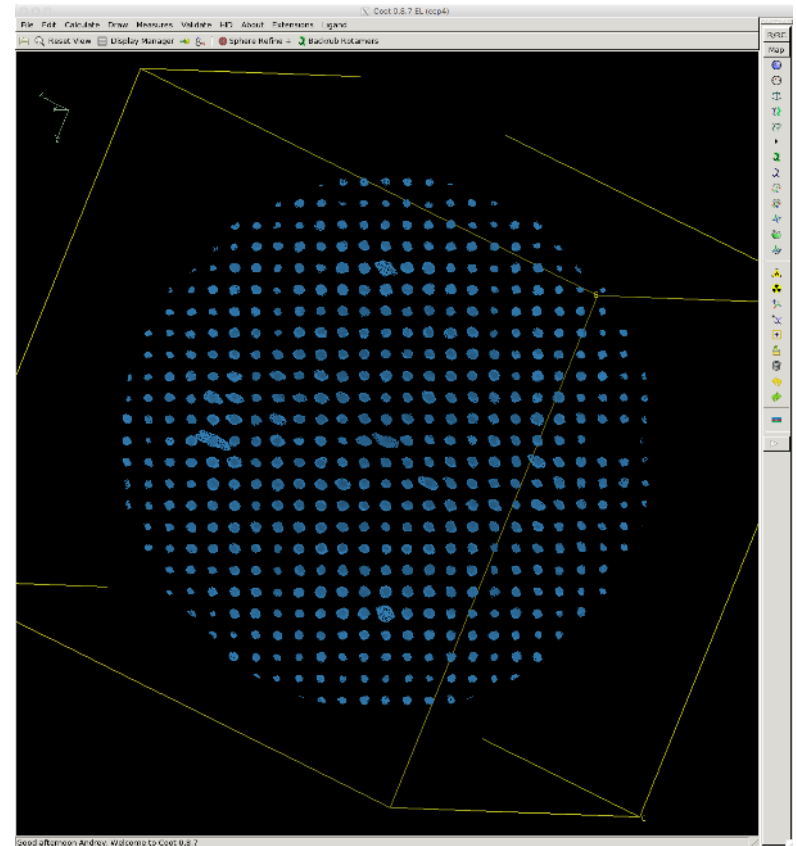
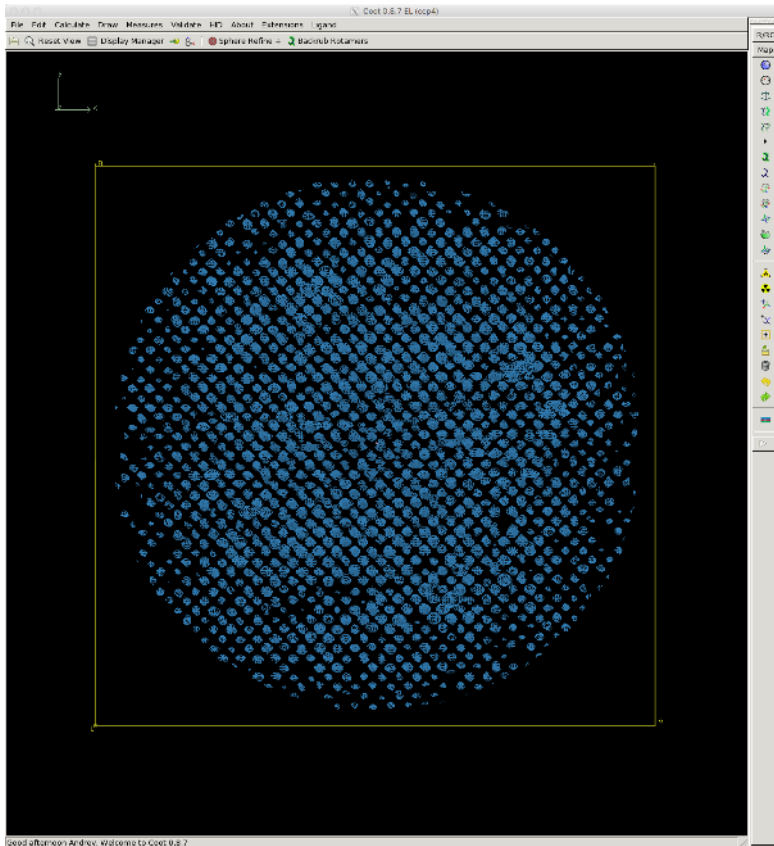
Image viewer

Cubic insulin, the experiment 1 from HZB MX tutorial



Sweep of images as 3D map

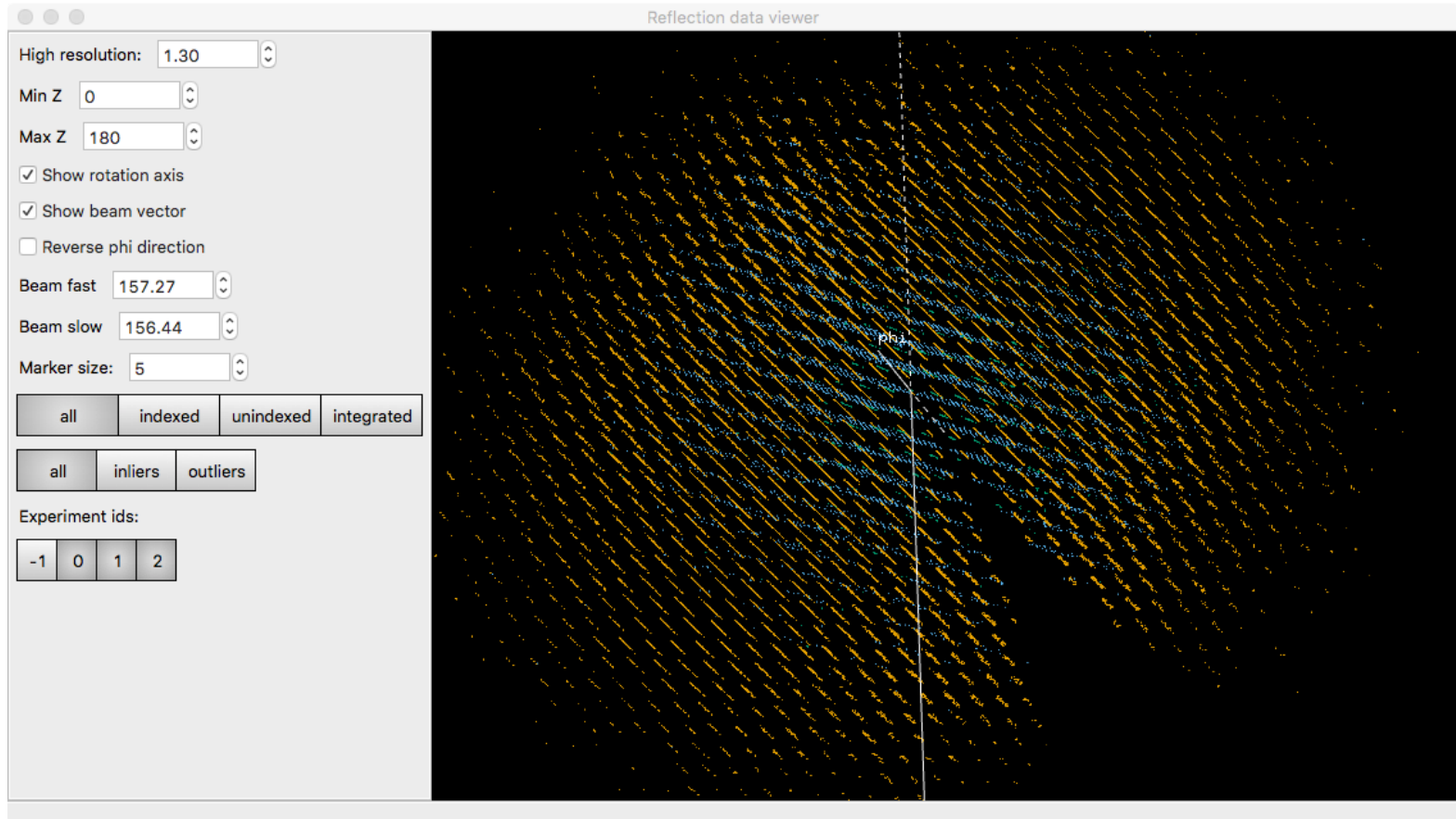
```
dials.rs_mapper data_00*.img  
coot --map output.ccp4
```



PyMol can be used as well

Sweep of images: spots positions in 3D

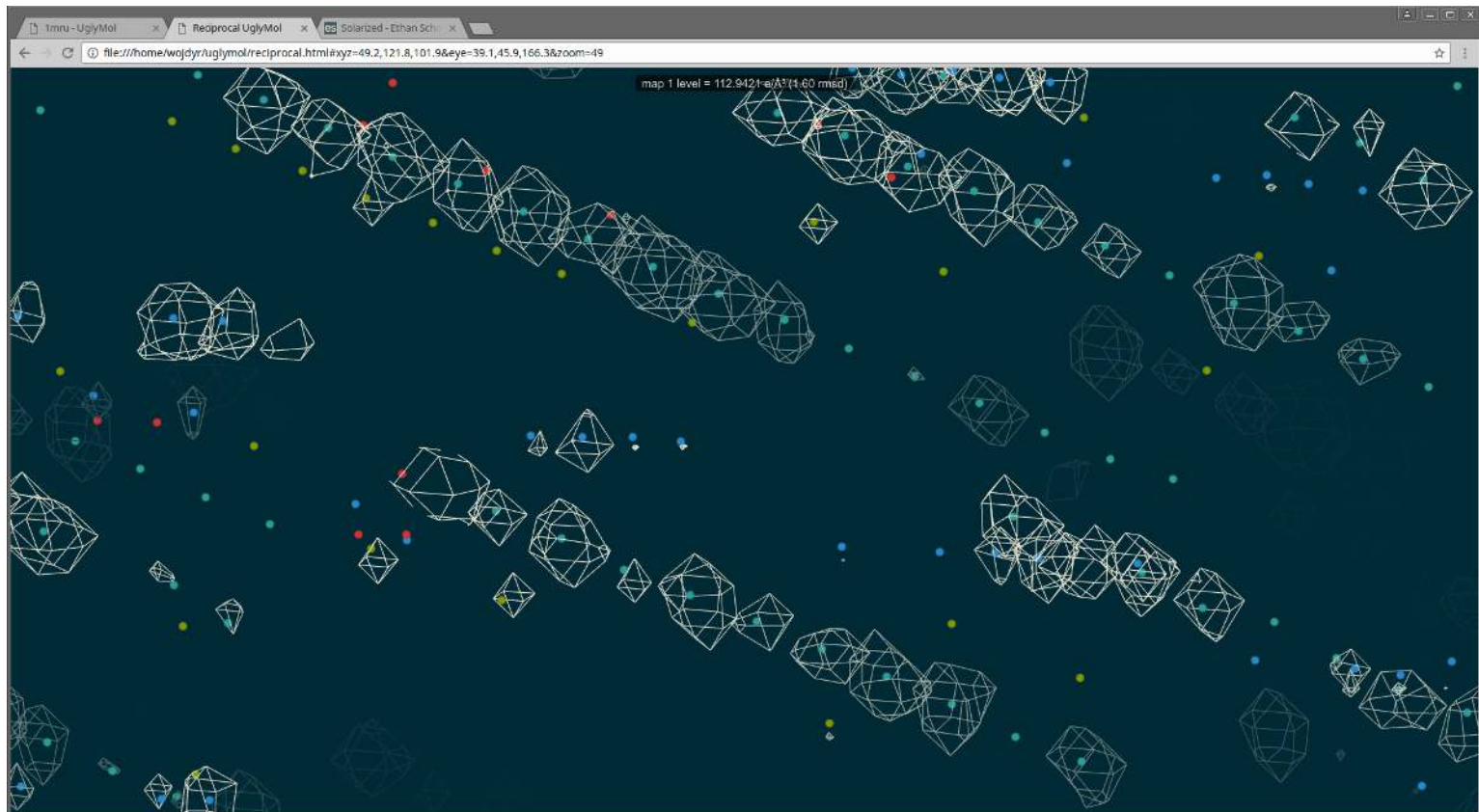
dials.reciprocal_lattice_viewer



Combined views

Combined view, intensities as maps and spots as dots:

- Uglymol in the result page of Xia2 task in CCP4 Cloud
- XDS + spot2pdb + dials.rs_mapper + Coot

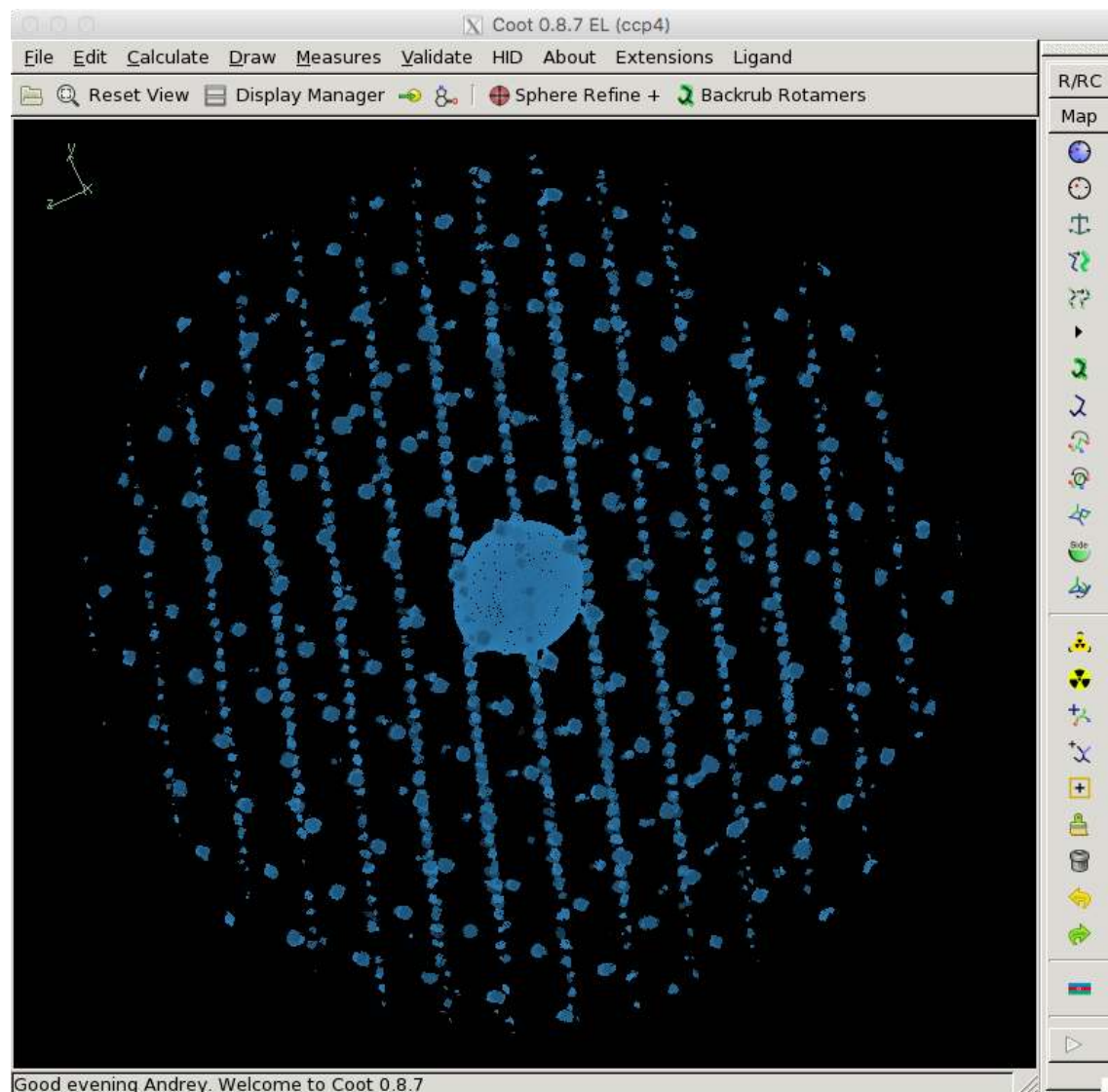
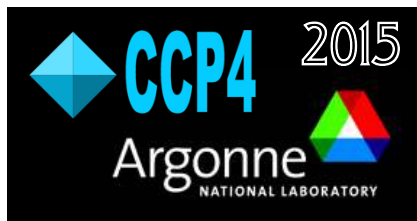


Inter-grown crystals (multi-lattice data)

Example of random crystal inter-growth

beta-lactamase OXA-163
PDB ID 4s2m

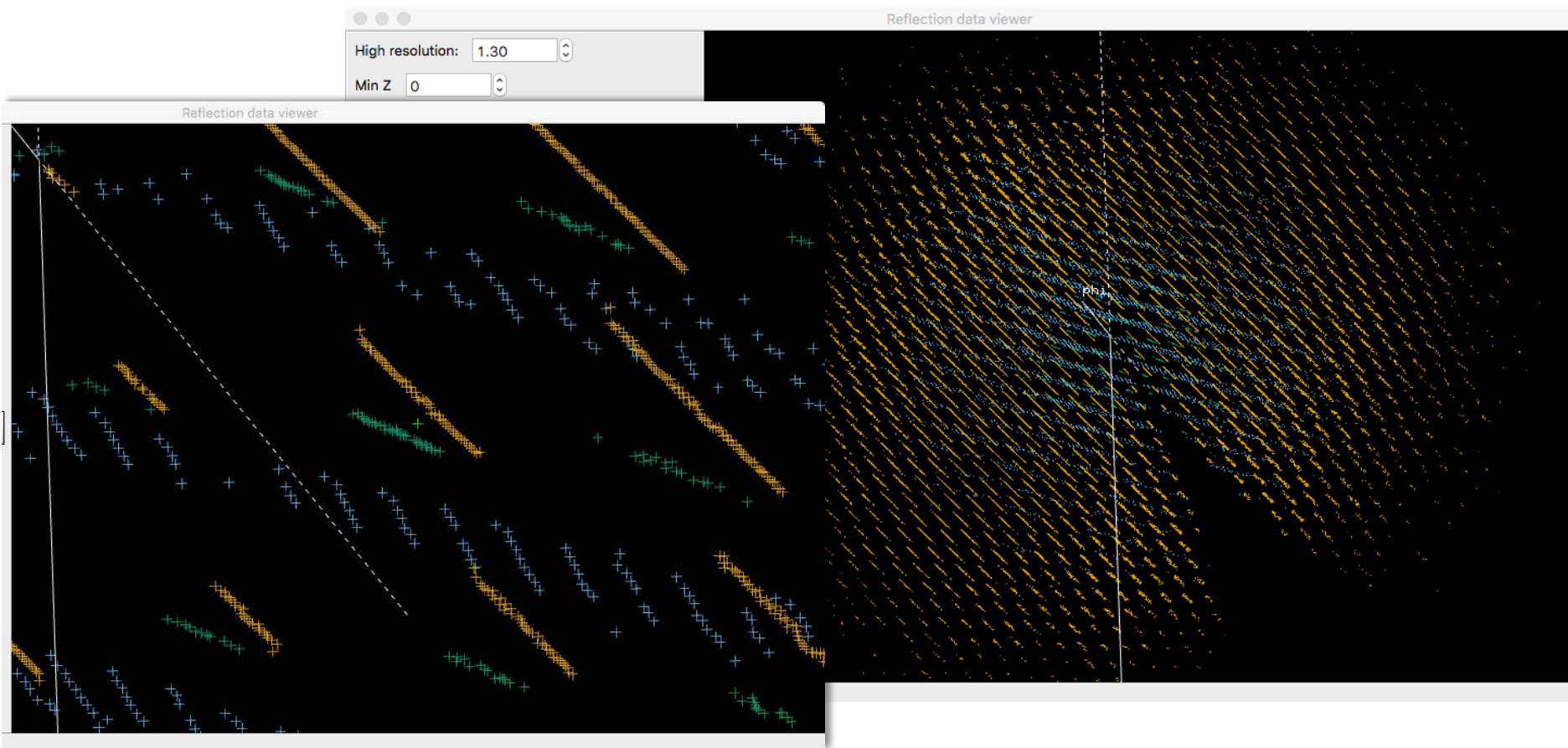
Data from Vlatko Stojanoski
Baylor College of Medicine



Example of random crystal inter-growth

dials.reciprocal_lattice_viewer

- different colour means different lattice
- individual lattices can be switched off and on



Example of random crystal inter-growth

Easy case:

- Lattices are mainly separated, with only very few reflection overlapping
- Signal from one lattice is substantially higher than from others

The intensities for the strongest lattice were processed,
structure solved and refined to $R=0.20$ $R\text{-free}=0.26$

An extreme case

Example from Leela Ruckthong

- How many lattices you can spot here?

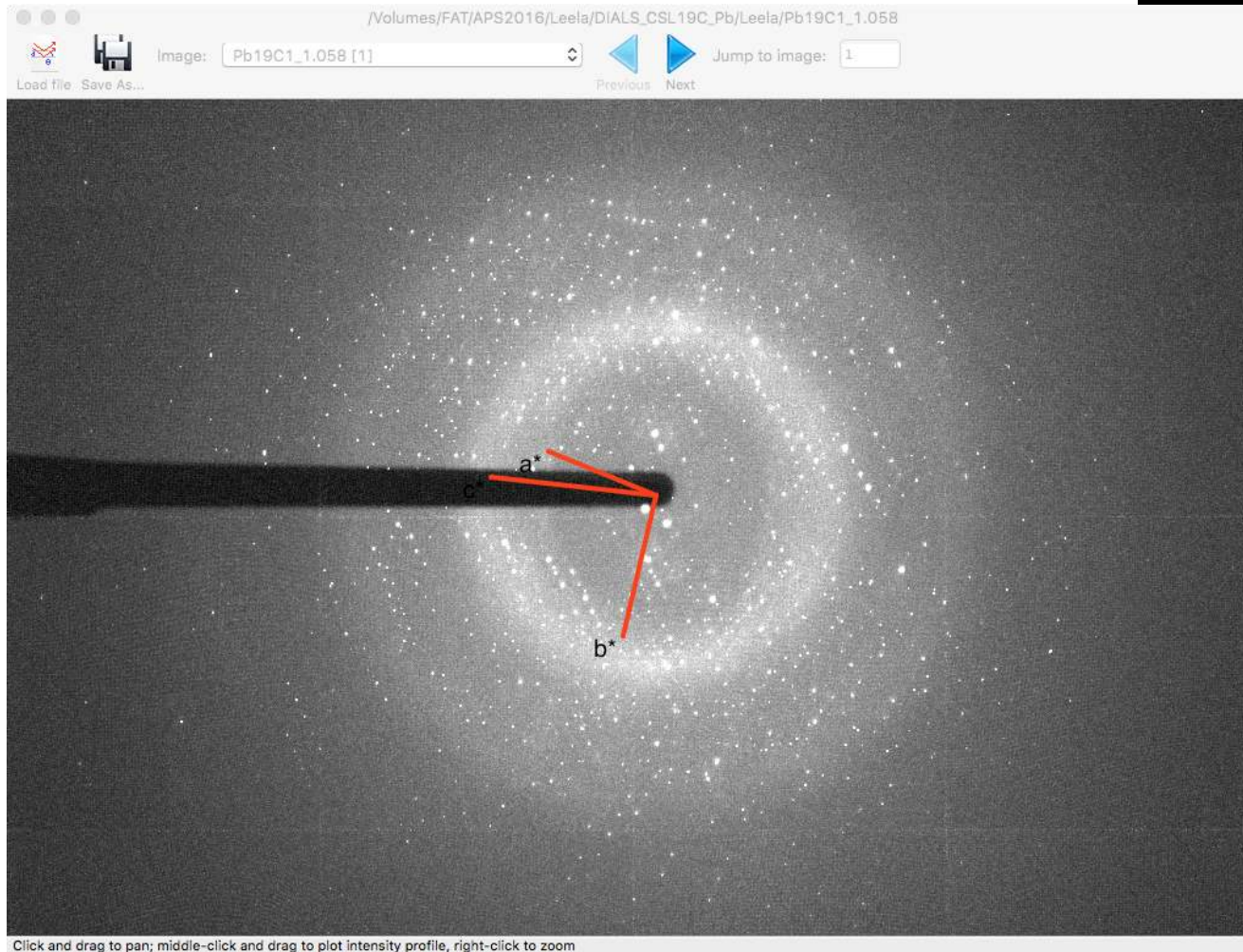
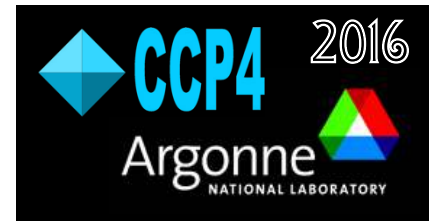




Image: Pb19C1_1.058 [1]



Jump to image: 1

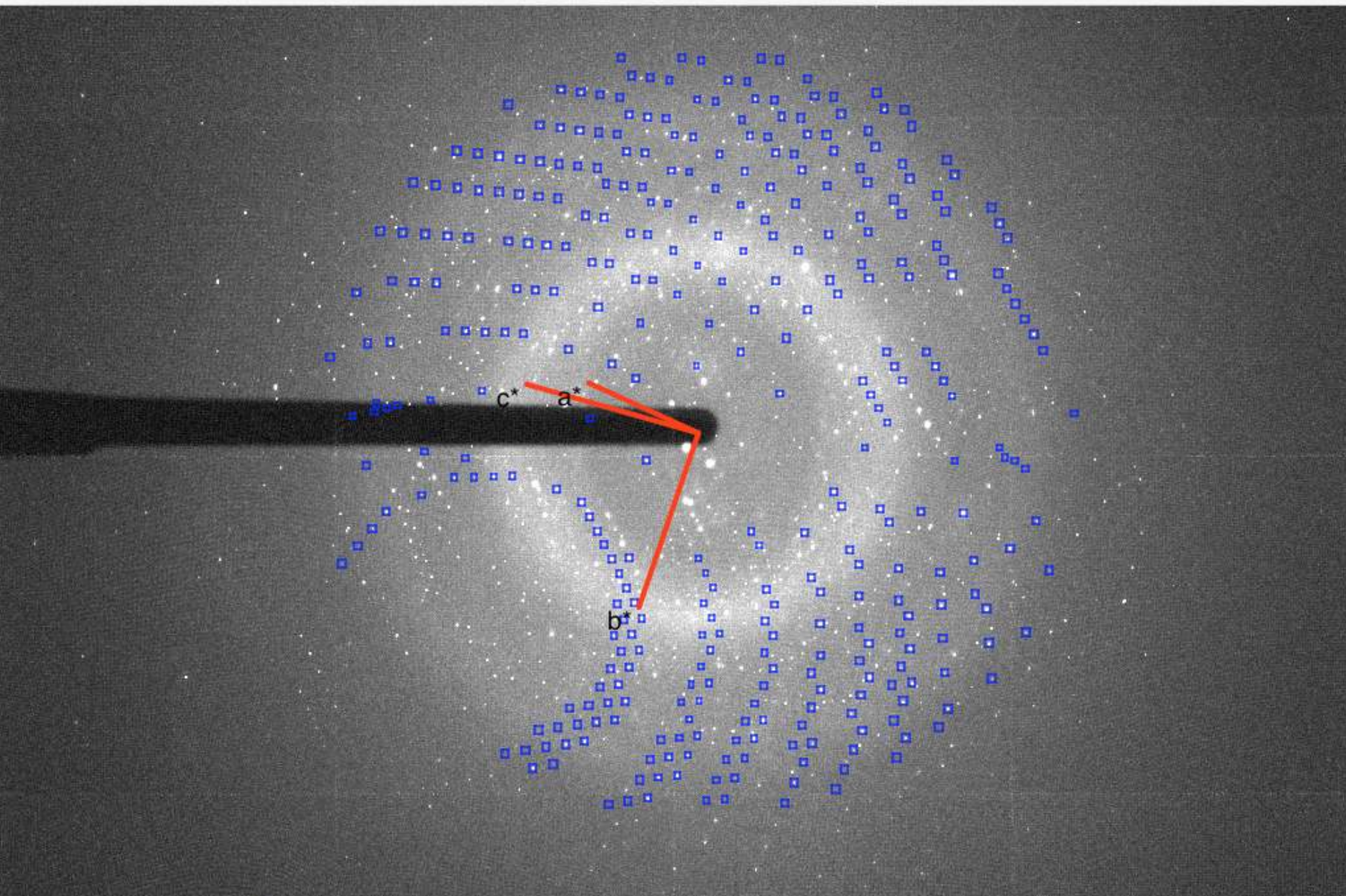




Image: Pb19C1_1.058 [1]



Jump to image: 1

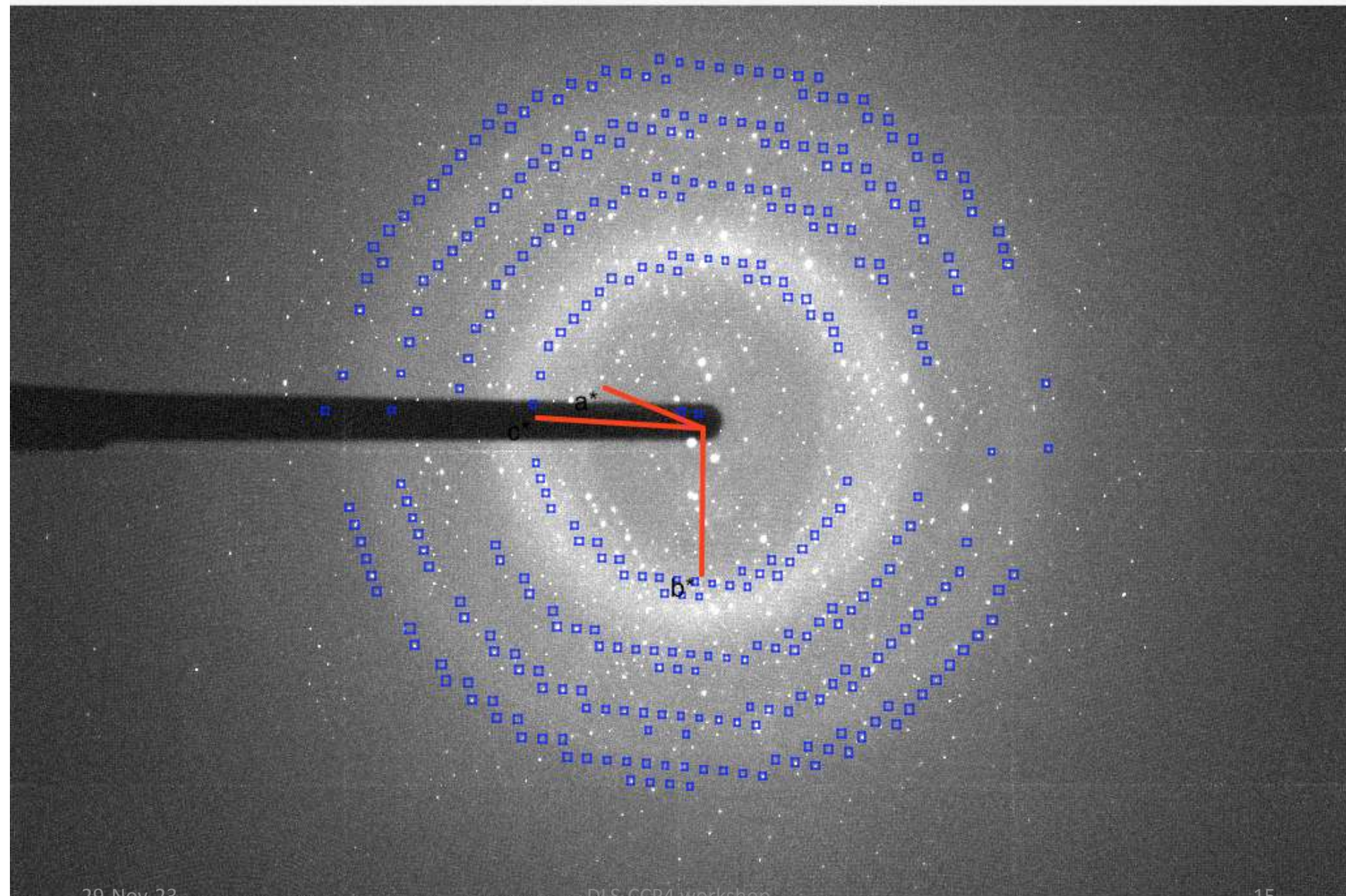




Image: Pb19C1_1.058 [1]



Jump to image: 1

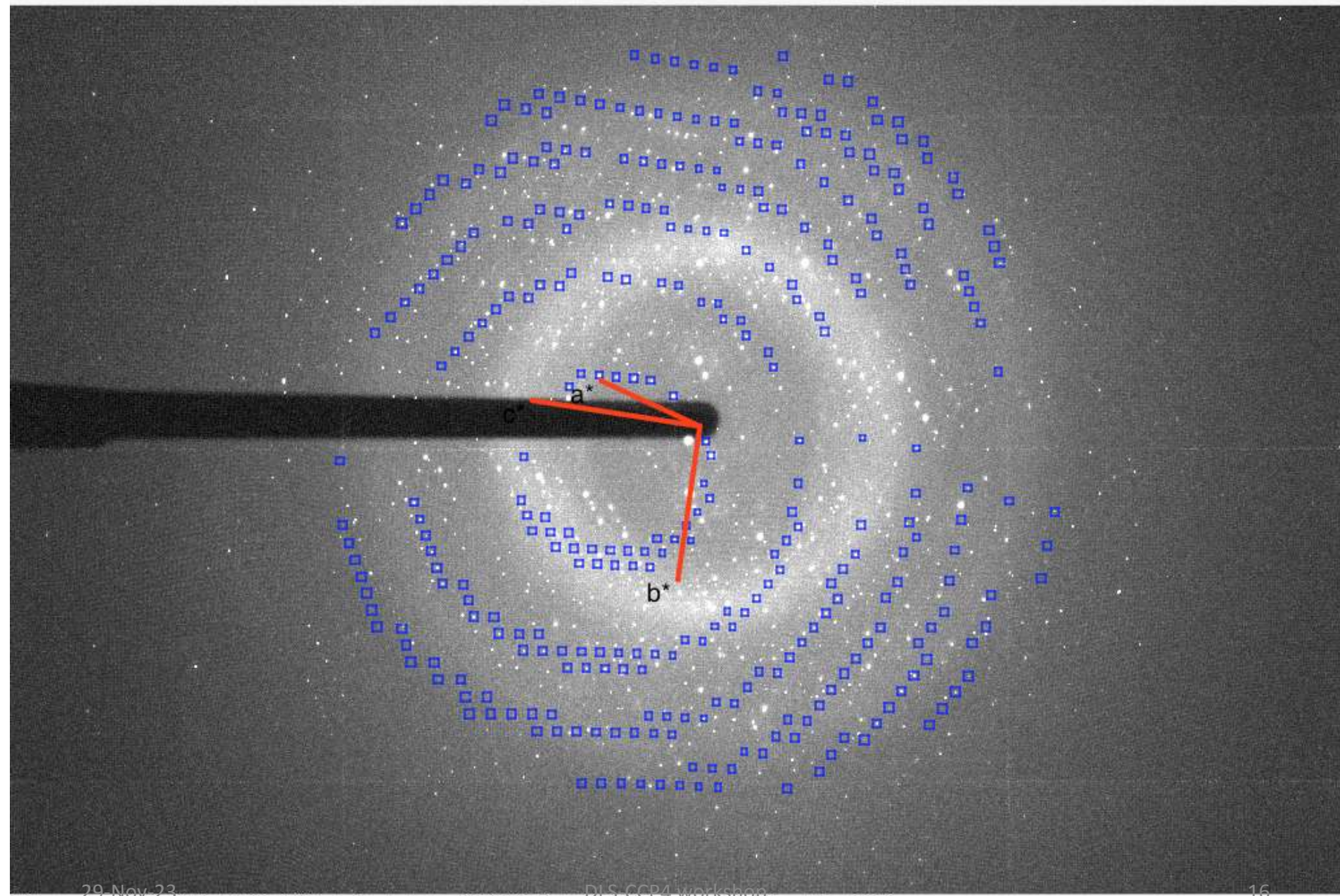




Image: Pb19C1_1.058 [1]



Jump to image: 1

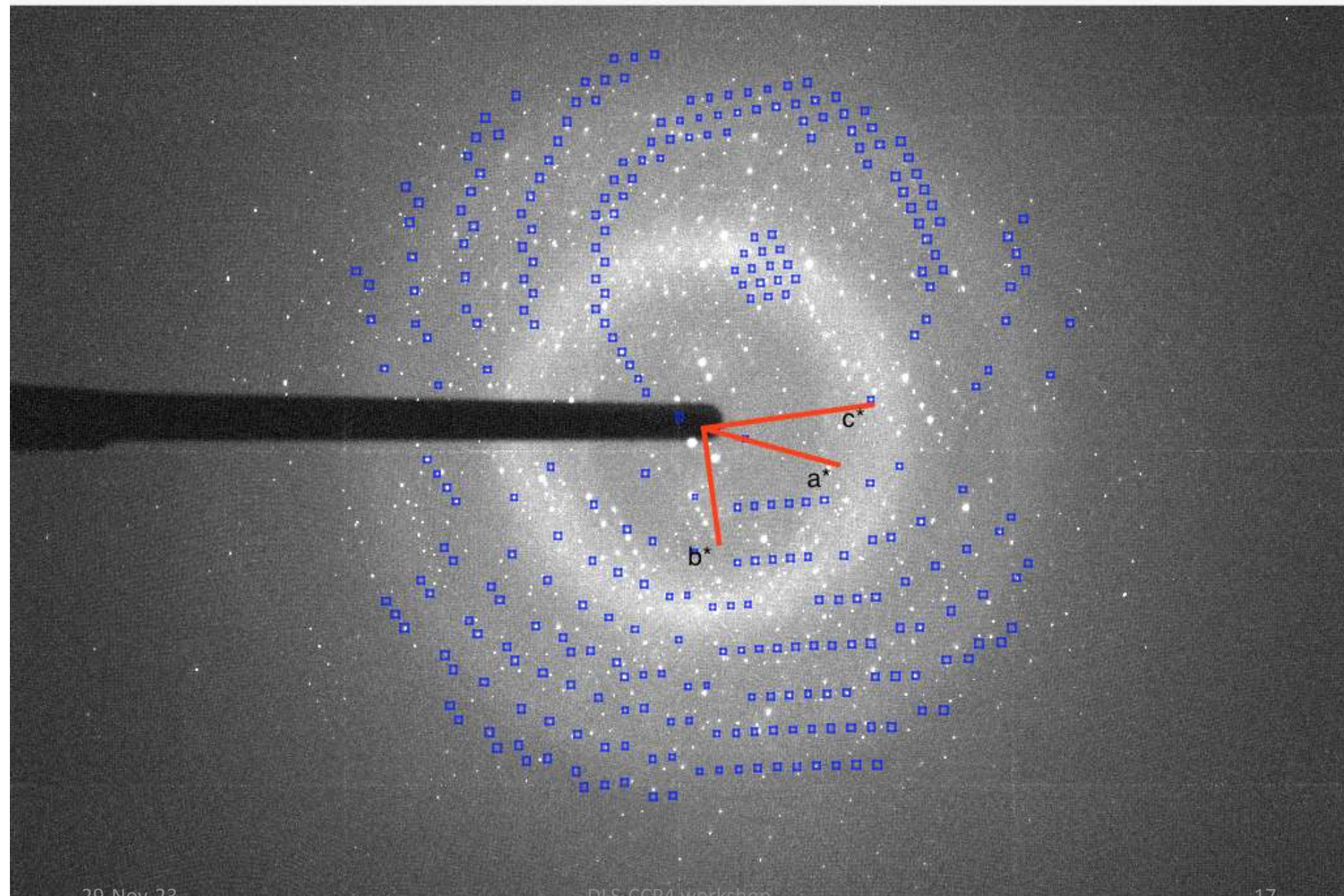




Image: Pb19C1_1.058 [1]



Jump to image: 1

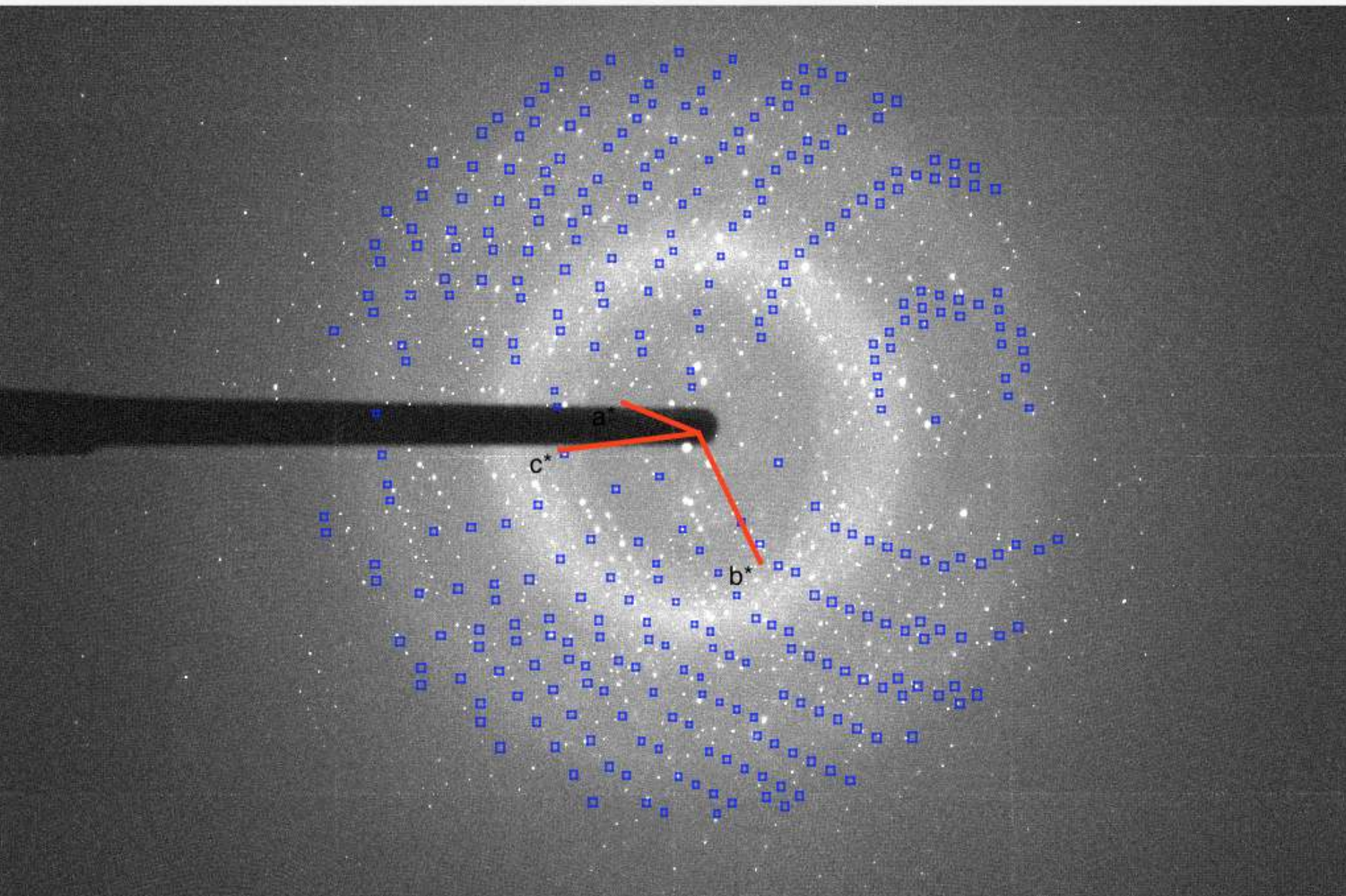
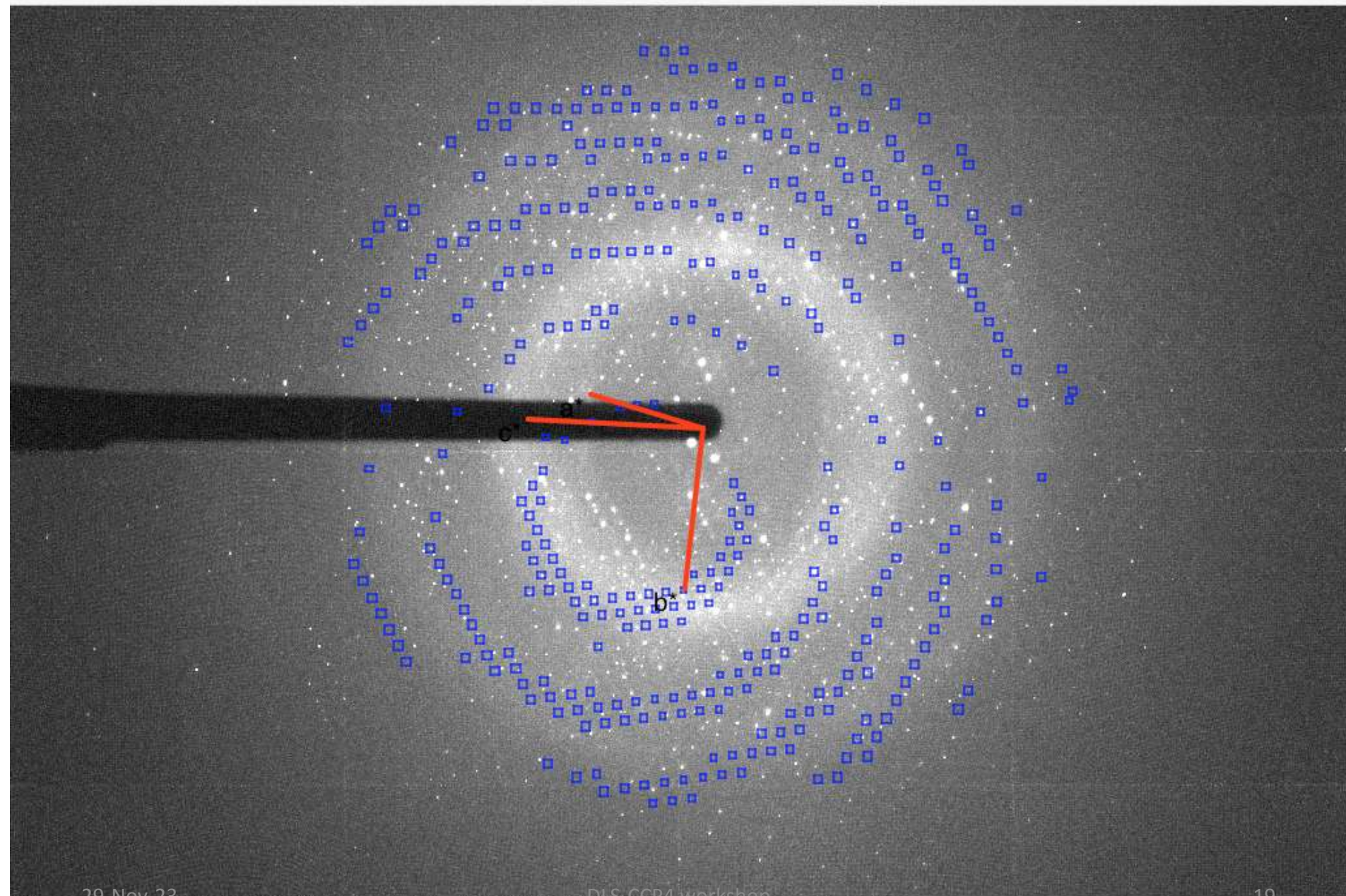


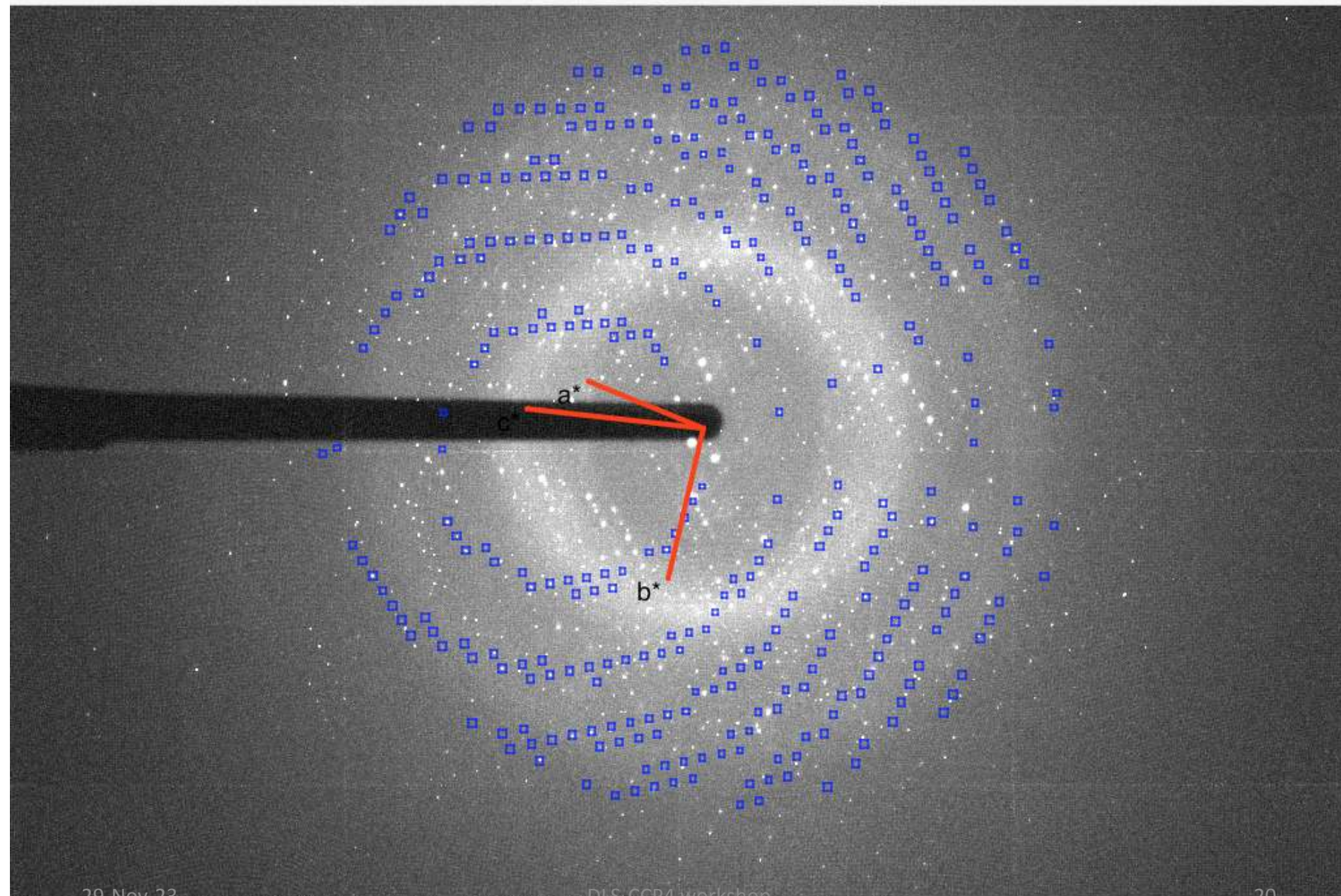


Image: Pb19C1_1.058 [1]



Jump to image: 1



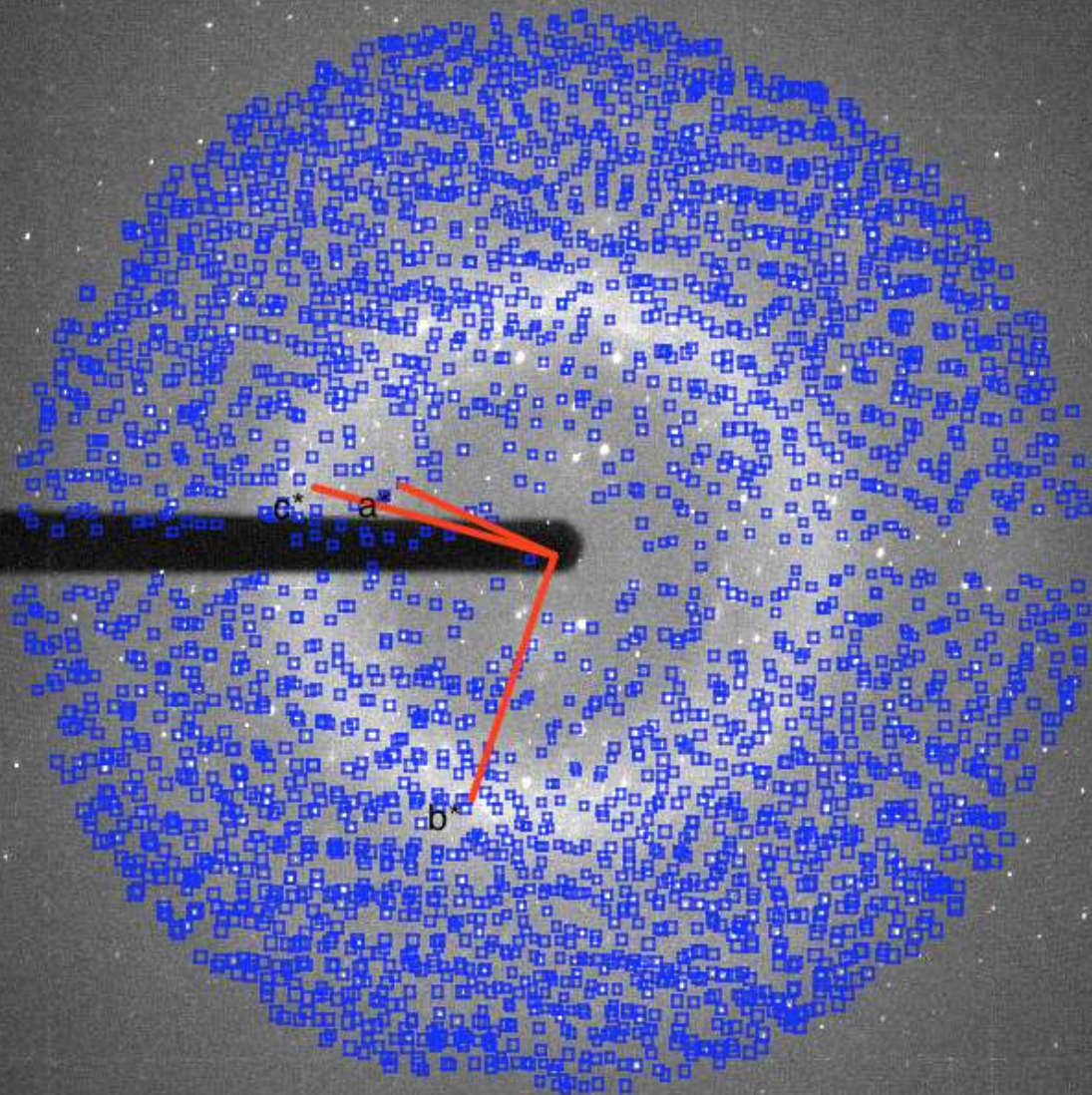


Load file Save As...

Image: Pb19C1_1.001 [1]

Previous Next

Jump to image: 1

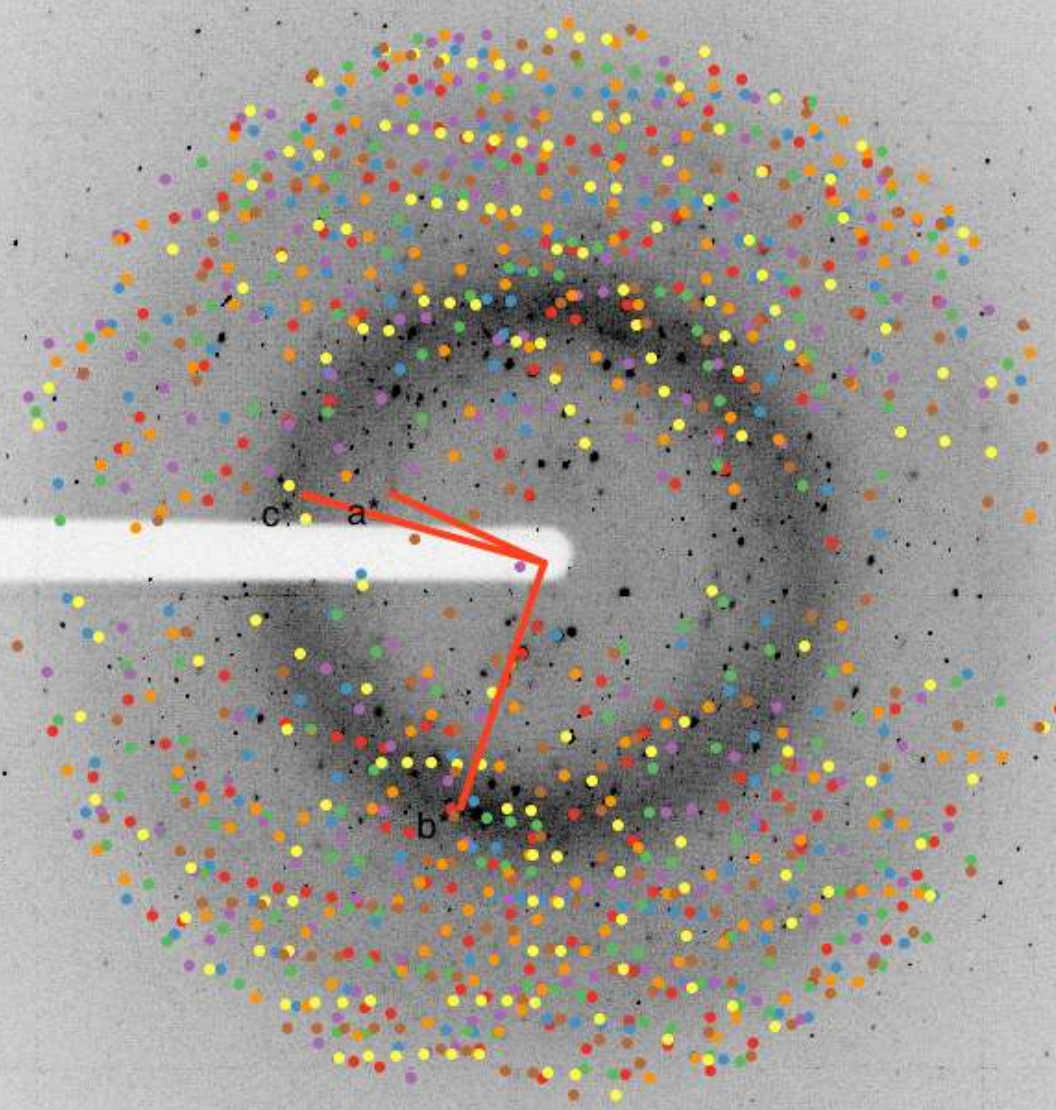


Load file Save As...

Image: Pb19C1_1.001 [1]

Previous Next

Jump to image: 1



Seven lattices: too many overlapping spots?

- Only the strongest single lattice gave reasonable merged data
 - » all others were incomplete or had much lower $I/\sigma(I)$
 - » merging data from several lattices did not work well
- Solved, refinement stats are not very good

Summary on multiple lattices

- Usually it is reasonable to use the data derived from one single lattice
- Sometimes completeness can be improved by merging datasets derived from two or more lattices
- Sometimes the second lattice found by Dials result in a dataset of higher resolution than the first one.
- DIALS: Indexing all the lattices together facilitates refinement of the parameters for each individual lattice (consider also `xia2.multiplex`)

Order-Disorder structures (OD-structures)

- Definition
- Example of an OD twin
- Example of allotwin

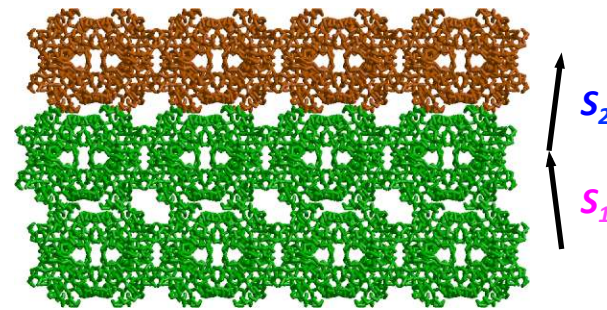
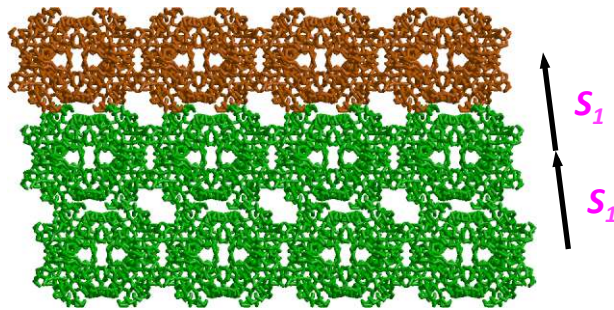
Actually disordered OD-structures:

- Relatively frequent
- Good for illustration purpose

Order-disorder structures (OD-structures)

- identical layers
- identical interfaces between the layers
- but: two or more ways of packing three adjacent layers

*) MX: "identical" means Ca r.m.s.d. < 1 Å

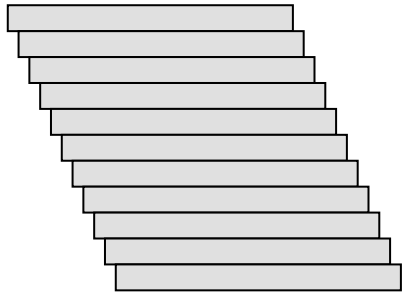


*) S_1 and S_2 are called stacking vectors

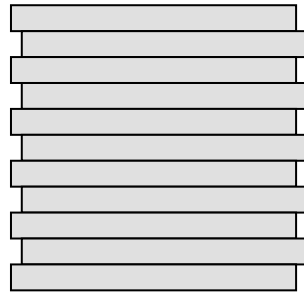
- two-dimensional periodicity
- a **potential** for disorder in the third dimension

OD-structures

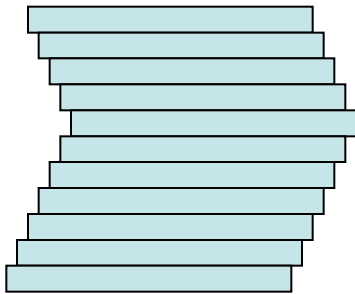
Single crystal



Single crystal

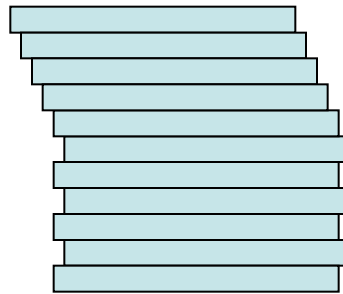


OD-twin



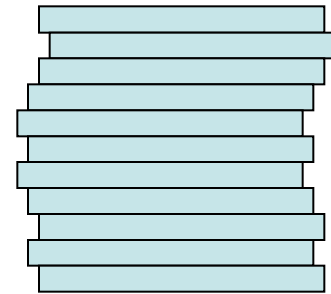
Example 1

Allotwin



Example 2

Partially
disordered
OD-structure



*Examples in the
next section*

Example 1: OD-twin

Morphological classification

OD-twin

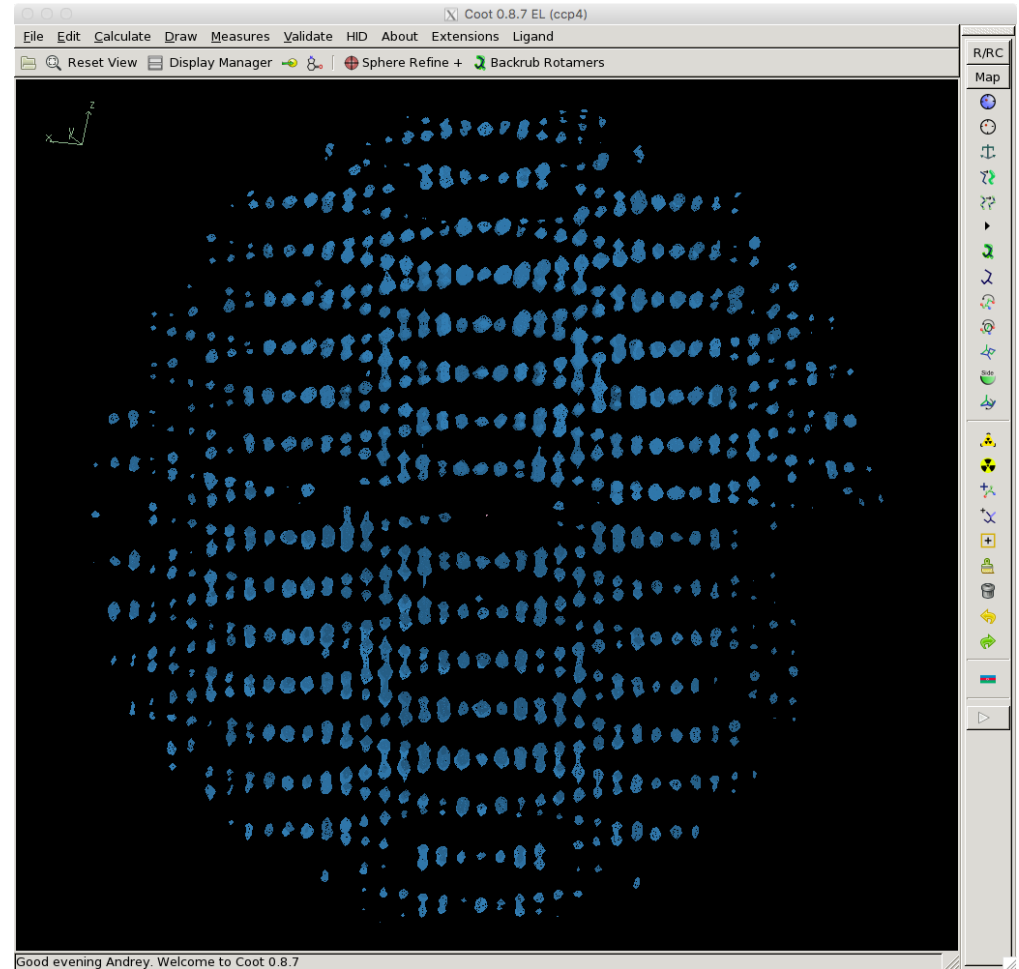
Geometrical classification

Twinning by reticular pseudo-merohedry

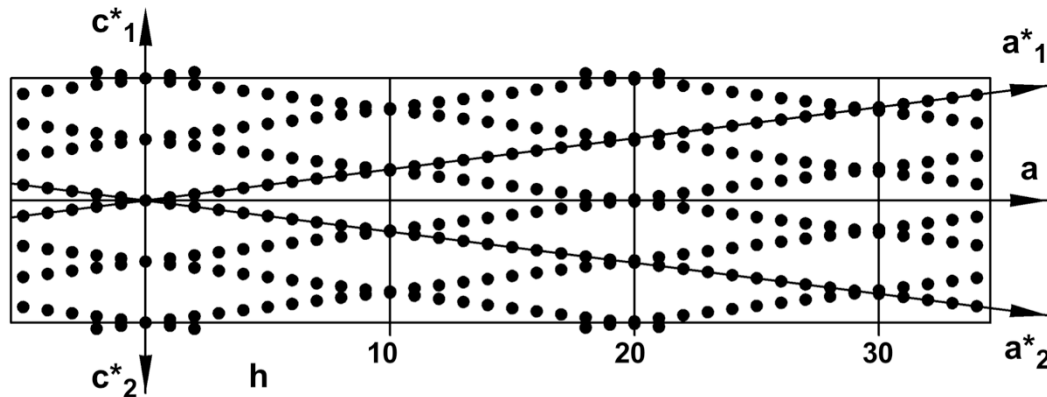
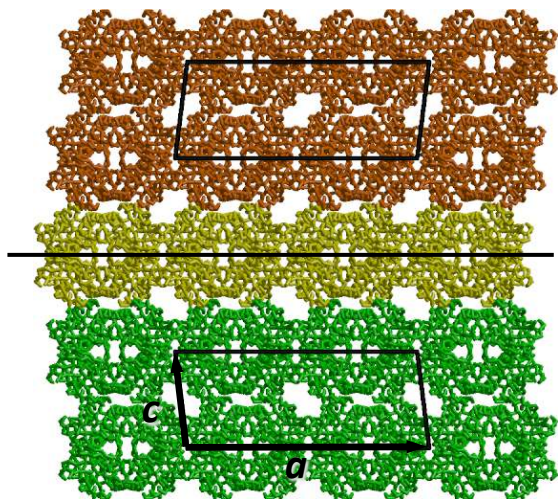
Synonym

Non-merohedral twinning

dials.rs_mapper + coot



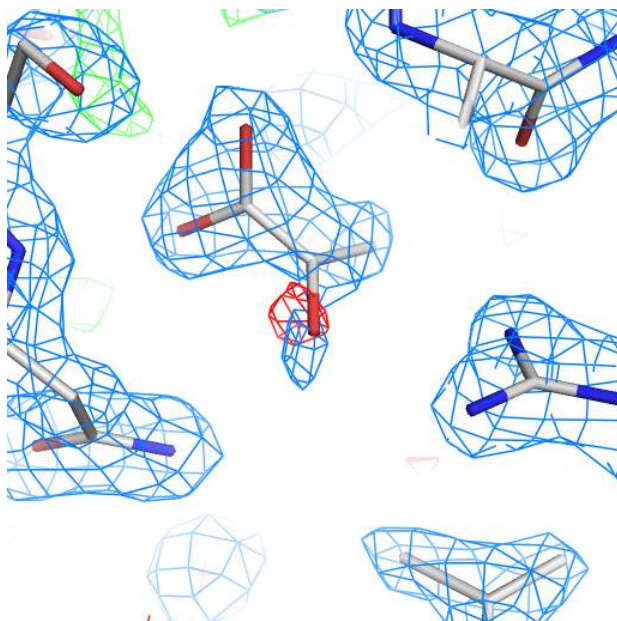
Real and reciprocal lattices



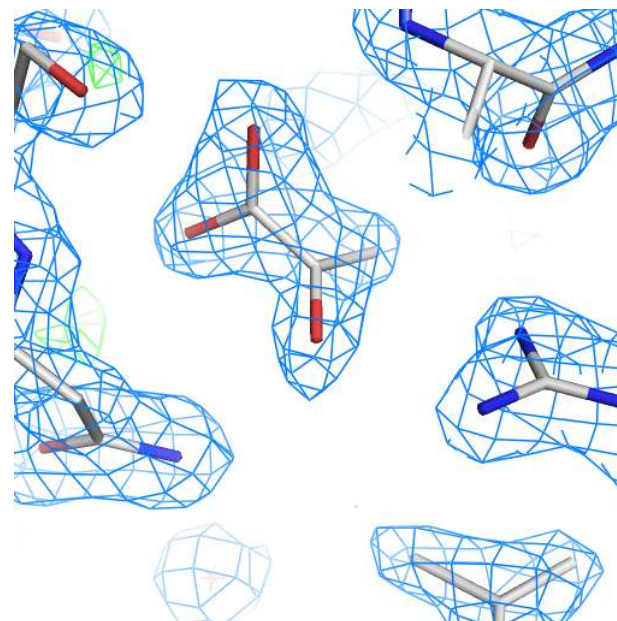
- Process data from one lattice and ignore twinning
- Process data from one lattice and demodulate the data
- Record total intensity of overlapping spots (SAINT, iMosflm) and deal with it at refinement (SHELXL)

Demodulation: improvement in the electron density

Visually, improvement from demodulation occurred only in the electron density for solvent molecules (here: L-lactate)

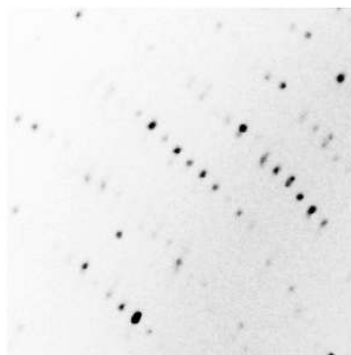
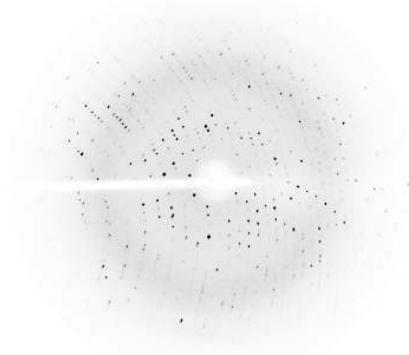


Before demodulation
R / R-free = 0.21 / 0.27



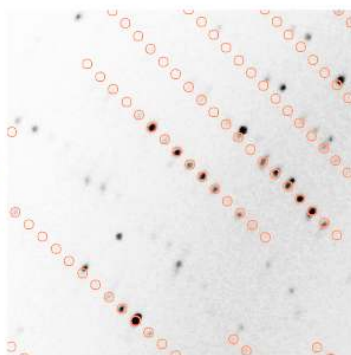
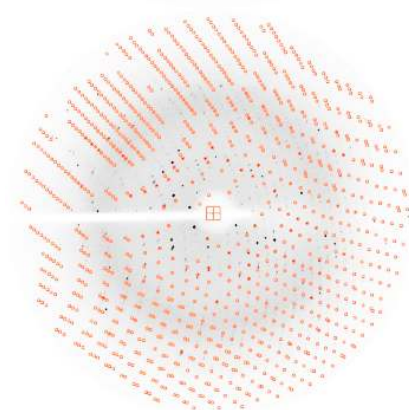
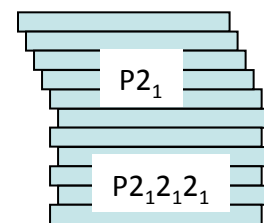
After demodulation
R / R-free = 0.16 / 0.23

Example 2: allotwin



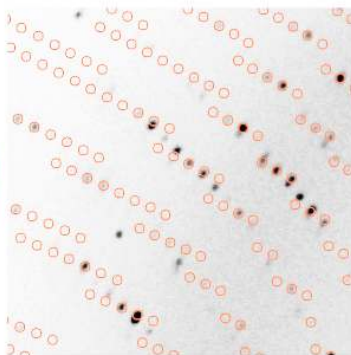
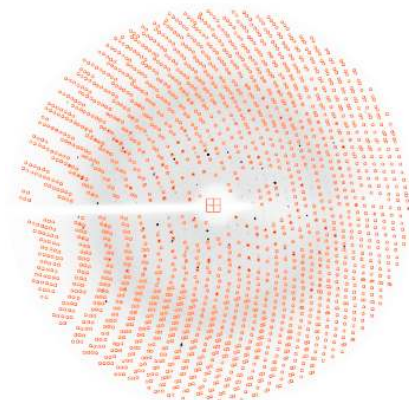
Crystals of Lon protease
Resolution 3Å

Dauter *et al.* (2005).
Acta Cryst. D61, 967-975.



$P2_1$

$a = 48.5 \text{ Å}$
 $b = 86.3 \text{ Å}$
 $c = 138.0 \text{ Å}$
 $\beta = 92.3^\circ$



$P2_12_12_1$

$a = 86.3 \text{ Å}$
 $b = 90.6 \text{ Å}$
 $c = 148.0 \text{ Å}$

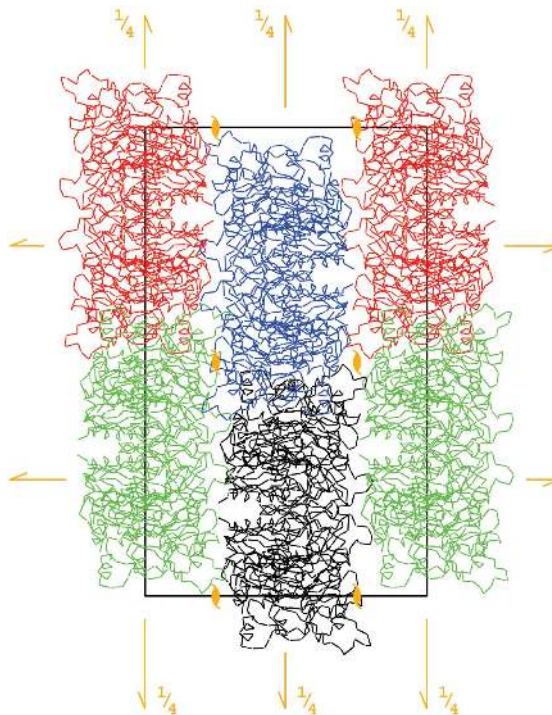
Example 2: allotwin

Crystals of Lon protease
Resolution 3Å

Dauter *et al.* (2005).
Acta Cryst. D **61**, 967-975.

Structures of both crystal
forms were solved from a
one allotwin crystal

PDB code 1z0t

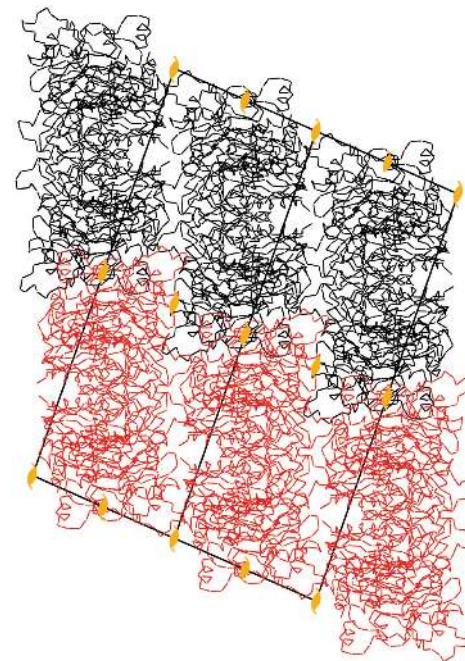


$P2_12_12_1$

R / R-free

0.19 / 0.35

PDB code 1z0v



$P2_1$

0.21 / 0.31

Example 2: allotwin

- More frequently, the presence of very different indexing solutions means that the indexing program is struggling rather than there are domains belonging to different space groups.
- 3D viewers will help to check what is actually happening.
- In any case, it is a warning sign if e.g. different programs suggest space groups belonging to different point groups (e.g. $C 2$ and $P 2_1 2_1 2_1$)

Example from ISPyB

Auto Processing							xia2 dials: ✓
Type	Resolution	Spacegroup	Mn<l/sig(i)>	Rmeas Inner	Rmeas Outer	Completeness	
xia2 dials	68.91 - 2.54	C 1 2 1	3.9	0.147	3.639	99.6	137.98 29.8
xia2 3dii	56.29 - 2.78	P 21 21 21	4.7	0.149	2.577	100.0	29.93 112.5
autoPROC							
fast_dp							
2x xia2.multiplex	92.39 - 2.23	C 1 2 1	4.4	0.146	5.270	100.0	137.70 29.8

xia2 dials ⚠

xia2 3dii ⚠

autoPROC

fast_dp

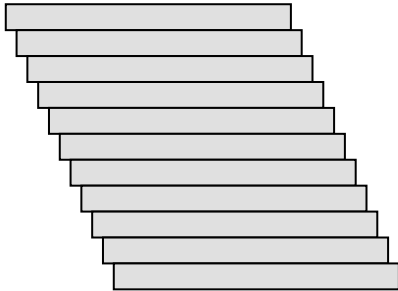
2x xia2.multiplex ⚠

Partially disordered OD-structures

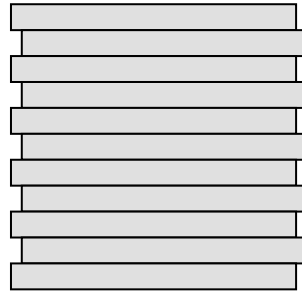
- Visualisation (detection)
- Ghost density
- Indexing
- Effect on structure solution

OD-structures

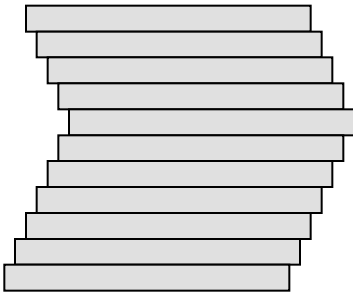
Single crystal



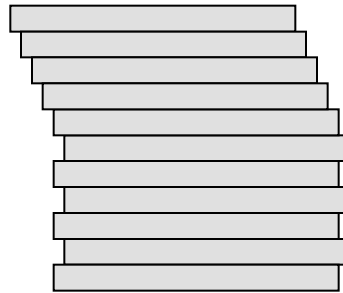
Single crystal



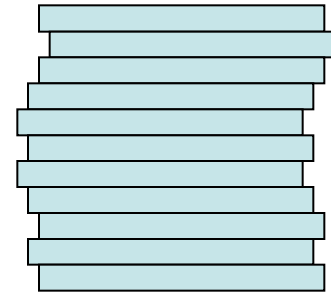
OD-twin



Allotwin

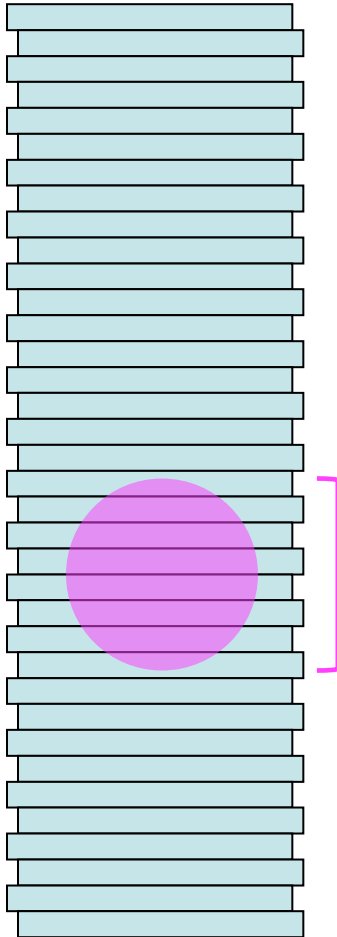


Partially
disordered
OD-structure

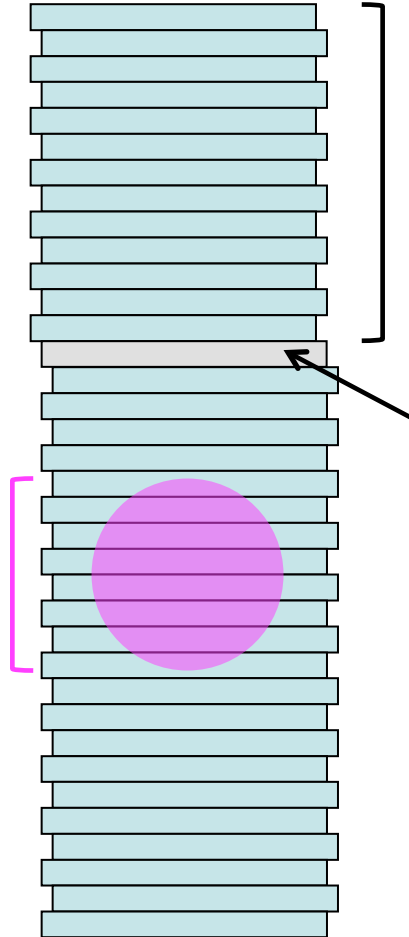


Partially disordered OD structures

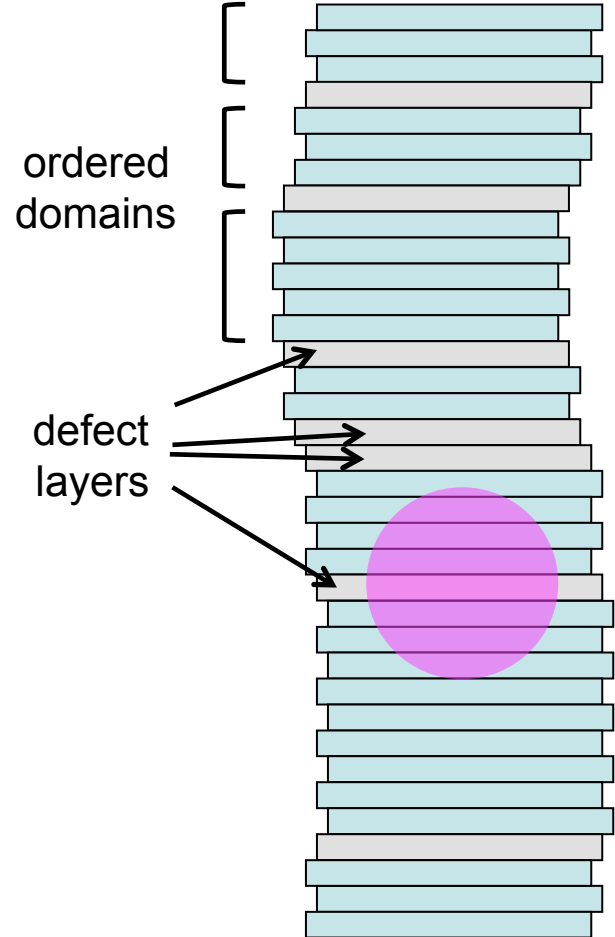
a true
single crystal



diffracts almost as
a single crystal

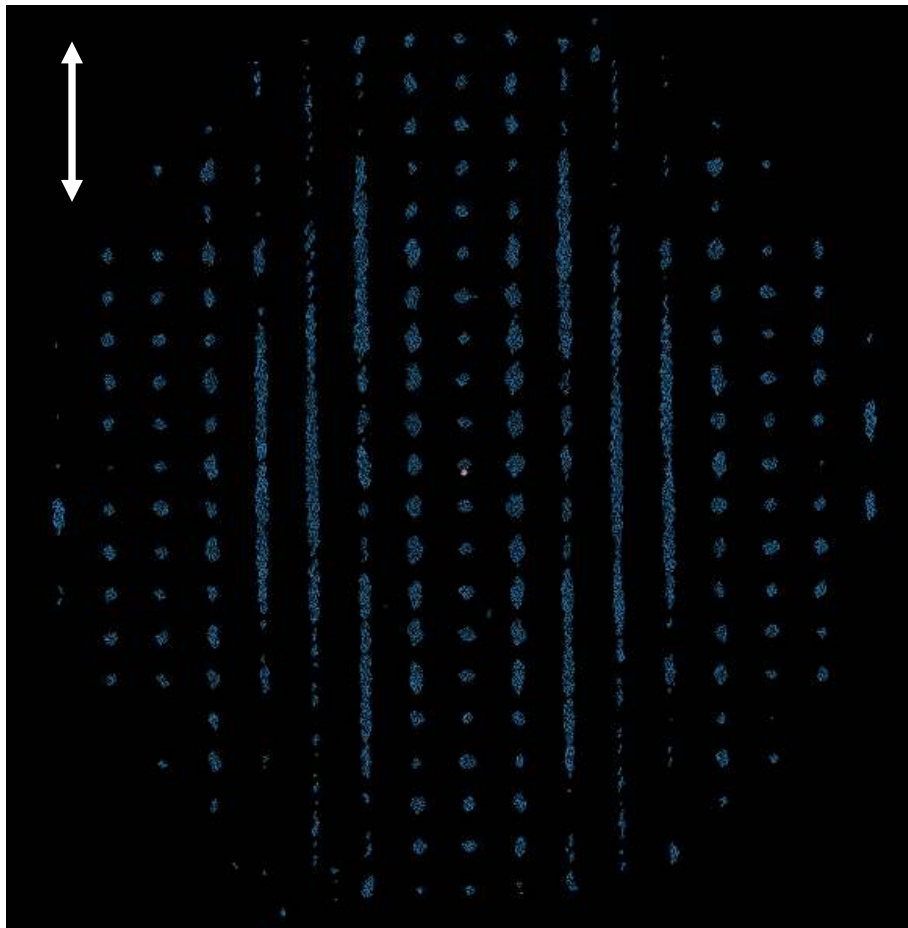


partially disordered
crystal

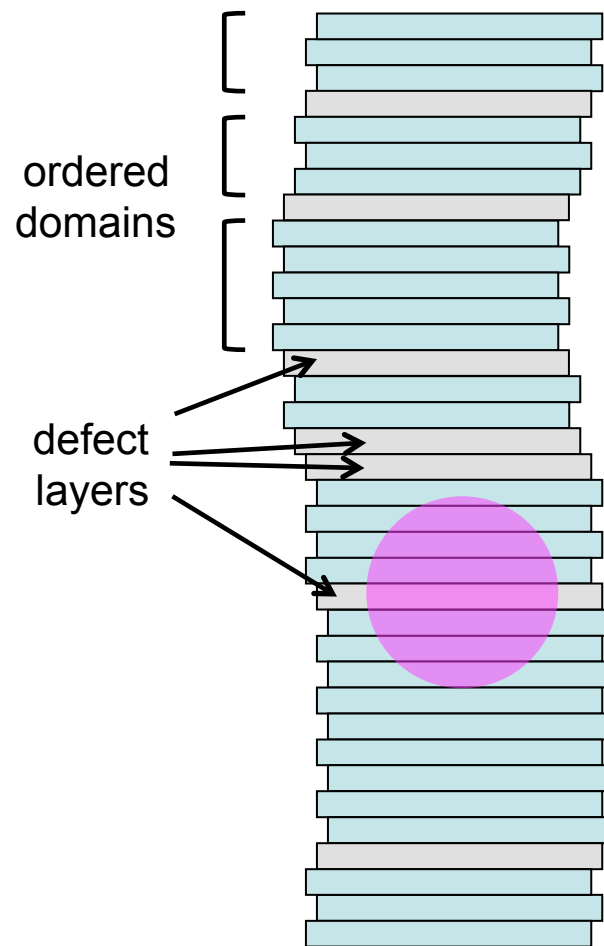


Diffraction of partially disordered structures

White arrow - direction in which
global periodicity is missing

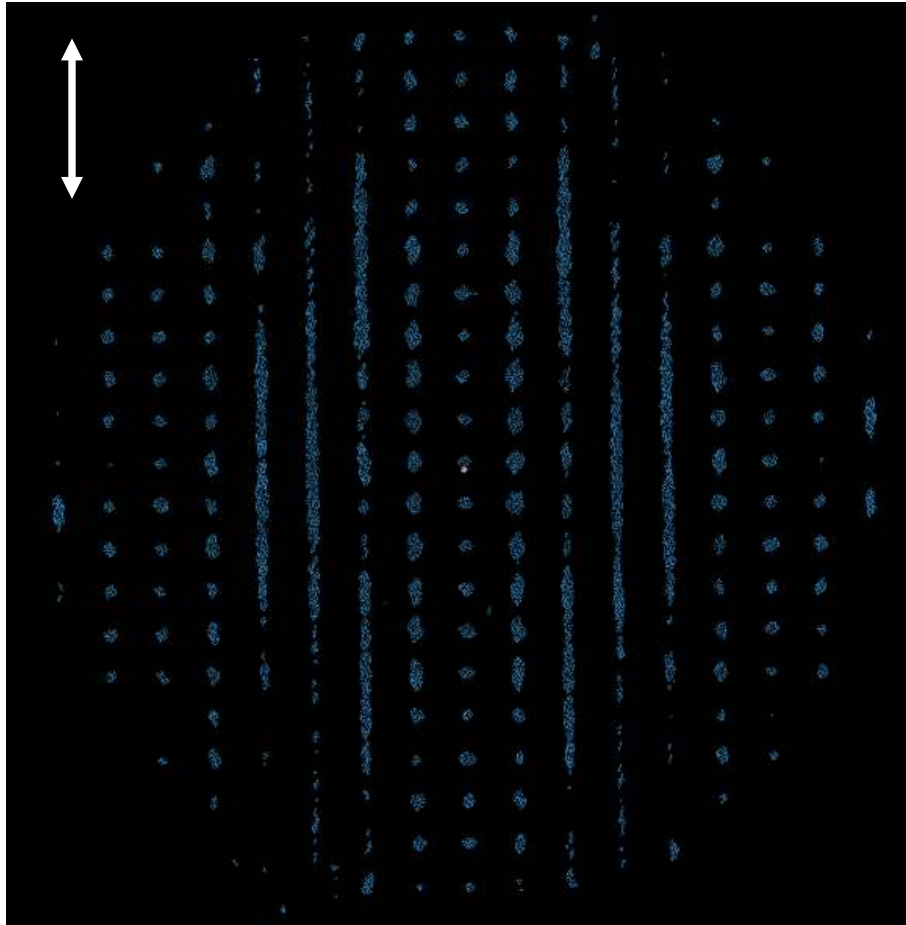


dials.rs_mapper + coot



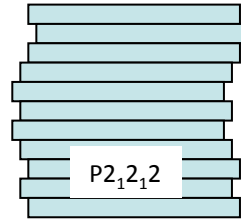
Example 1: ghost density

White arrow - direction in which global periodicity is missing



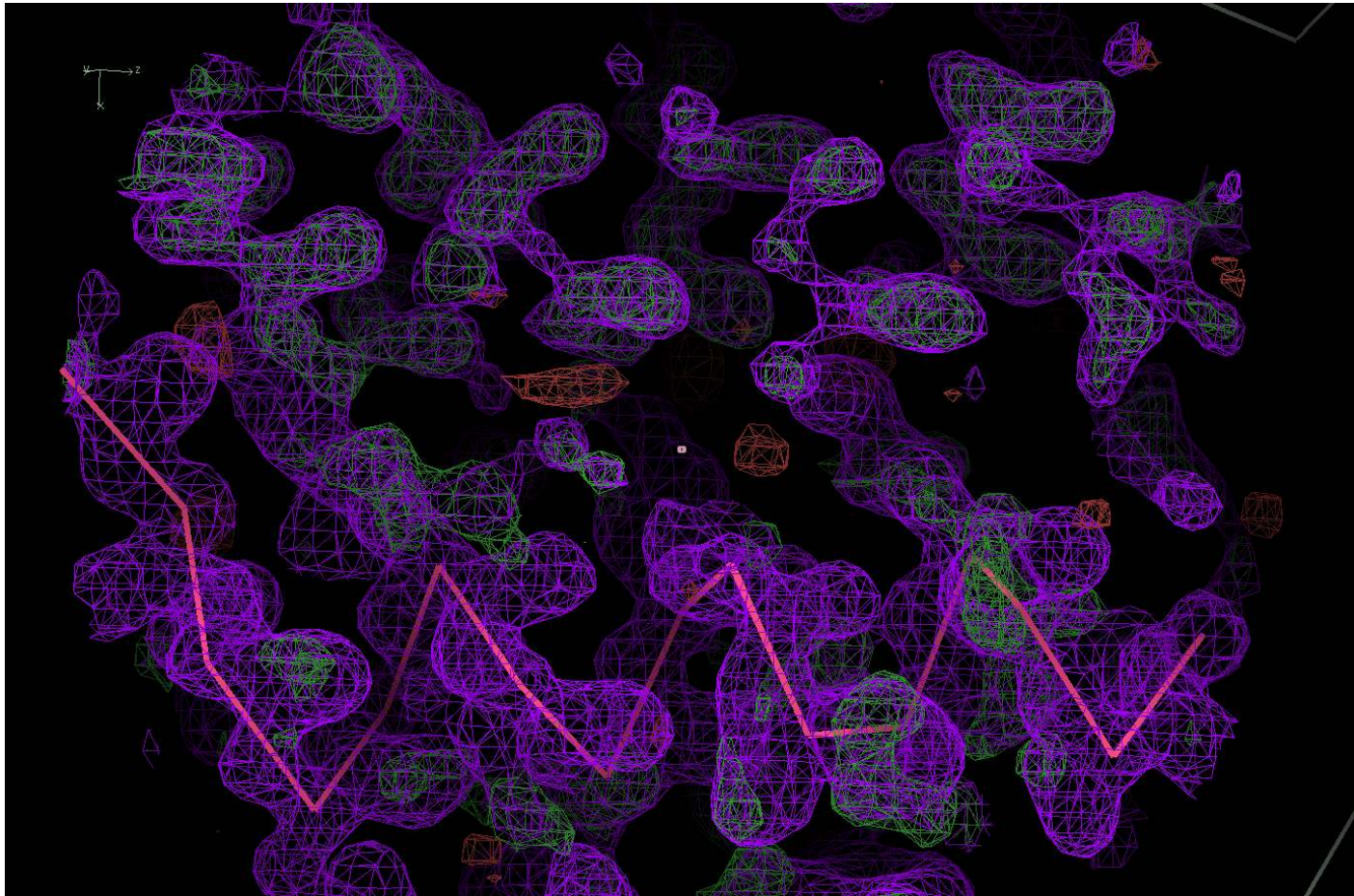
dials.rs_mapper + coot

An example from **Rafael Ciges**,
Biomedical Institute of Valencia

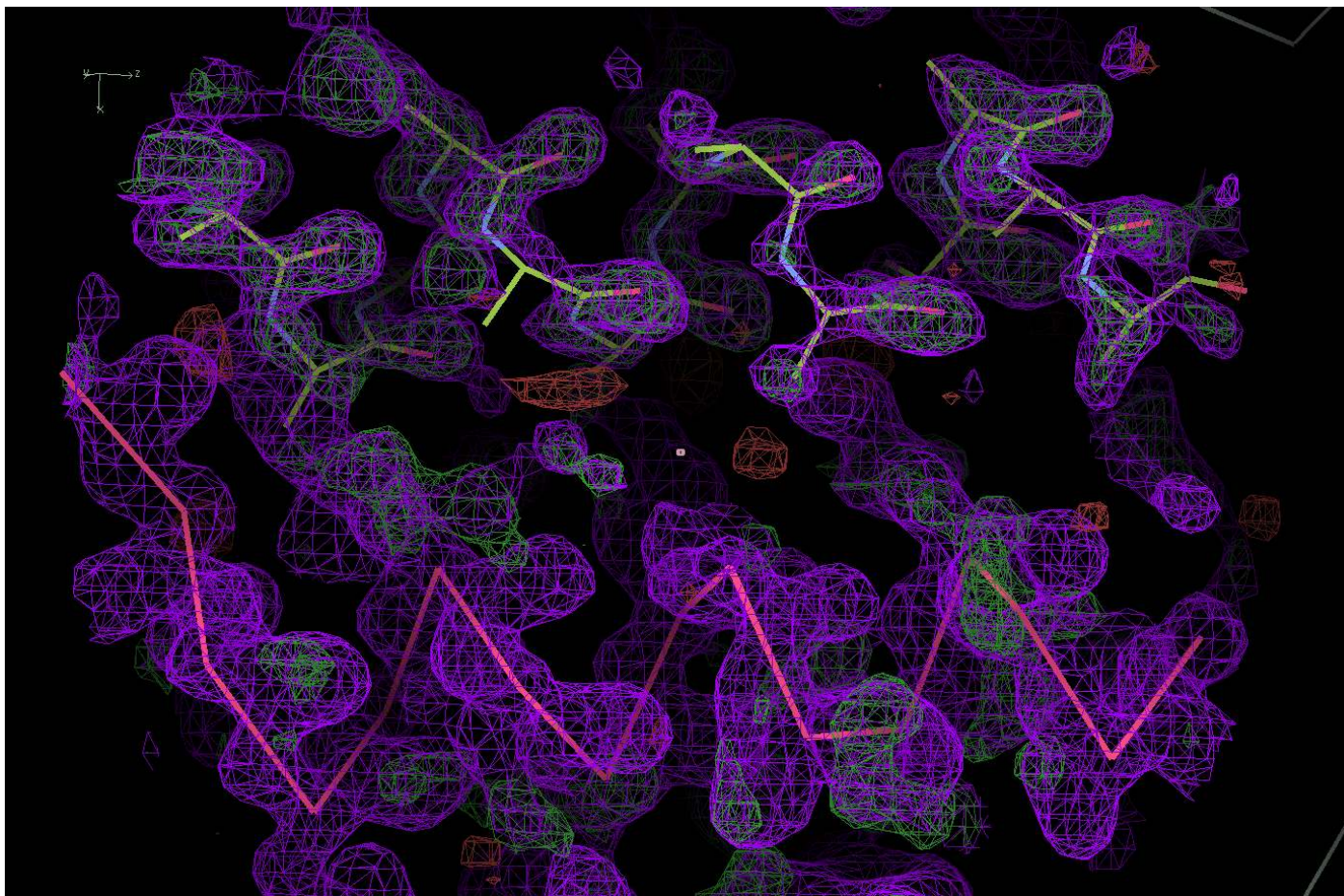


- Space group $P2_12_12$
- Resolution 1.2Å
- Structure was solved with MR
- Preliminary refinement $R_{\text{free}} = 0.35$
- Extra residues were expected compared to MR model

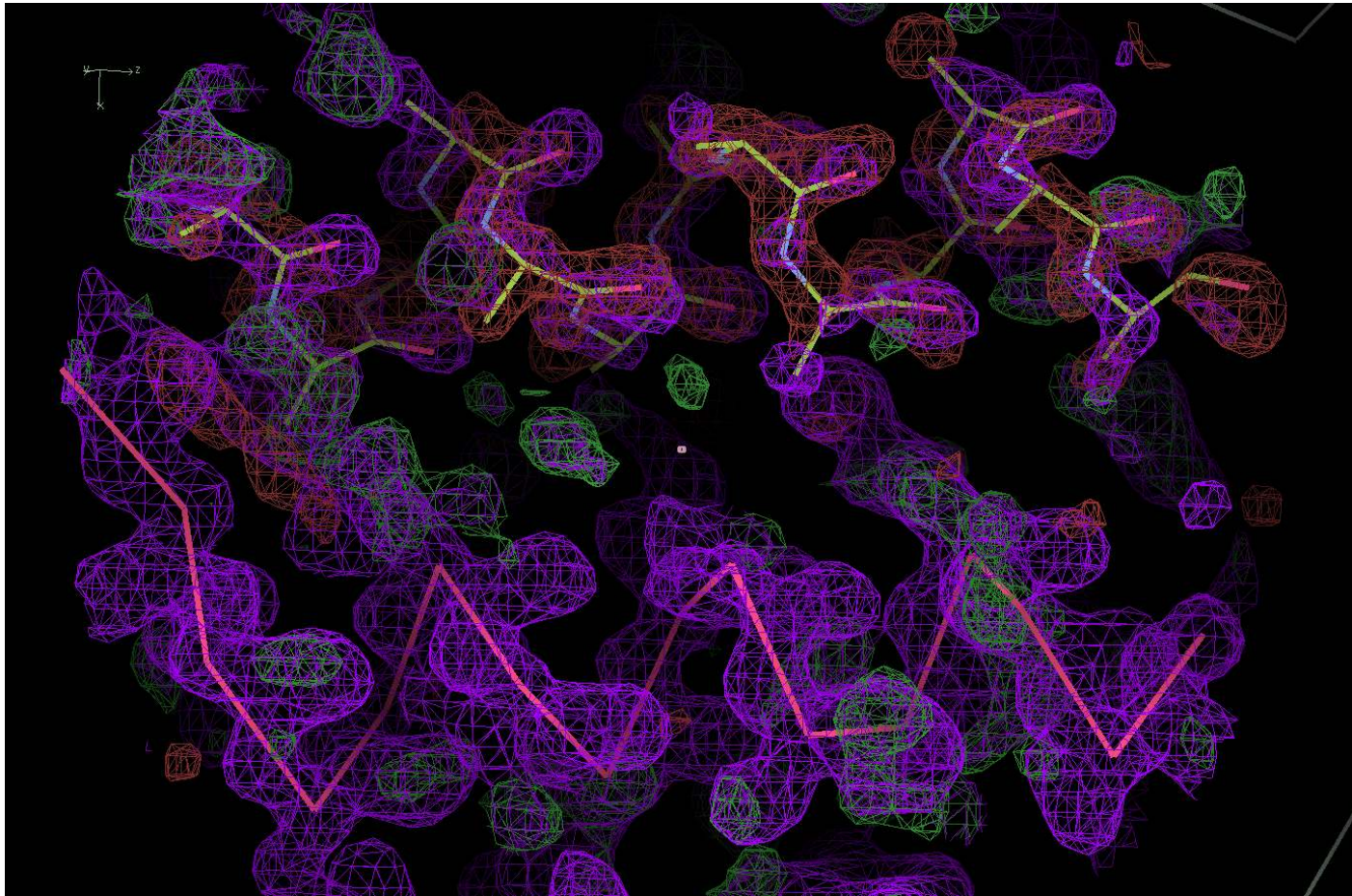
Example 1: after initial refinement



Example 1: helix added



Example 1: after refinement with extra helix

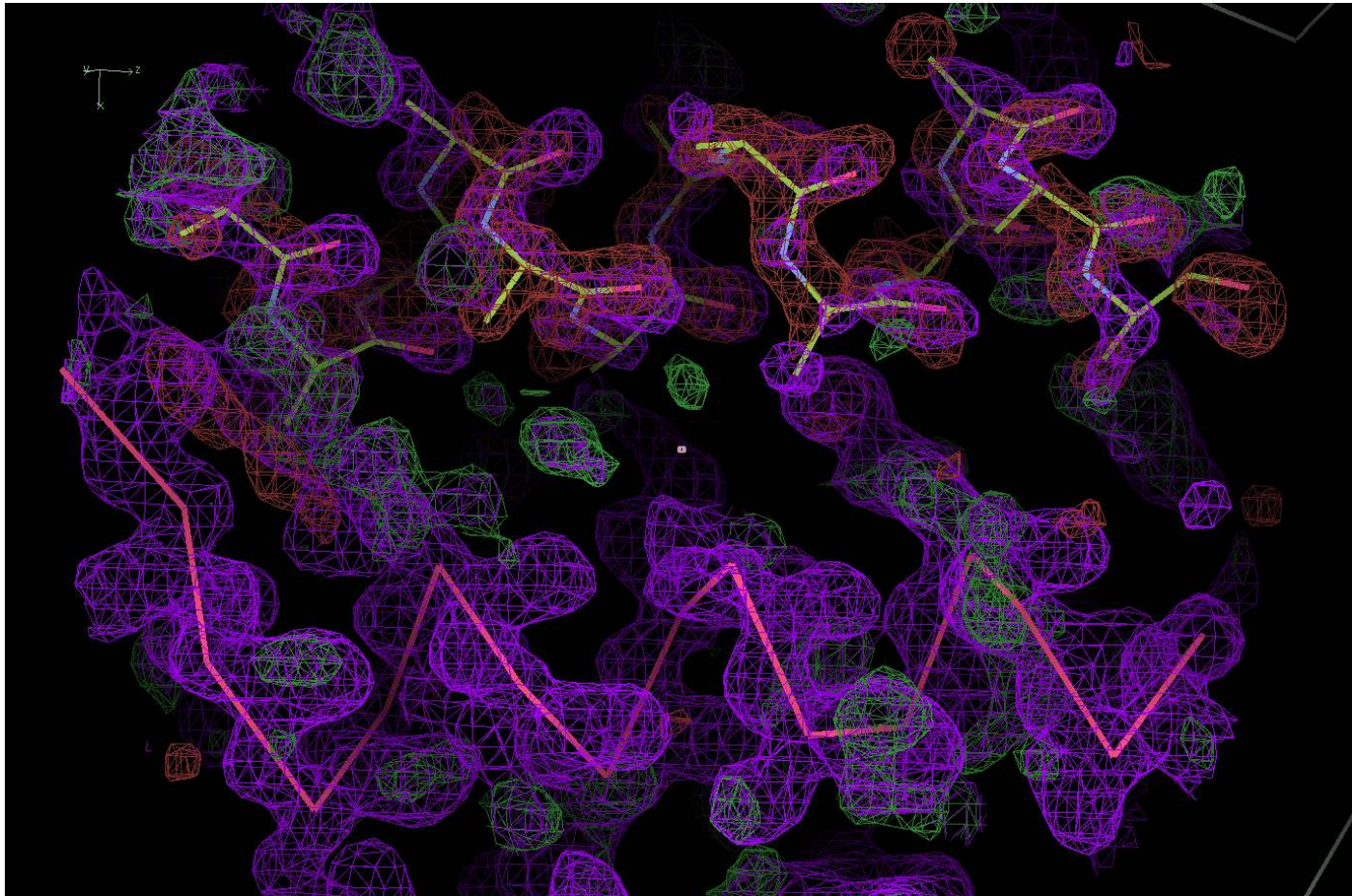


Example 1: demodulation of intensities

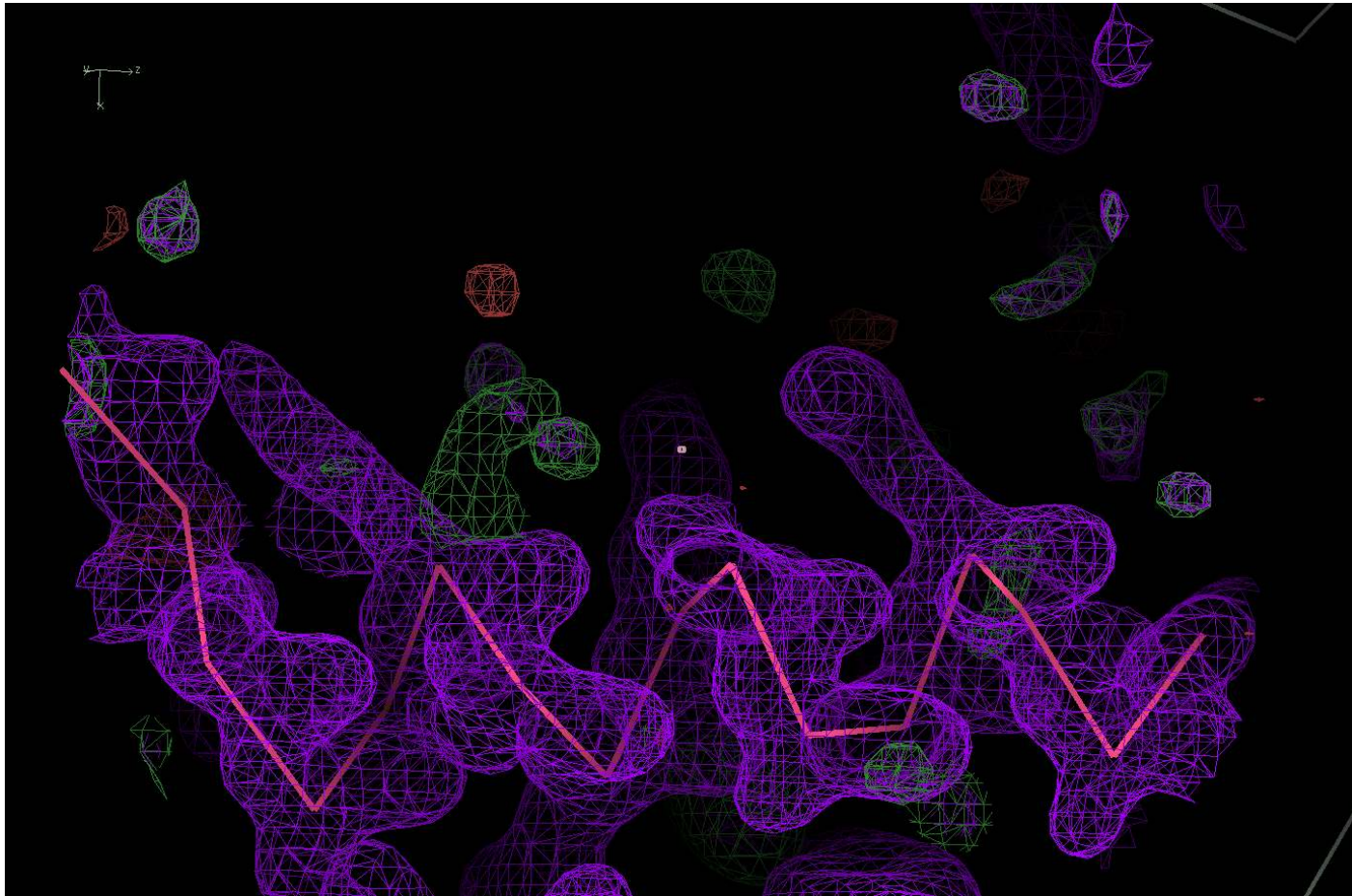
- Data were demodulated to approximately account for disorder

	R	R-free
Original data	0.33	0.34
Corrected data	0.25	0.26

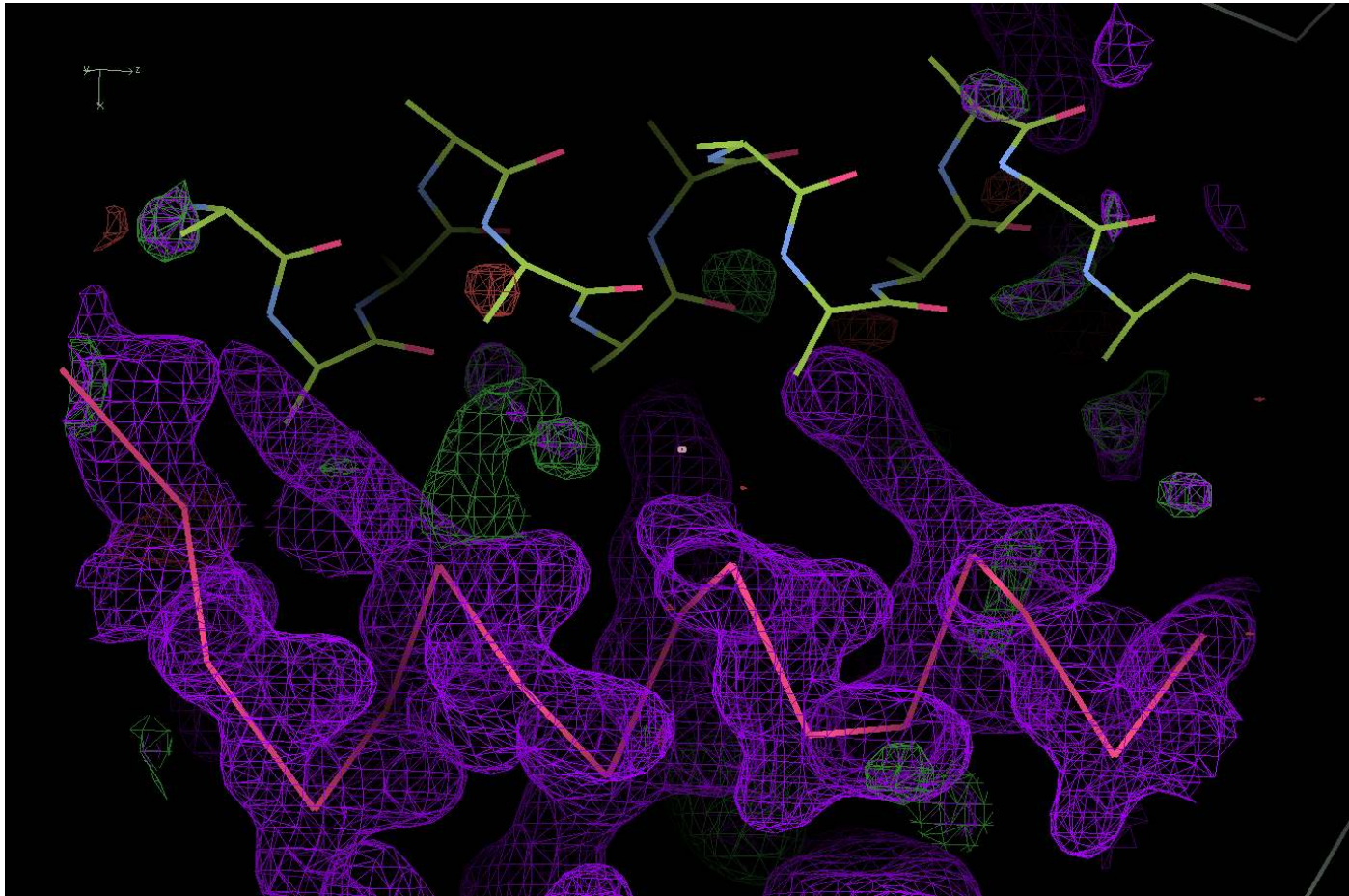
Example 1: after refinement with extra helix



Example 1: after refinement against demodulated data ...



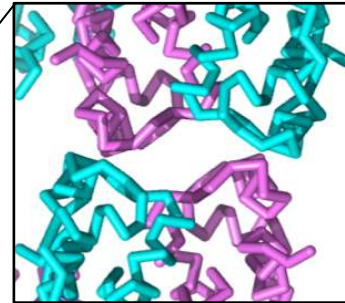
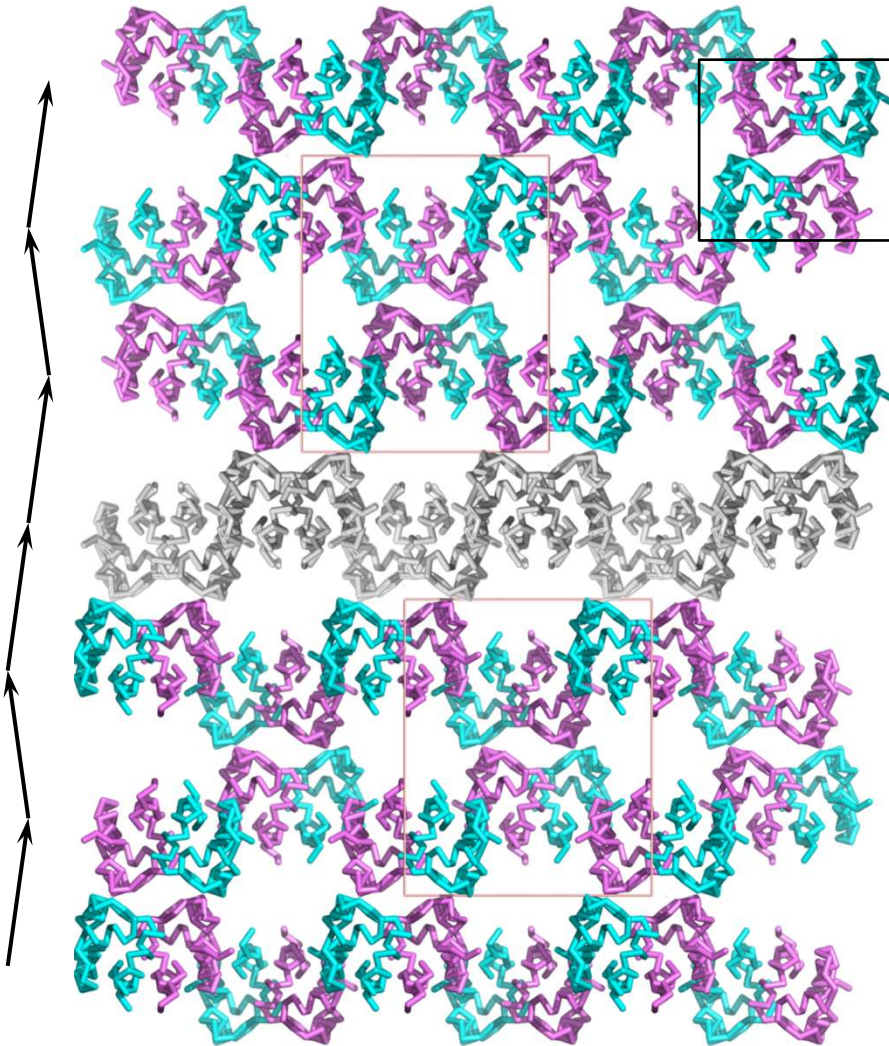
Example 1: ... there is no ED for the extra helix



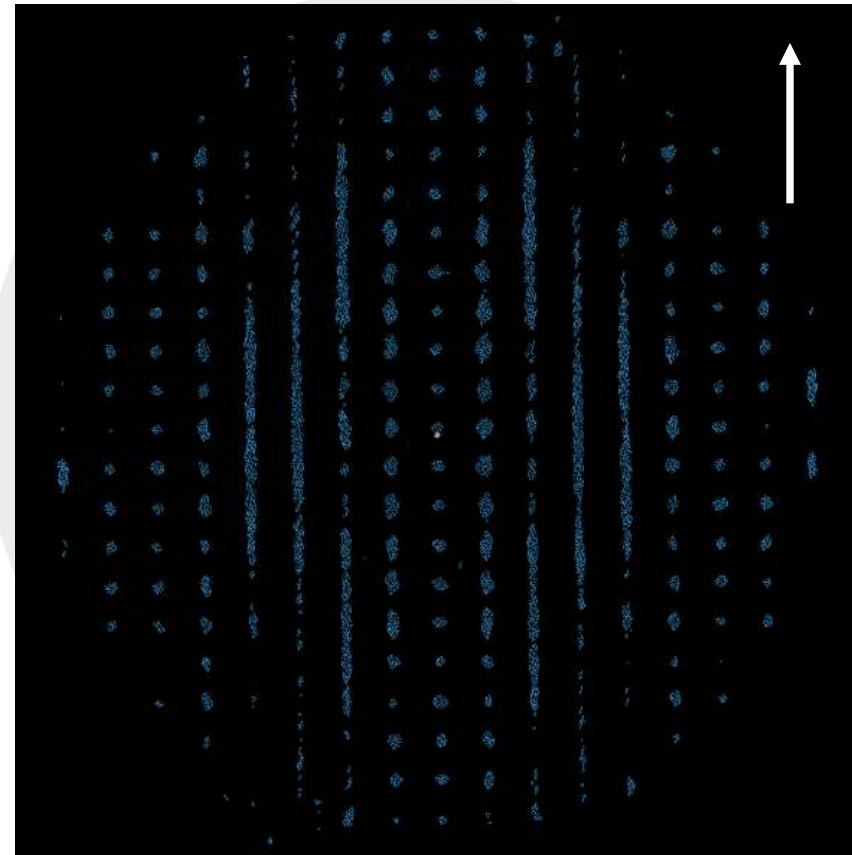
Example 1: ghost density

Two adjacent domains

Packing ambiguity



White arrow -
direction in which
global periodicity is
missing



Example 1: Summary

- Partial disorder in OD structures results in a ghost density
- Structure can be solved and refined ignoring partial disorder
- Demodulation procedure may remove ghost density and therefore help with interpretation of the ED maps
 - » Not always badly needed
 - » Not always works
 - » There are several bespoke scripts around
- Atomic model usually indicate reasons for partial disorder

Example 2: auto-indexing failure

Fast DP @ DIAMOND
(be cautious it is fast first of all)

$R_{\text{meas}} = 0.12$?
 $\text{CC}(1/2) = 0.3$ at 1.56 \AA

Refinement

$R_{\text{cryst}} = 0.33$?
 $R_{\text{free}} = 0.36$

Molecular Replacement ✓

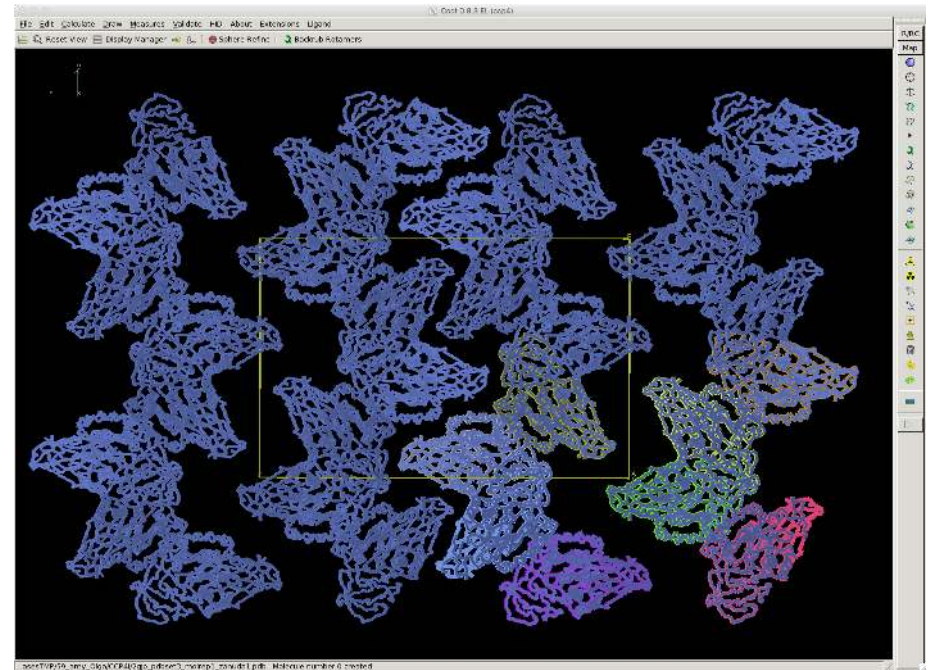
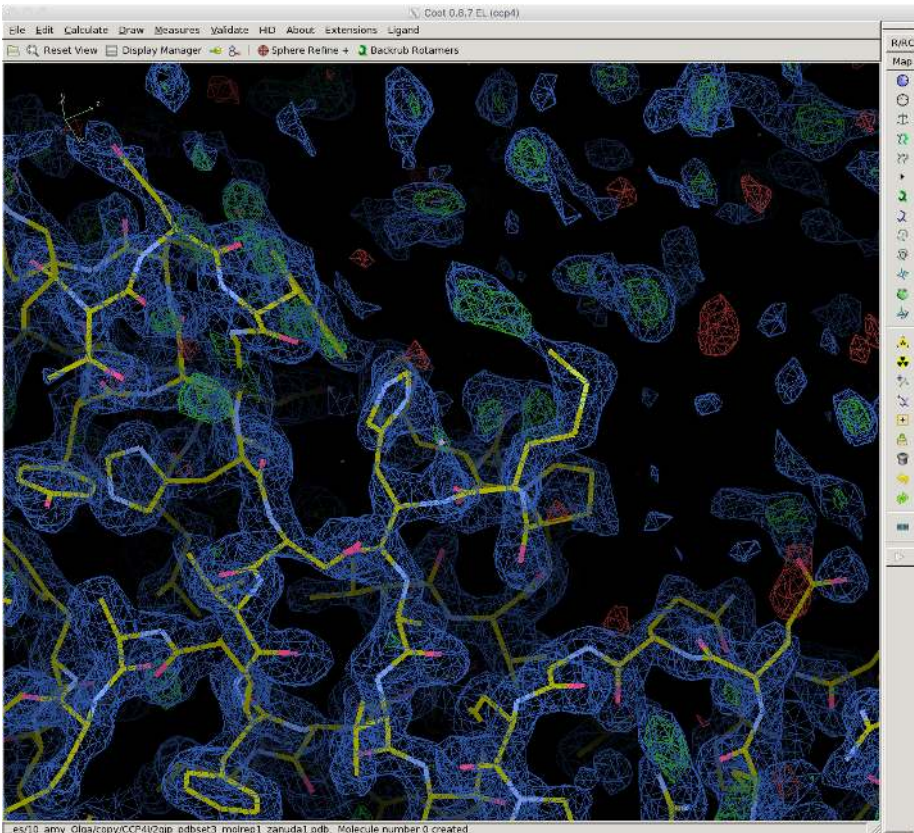
--- Peaks of Rotation Function ---

	theta	phi	chi	Rf/sigma
1	63.62	174.24	148.98	13.70
2	80.19	-58.05	61.61	13.63
3	149.48	-148.30	170.26	13.34
4	107.22	84.22	129.22	13.04
5	87.46	75.99	136.16	12.18
6	111.97	-14.20	175.28	12.10
7	157.20	173.73	153.99	11.25
8	58.77	-96.16	51.96	11.24
9	75.76	-63.11	54.46	6.21
10	102.46	82.67	133.90	5.83

Example 2: evidences of wrong indexing

Maps ?

Crystal Packing X

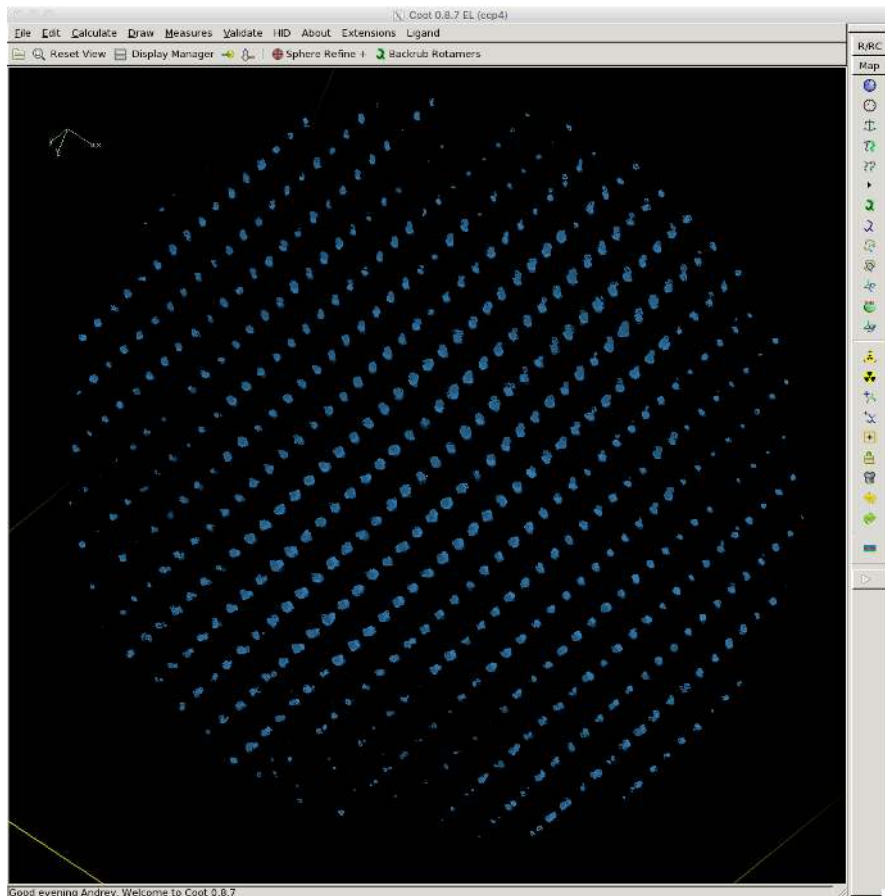


Wrong indexing?

Example 2: evidences of partial disorder

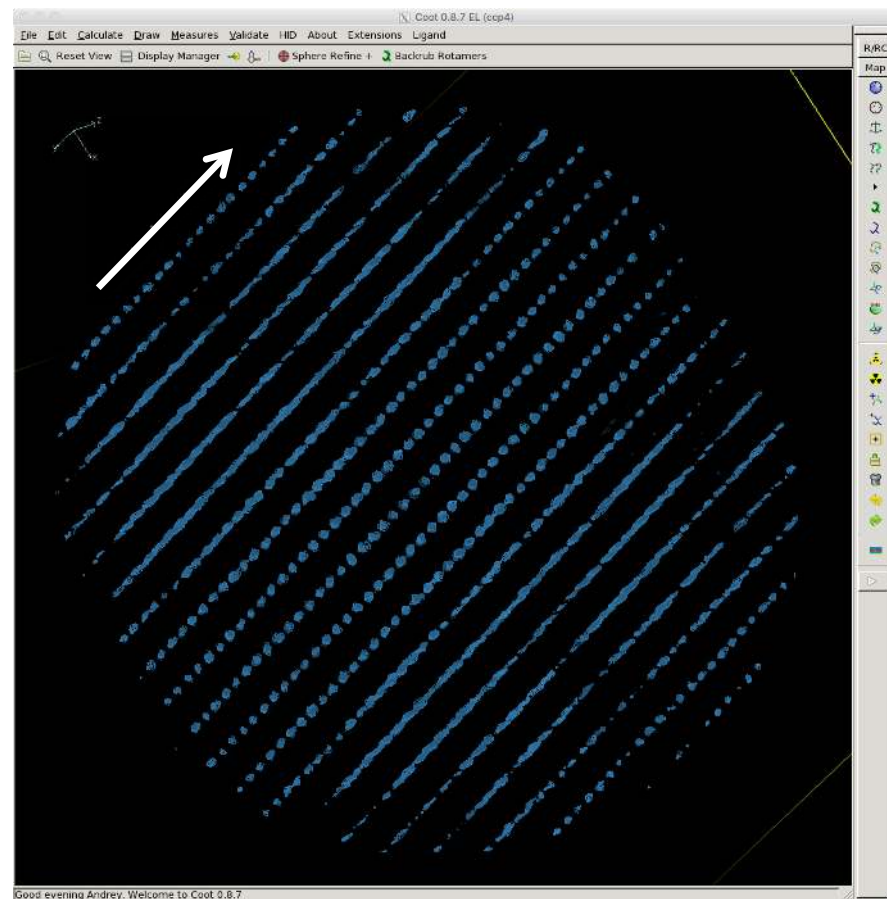
front view

There is global 2D translational symmetry in the plane of figure

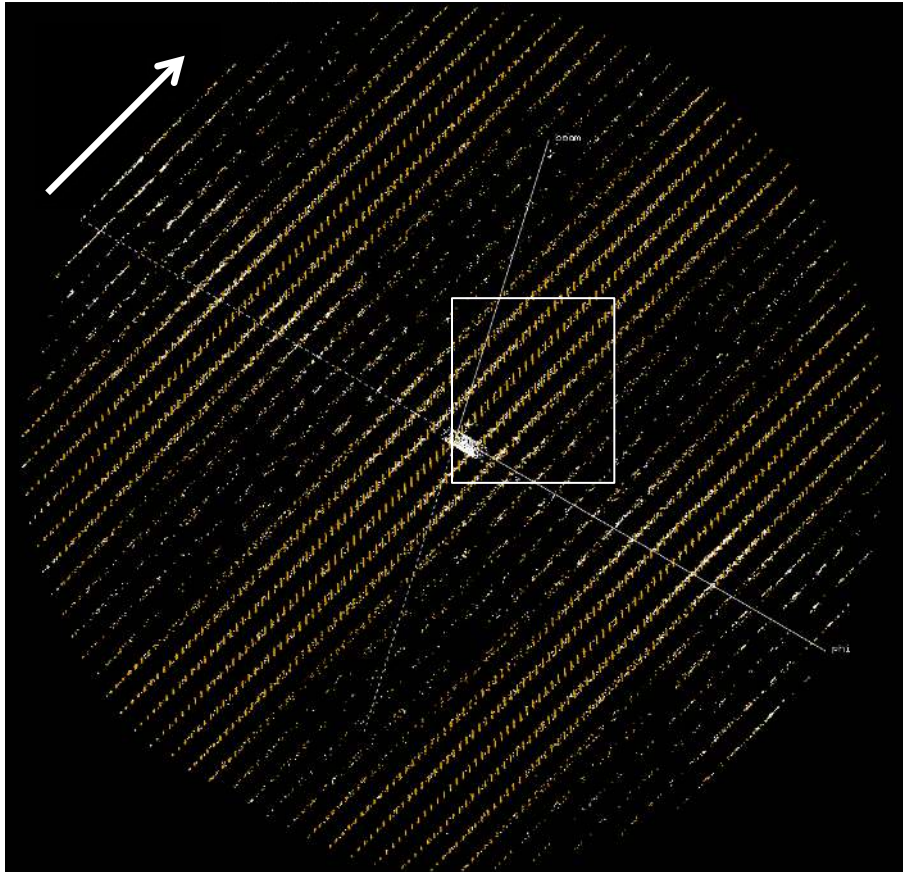


side view

White arrow indicates direction in which translational symmetry is not global (only within individual domains)



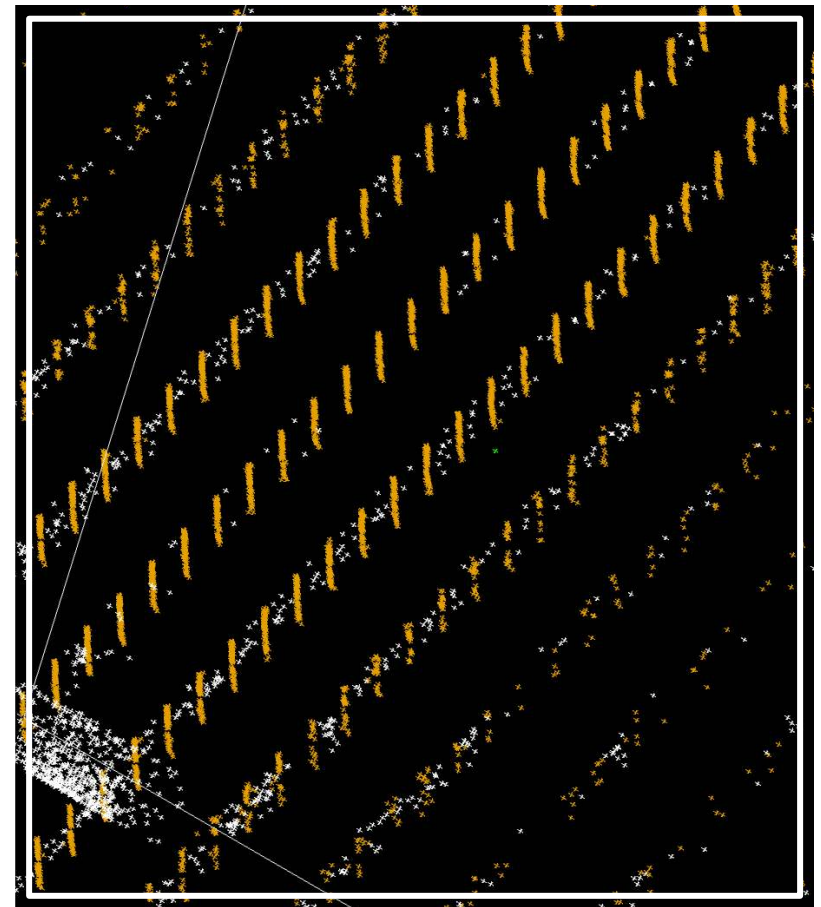
Example 2: correct indexing



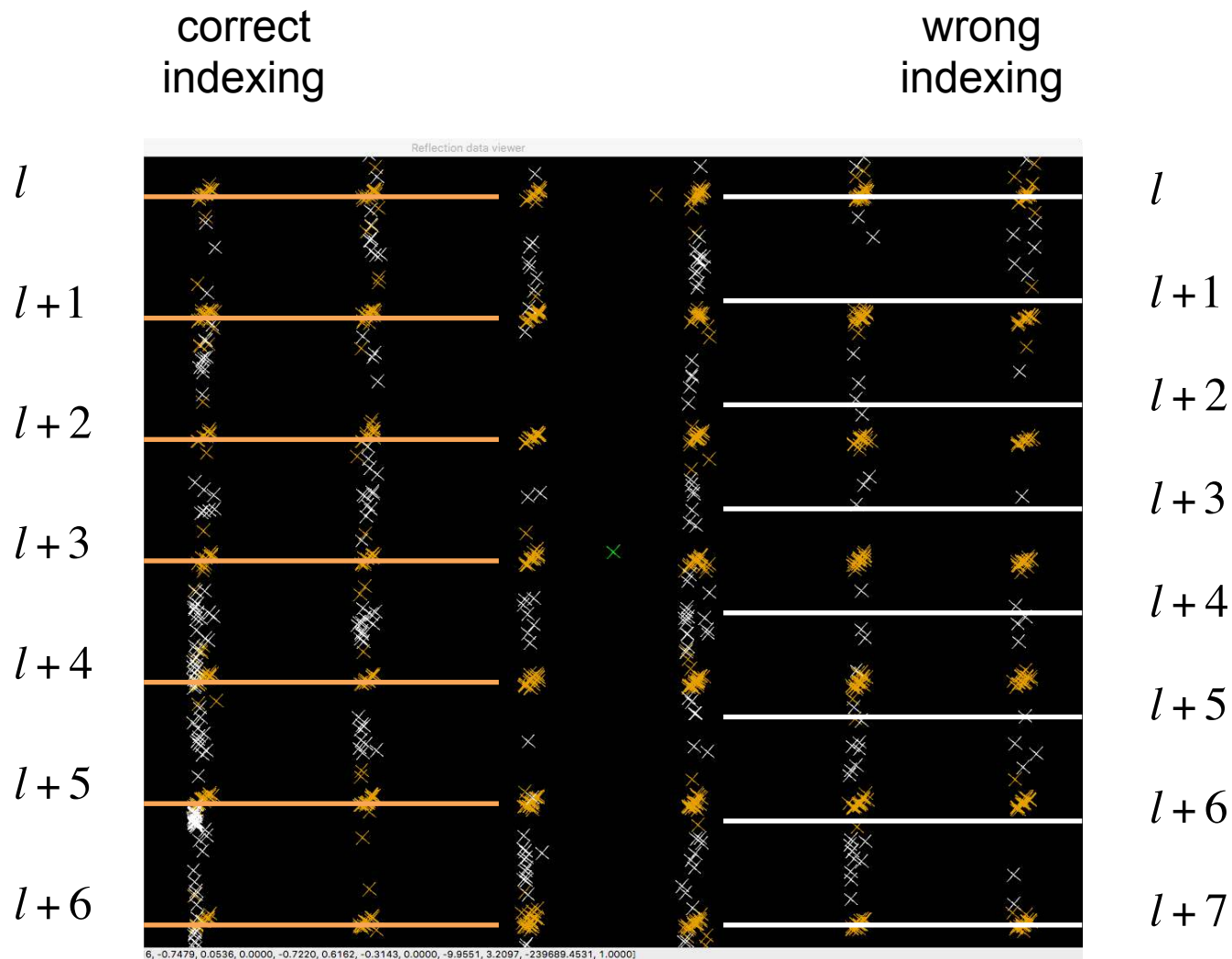
White arrow indicates direction in which translational symmetry is not global (only within individual domains)
There are also areas with less spots

White “spots” are not indexed; actually, these are tails of diffuse reflections

Indexing program may take them for real spots and produce wrong result.



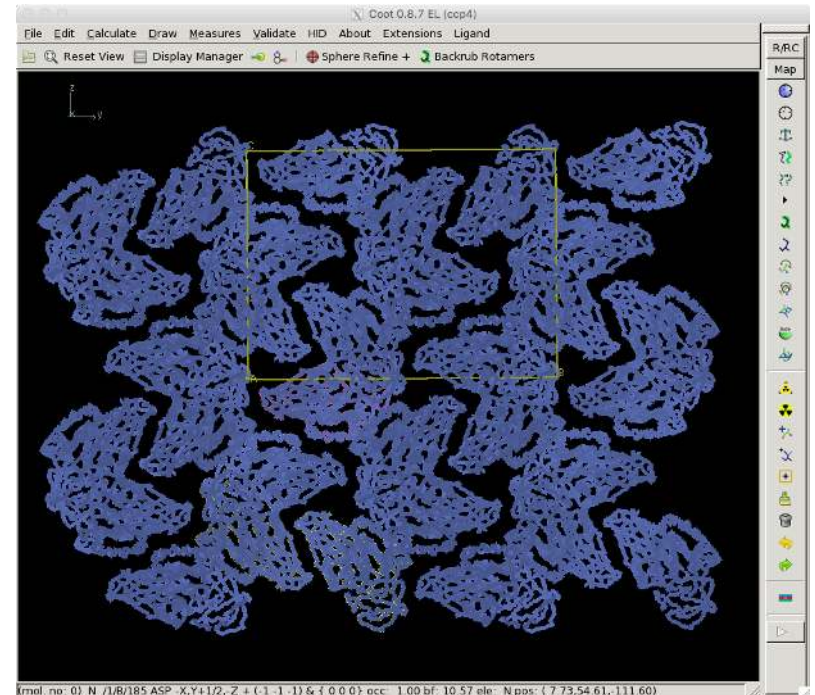
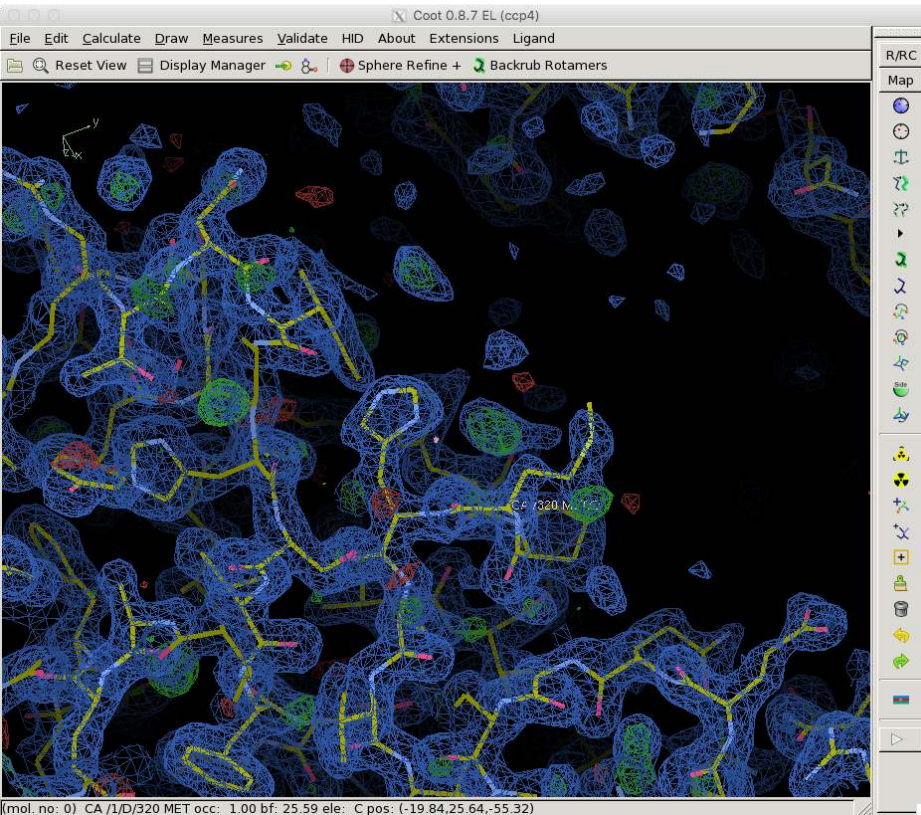
Example 2: what initially was wrong



Example 2: happy end

Maps ✓

Crystal Packing ✓



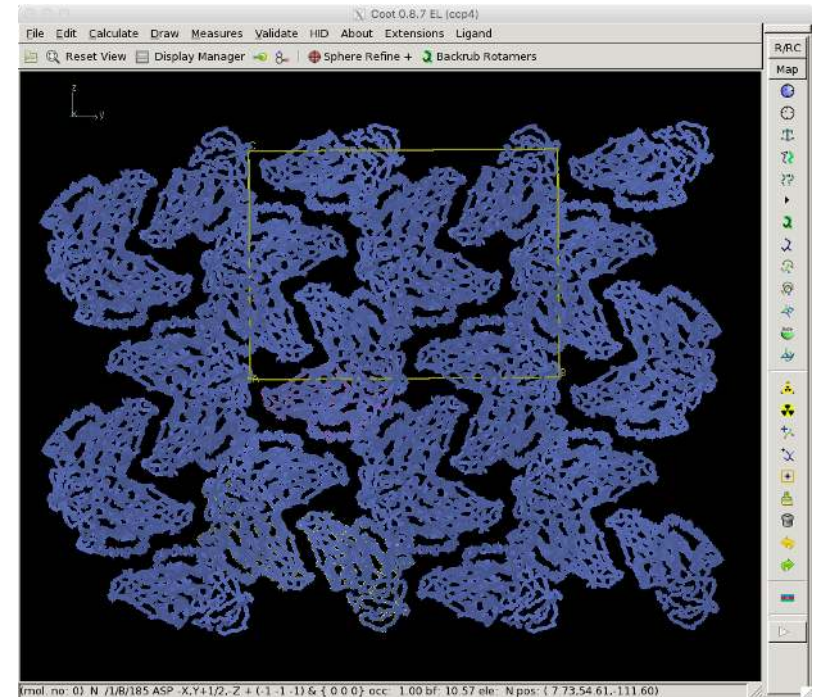
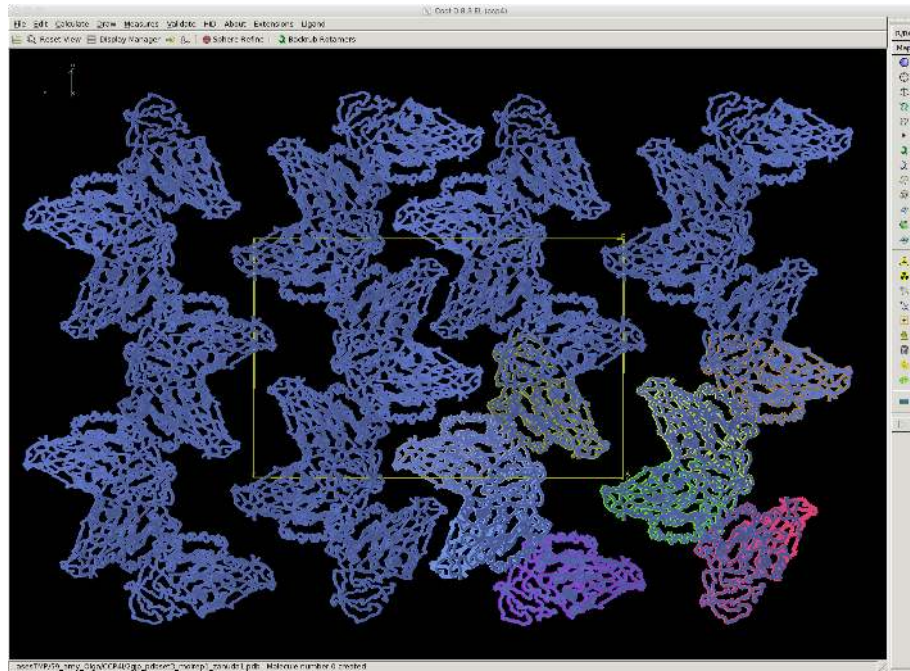
Refinement



$$R_{\text{cryst}} = 0.23$$

$$R_{\text{free}} = 0.26$$

Example 2: wrong and correct



Example 2: Summary

- Partial disorder a frequent reason of indexing failure
- Use 3d viewers for diagnostics
- **Warning:** high contrast in MR can be obtained even for wrongly indexed data provided that the search model is highly similar to the target
- Look at crystal packing and solvent area

Partial disordered OD structures

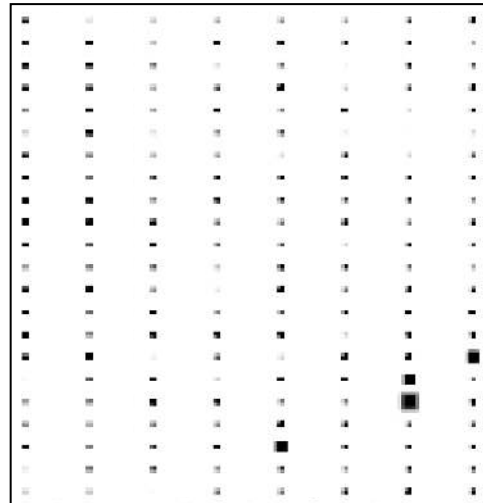
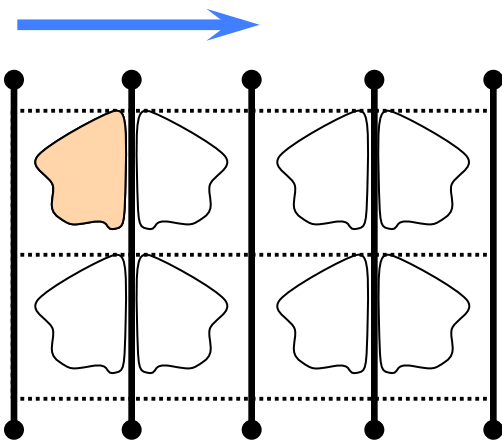
- Signs: (some) rows of reflections have tails or merge together
- Data processing
 - Indexing can go wrong (use higher "gain" parameter, merge several adjacent images together etc. to get it right)
- Structure solution:
 - Molecular Replacement - yes
 - Experimental phasing – might be problematic
- Refinement / model building:
 - Some features of electron density may be non-interpreted (ghost density)
 - Expect higher R-factors
- Crystals with translocation defects
 - Term usually used in MX for partially disordered OD-structures of type I

Pseudo-translation

- Presence of weak reflections
- Effect on indexing

Pseudotranslation

Crystallographic translation



No pseudotranslation

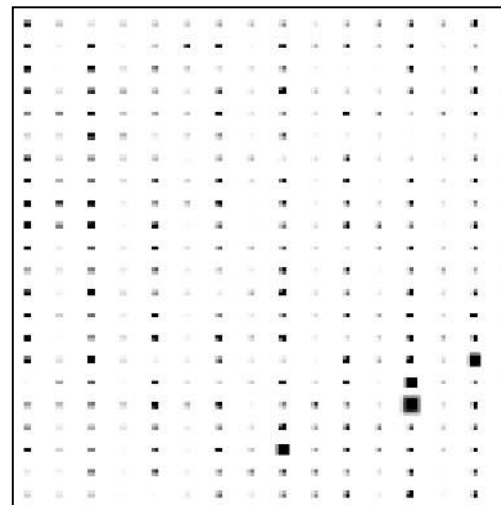
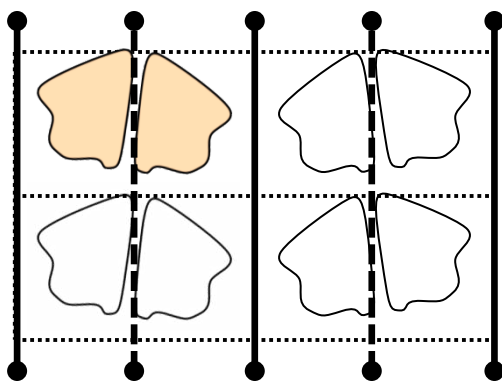
$$c$$

$$c^*$$

Crystallographic translation



Pseudo-translation



Pseudotranslation

$$c' = 2c$$

$$c'^* = c^* / 2$$

Planes $2L+1$ contain weak reflections

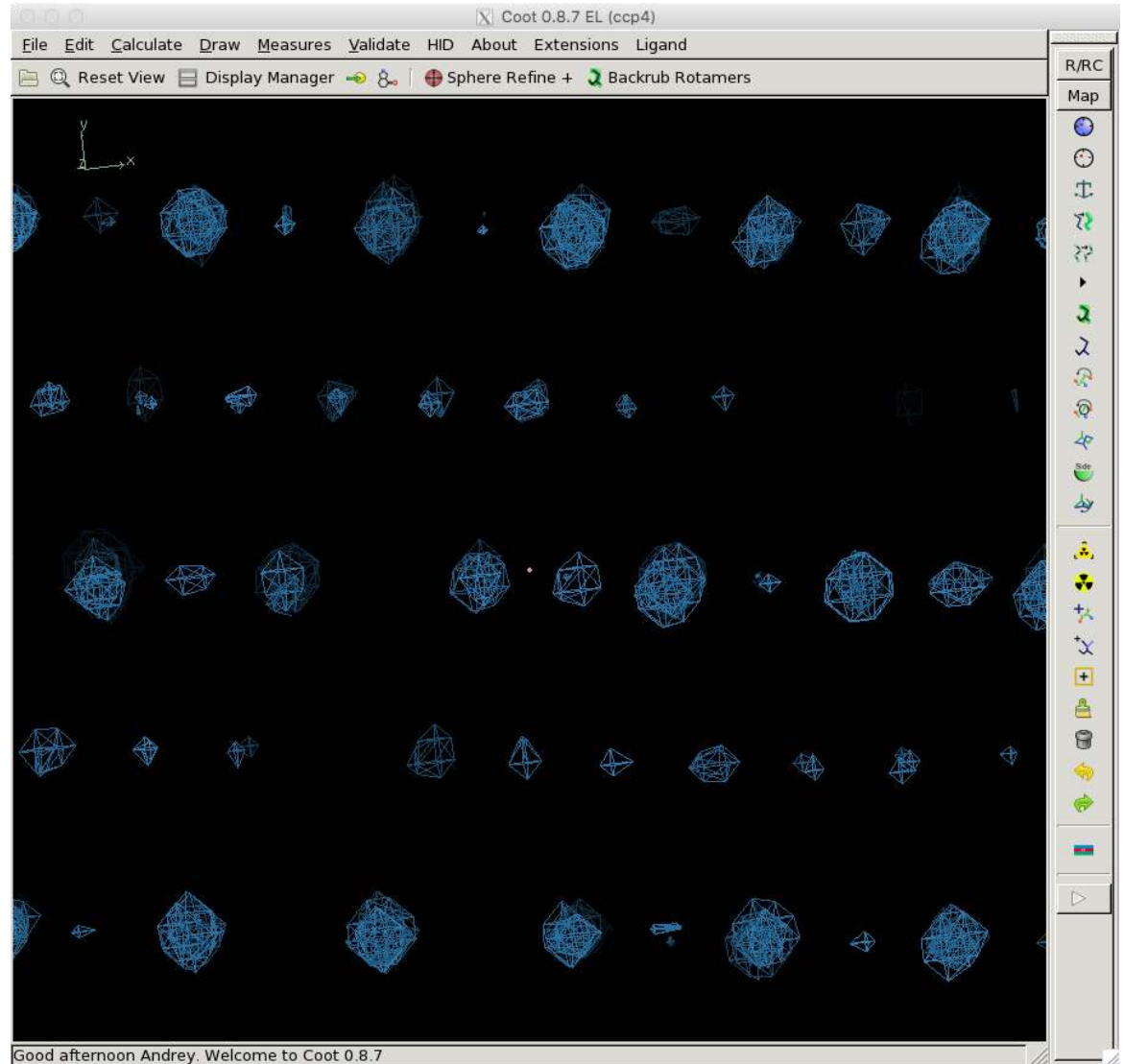
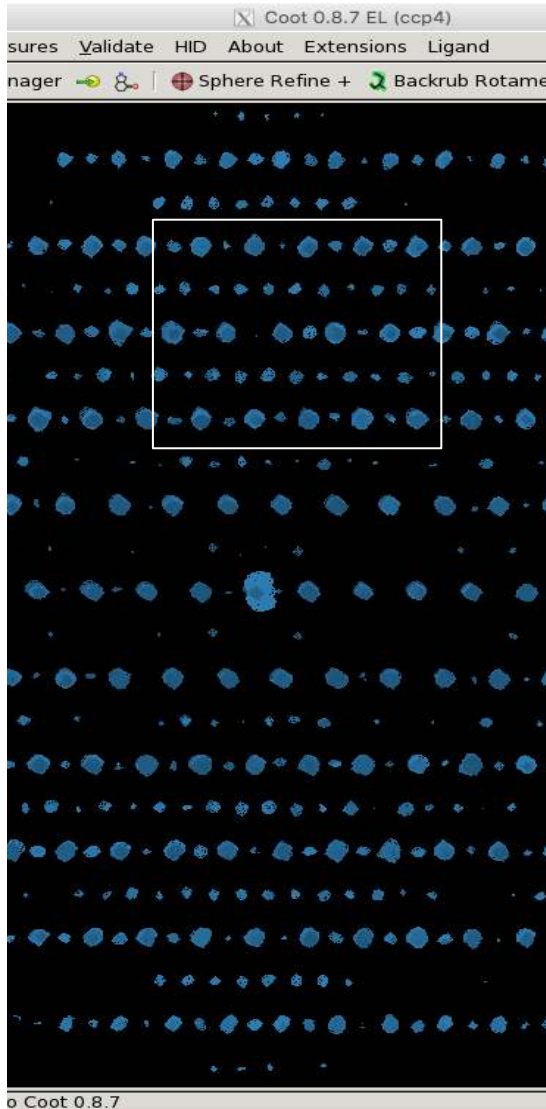
Example: two pseudo-translation vectors

	space group	a (Å)	b (Å)	c (Å)
All reflections	$C 2 2 2$	74.9	122.8	125.0
Strong reflections only	$I 2 2 2$	37.5	61.4	125.0

Example from Victor Lamzin, YSBL-DESY

» structure solved using SAD

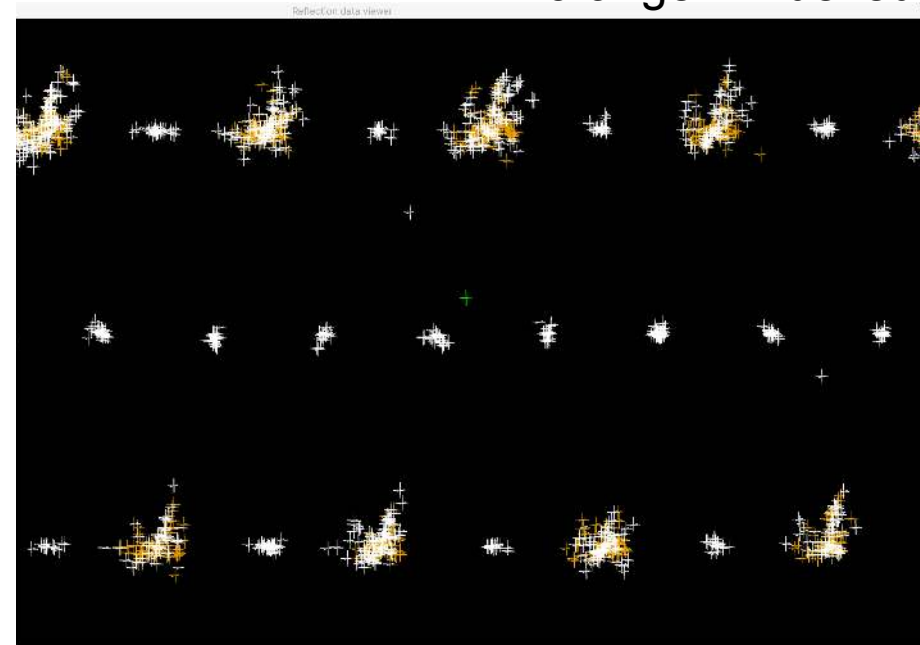
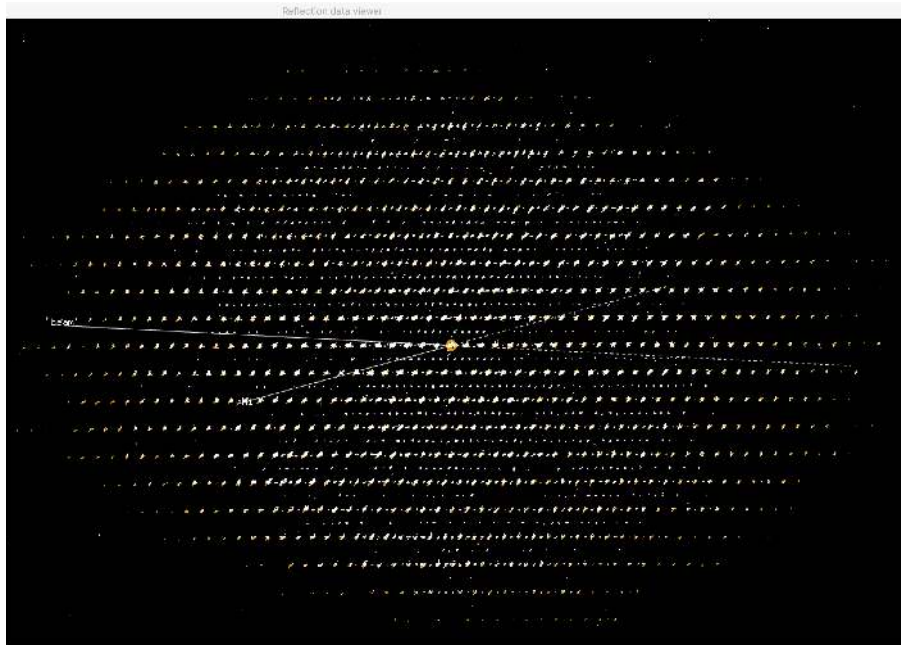
Example: two pseudo-translation vectors



Example: two pseudo-translation vectors

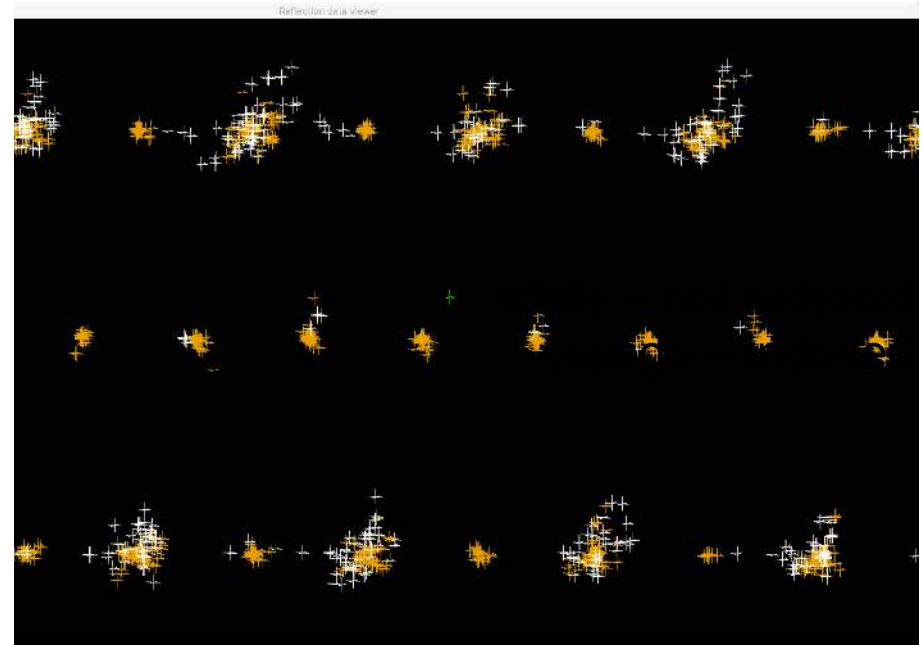
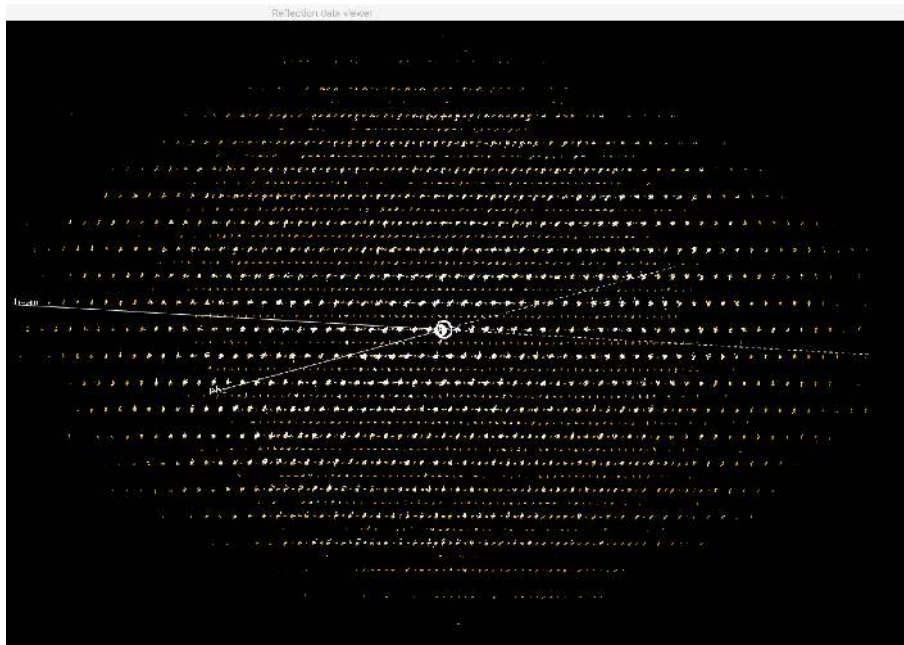
Images imported as they were, oscillation 0.1°

white – not indexed
orange – indexed



Example: two pseudo-translation vectors

Merged each 5 adjacent images to make oscillation 0.5° , then imported



Weak reflections may confuse indexing programs

Visual control using 3D viewers is useful

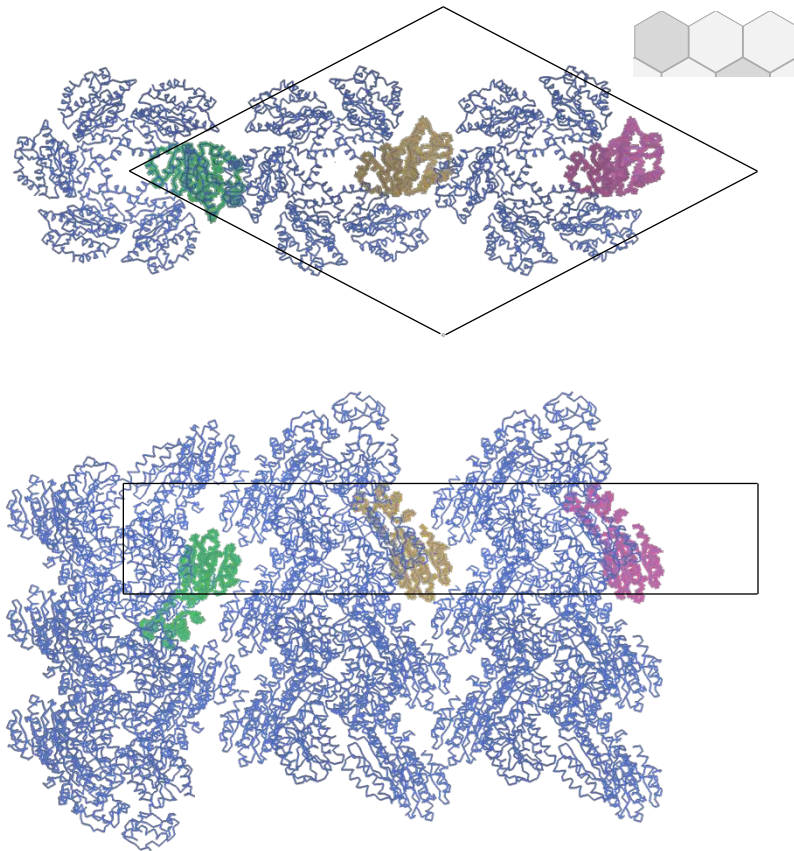
- check if pseudo-translation is not overlooked
- check if pseudo-translation is not an indexing artefact

How important is it to use weak reflections?

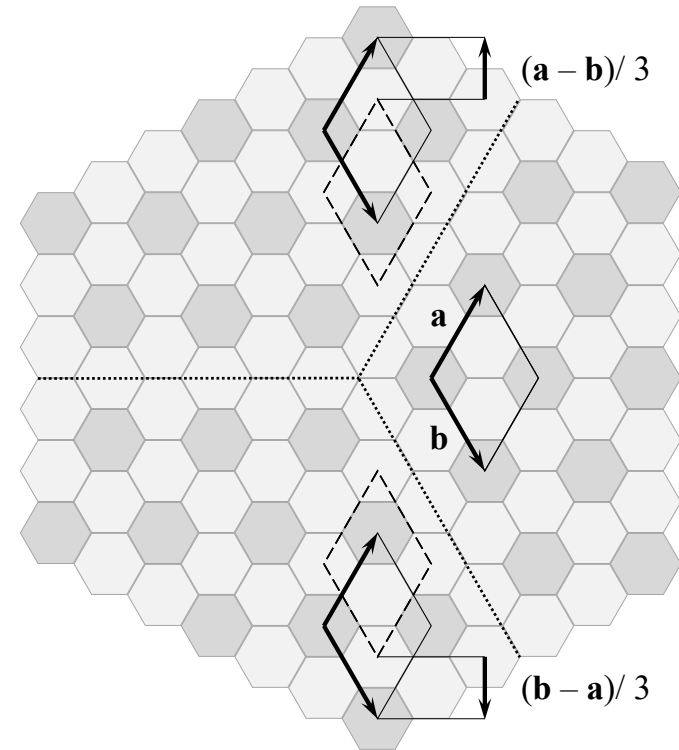
- usually improve both density and refinement stats
- sometimes ignored to simplify the first steps of structure solution and used later
- there are examples when these only make refinement stats worse
 - » the last example - "fake pseudo-translation" - suggests a possible reason

Fake pseudotranslation

An example from
Maria Jose Pedroza Romo and **James Moody**
 Brigham Young University, Utah

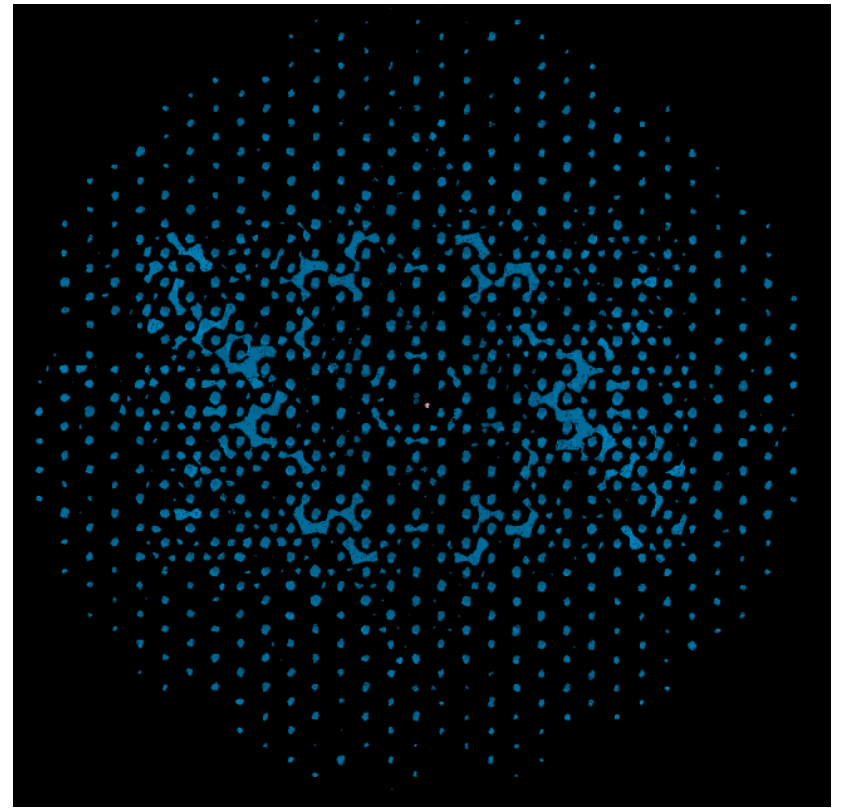
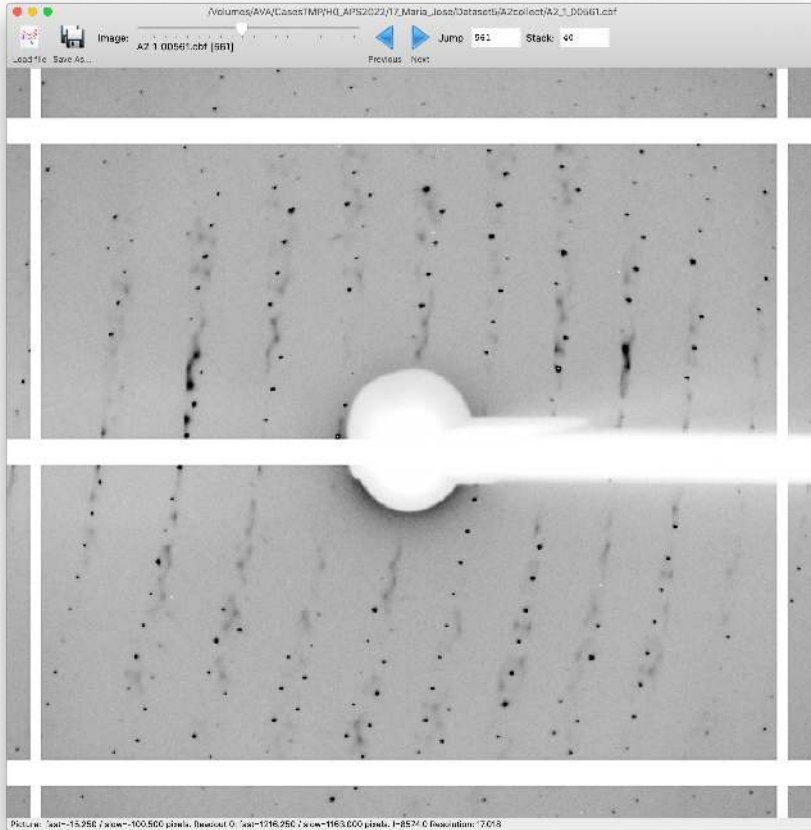


“Pseudotranslation” at the level of
 crystal domains



“strong” reflections :	$h - k = 3n$	$I_{\text{obs}} = I_{\text{dom}}$
“weak” reflections :	$h - k = 3n \pm 1$	$I_{\text{obs}} = \frac{V_{\text{dom}}}{V_{\text{coh}}} I_{\text{dom}}$

4. Fake pseudotranslation



MR and initial refinement:

“strong” reflections (completeness 33%), $R=0.26$, $R_{\text{free}}=0.28$

Rescaling:

“weak” reflections to F_{calc}

Final refinement:

“strong” + “weak” (completeness 100%), $R=0.27$, $R_{\text{free}}=0.31$

END

You are welcome to discuss your problematic data