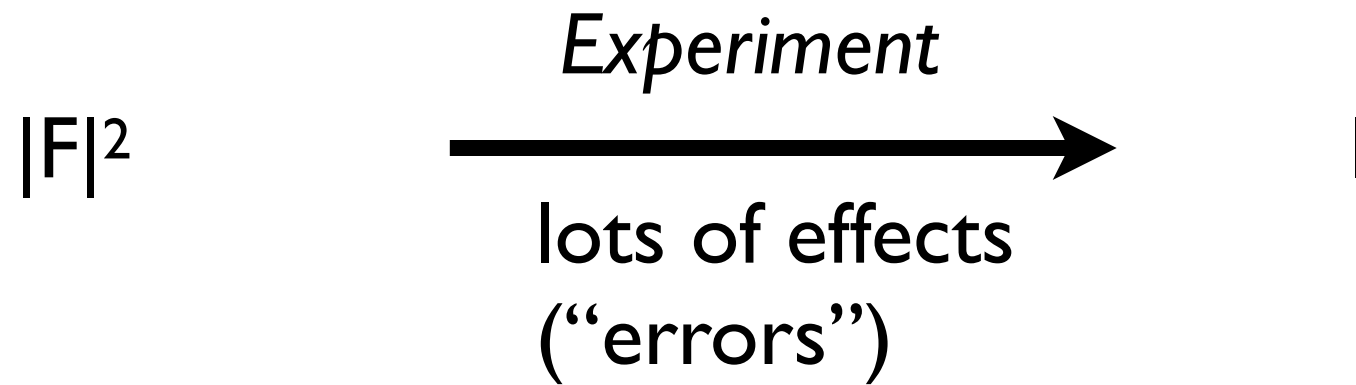


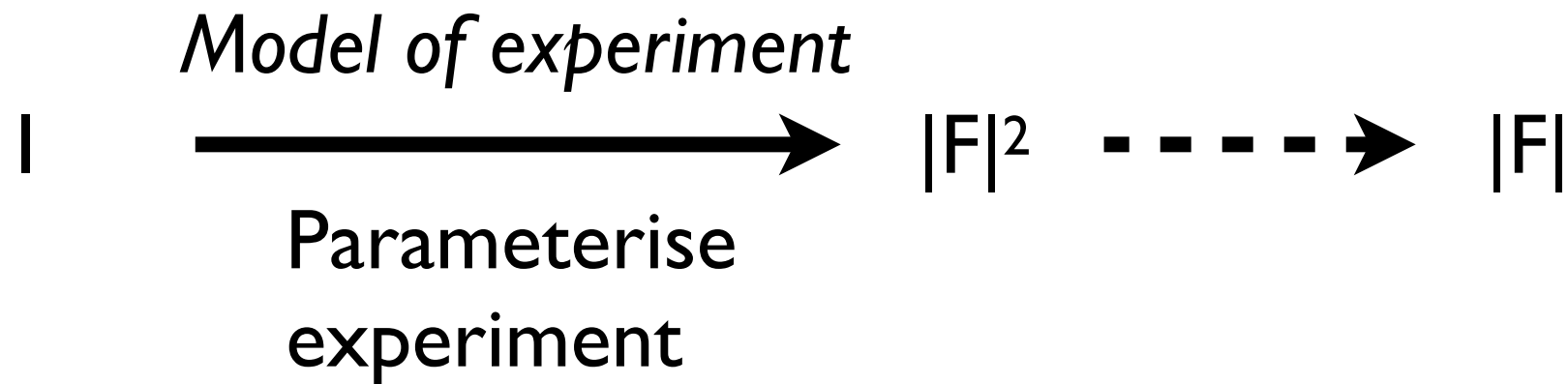
Data Reduction

Space groups, scaling and data quality

Scaling and Merging



Our job is to invert the experiment: we want to *infer* $|F|^2$ and $|F|$ from our measurements of intensity I

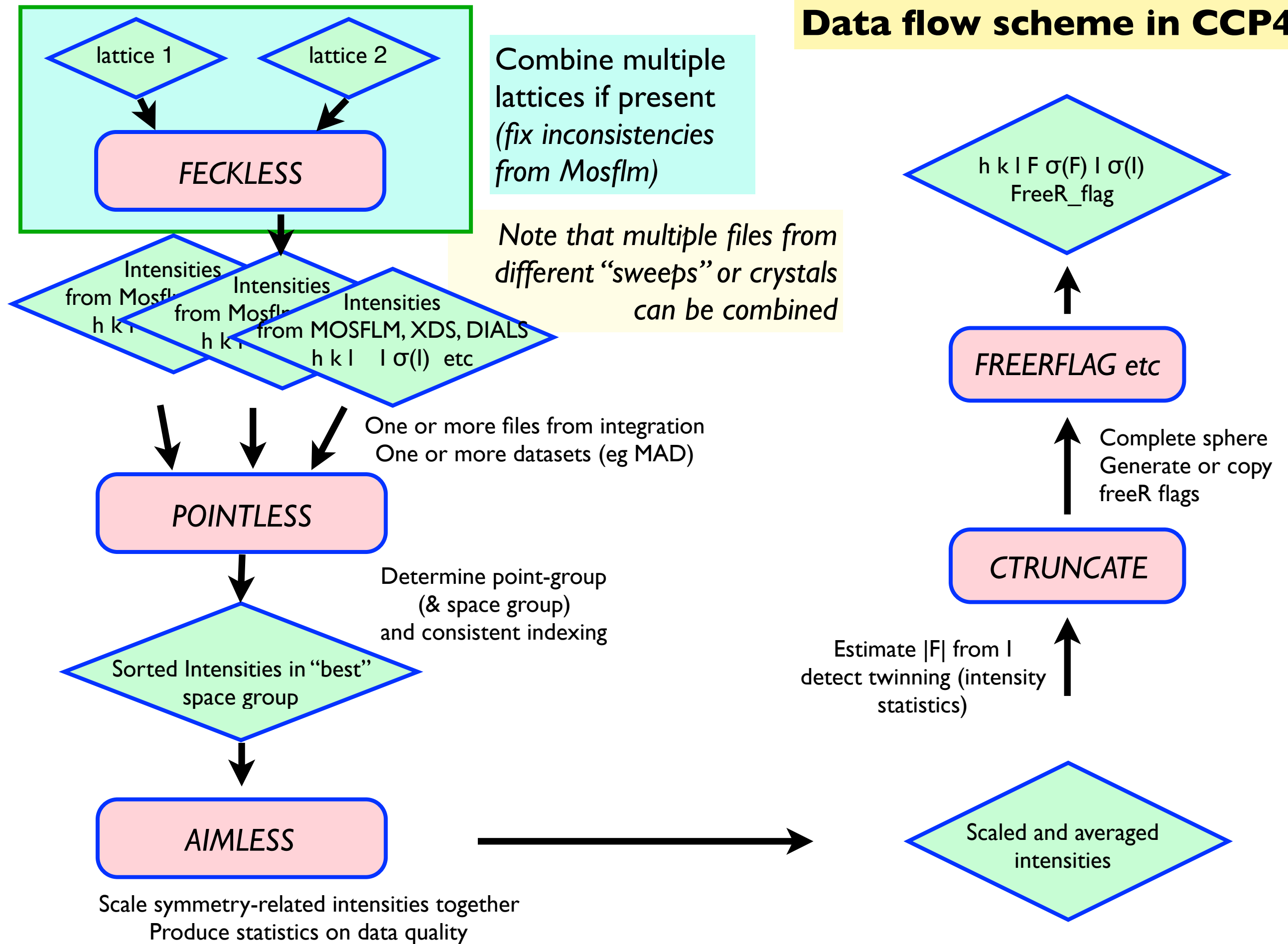


Overview of data reduction process

1. Determine point group and if possible space group
 - *we need the point group to scale the data*
 - *too low symmetry makes solving the structure harder, (though not impossible)*
2. Scale data to make it internally consistent
analyse for:-
 - *maximum resolution*
 - *radiation damage*
 - *data quality*
3. Analyse for pathologies, and estimate amplitude
 - *twinning*
 - *translational non-crystallographic symmetry*

NB I am discussing data from one or a few crystals, not from hundreds of crystals, nor serial crystallography

Data flow scheme in CCP4



How to start from ccp4i2

Start iMosflm

Start DUI

Follow-on to run
data reduction

Just click "Run"

Integrate X-ray images



Automated integration of images with DIALS using xia2
Select a directory containing images and integrate them



Automated integration of images with XDS using xia2
Select a directory containing images and integrate them



Integrate images with Mosflm
Launch iMosflm and capture output



DIALS Image Viewer
DIALS Image Viewer



DIALS Reciprocal Lattice Viewer
DIALS Reciprocal Lattice Viewer



Integrate images with Dials
Launch DUI and capture output

X-ray data reduction and analysis

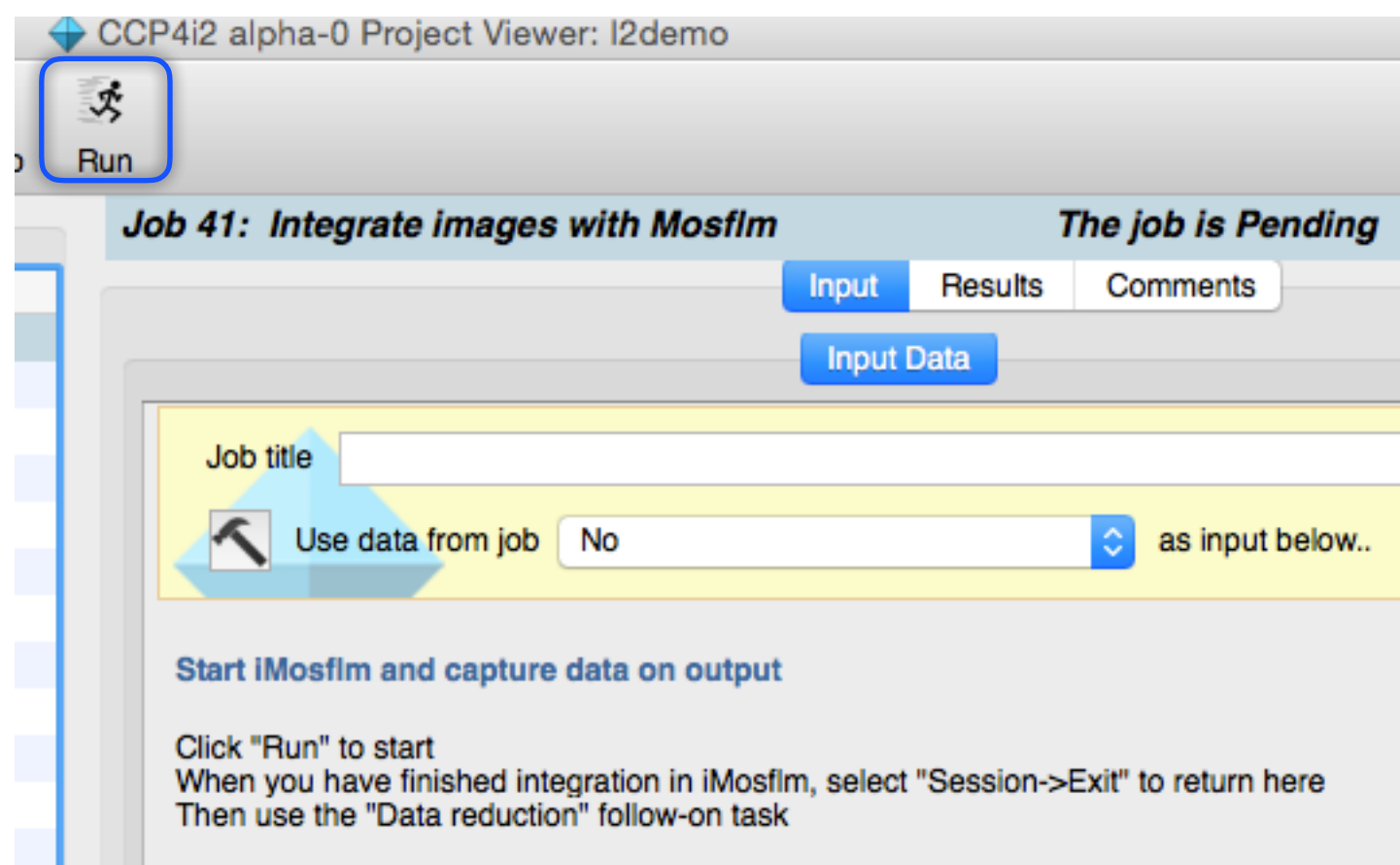


Data reduction - AIMLESS
Scale and analyse unmerged data and suggest space group (Pointless, Aimless, Ctruncate, FreeRflag)



Generate a Free R set
Generate a Free R set for a complete set of reflection indices to a given resolution (FreeRflag)

Run xia2 with
DIALS or XDS



Data reduction task

Import one or more files

Identify dataset
(short names without spaces)

Input files can be:

- MTZ file from DIALLS
- MTZ file from MOSFLM
- XDS_ASCII.HKL from XDS (already scaled so maybe switch off scaling)
- INTEGRATE.HKL from XDS (unscaled)
- Output from XDS/XSCALE (already scaled)
- Scalepack unmerged file (already scaled)
- SAINT .RAW file

Job 40: Data reduction - AIMLESS **The job is Pending**

Input Results Comments

Input Data Important Options Additional Options

Job title

 Use data from job 39 Integrate images with Mosfilm as input below..

 Show list Select unmerged data files

 ..must be selected

Crystal name dataset name

Batches in file:

Exclude batches from calculations and output

Resolution range (Å) to Maximum resolution in files 0.00Å

☐ use explicit resolution range in symmetry determination as well as in scaling

Options for symmetry determination Determine Laue group and space group

Optional input data

1. Reference data to resolve indexing ambiguity and space group

☐ use reference data in analysis against Batch after scaling

Reference data are Reflection list and is optionally defined in next line

 Reflections ..is not used

2. Optional existing FreeR set, define to copy or extend if necessary

 Free R set ..is not used

Symmetry determination, point group and space group (POINTLESS)

The crystal symmetry may impose constraints on the unit cell dimensions, according to the crystal class (the Bravais lattice): cubic, hexagonal/trigonal, tetragonal, orthorhombic, monoclinic, or triclinic, + lattice centring P, C, I, R, or F. For example, in the tetragonal system $a=b$, and all angles = 90°

Indexing in MOSFLM, XDS, DIALS, etc only gives a unit cell, which implies possible lattice symmetry, due to the constraints of unit cell dimensions. But to determine the point group we need to look at the intensities, as rotational and screw symmetry in real space leads to rotational symmetry in reciprocal space

Note that POINTLESS (and other programs) will find **symmetry** in the diffraction pattern, but this symmetry may or may not be *crystallographic* (rather than non-crystallographic pseudo symmetry)

Stages of space group determination in POINTLESS

1. from the cell dimensions, determine the maximum possible lattice symmetry, with some tolerance (ignoring any input symmetry)
2. for each possible rotation operator, score potentially related observations pairs for agreement (correlation coefficients and R-factor)
3. score all possible combinations of operators to determine the point group (point groups from the maximum down to P1)
4. score axial systematic absences to detect screw axes, hence space group (note that axial observations are sometimes unobserved)

Symmetry determination, point group and space group (POINTLESS)

Stage 1: score individual symmetry operators in the maximum lattice group

Maximum possible lattice symmetry determined from cell dimensions
pseudo-cubic example, $a \approx b \approx c$, angles $\approx 90^\circ$

Compare pairs of observations related by each possible rotational operator, using correlation coefficients and R-factors on normalised intensities $|E|^2$

Analysing rotational symmetry in lattice group $P\ m\ \bar{3}\ m$

Scores for each symmetry element

Nelmt	Lklhd	Z-cc	CC	N	Rmeas	0.950	Symmetry & operator (in Lattice Cell)		
1	0.955	9.70	0.97	13557	0.073		identity		
2	0.062	2.66	0.27	12829	0.488		2-fold	(1 0 1)	{+l,-k,+h}
3	0.065	2.85	0.29	10503	0.474		2-fold	(1 0 -1)	{-l,-k,-h}
4	0.056	0.06	0.01	16391	0.736		2-fold	(0 1 -1)	{-h,-l,-k}
5	0.057	0.05	0.00	17291	0.738		2-fold	(0 1 1)	{-h,+l,+k}
6	0.049	0.55	0.06	13758	0.692		2-fold	(1 -1 0)	{-k,-h,-l}
7	0.950	9.59	0.96	12584	0.100	***	2-fold k	(0 1 0)	{-h,+k,-l}
8	0.049	0.57	0.06	11912	0.695		2-fold	(1 1 0)	{+k,+h,-l}
9	0.948	9.57	0.96	16928	0.136	***	2-fold h	(1 0 0)	{+h,-k,-l}
10	0.944	9.50	0.95	12884	0.161	***	2-fold l	(0 0 1)	{-h,-k,+l}
11	0.054	0.15	0.01	23843	0.812		3-fold	(1 1 1)	{+l,+h,+k} {+k,+l,+h}
12	0.055	0.11	0.01	24859	0.825		3-fold	(1 -1 -1)	{-l,-h,+k} {-k,+l,-h}
13	0.055	0.14	0.01	22467	0.788		3-fold	(1 -1 1)	{+l,-h,-k} {-k,-l,+h}
14	0.055	0.12	0.01	27122	0.817		3-fold	(1 1 -1)	{-l,+h,-k} {+k,-l,-h}
15	0.061	-0.10	-0.01	25905	0.726		4-fold h	(1 0 0)	{+h,-l,+k} {+h,+l,-k}
16	0.060	2.53	0.25	23689	0.449		4-fold k	(0 1 0)	{+l,+k,-h} {-l,+k,+h}
17	0.049	0.56	0.06	25549	0.653		4-fold l	(0 0 1)	{-k,+h,+l} {+k,-h,+l}

Only orthorhombic symmetry operators are present
High CC, low R_{meas}

Stage 2: score possible point groups

All possible combinations of rotations are scored to determine the point group.

Good scores in symmetry operations which are absent in the sub-group count against that group.

Example: C-centred orthorhombic which might been hexagonal

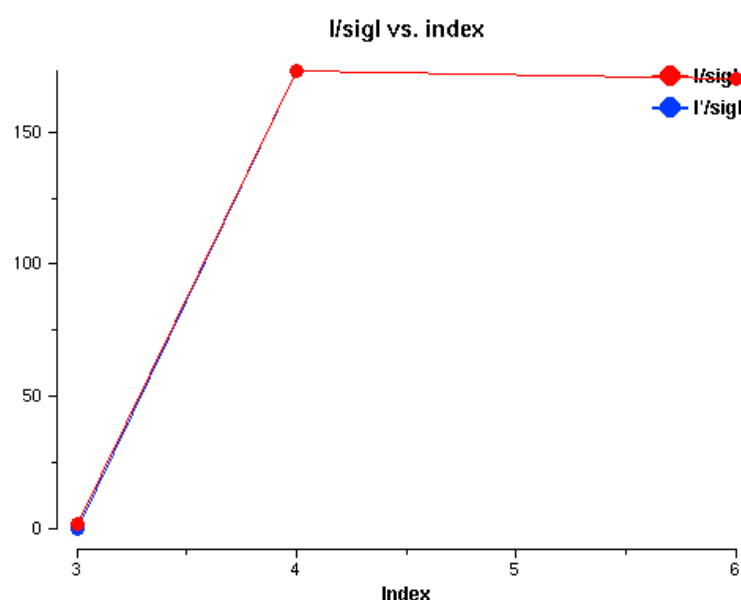
	Laue	Group		Lklhd	NetZc	Zc+	Zc-	CC	CC-	Rmeas	R-	Delta	ReindexOperator
= 1	C	m m m	***	0.989	9.45	9.62	0.17	0.96	0.02	0.08	0.76	0.0	[h,k,l]
2	P	1 2/m 1		0.004	7.22	9.68	2.46	0.97	0.25	0.06	0.56	0.0	[-1/2h+1/2k, -l, -1/2h-1/2k]
3	C	1 2/m 1		0.003	7.11	9.61	2.50	0.96	0.25	0.08	0.55	0.0	[h,k,l]
4	C	1 2/m 1		0.003	7.11	9.61	2.50	0.96	0.25	0.08	0.55	0.0	[-k, -h, -l]
5		P -1		0.000	6.40	9.67	3.27	0.97	0.33	0.06	0.49	0.0	[1/2h+1/2k, 1/2h-1/2k, -l]
6	C	m m m		0.000	1.91	5.11	3.20	0.51	0.32	0.34	0.51	2.5	[1/2h-1/2k, -3/2h-1/2k, -l]
7		P 6/m		0.000	1.16	4.59	3.43	0.46	0.34	0.41	0.46	2.5	[-1/2h-1/2k, -1/2h+1/2k, -l]
8	C	1 2/m 1		0.000	1.51	5.15	3.64	0.52	0.36	0.33	0.47	2.5	[1/2h-1/2k, -3/2h-1/2k, -l]
9	C	1 2/m 1		0.000	1.51	5.15	3.64	0.51	0.36	0.33	0.47	2.5	[-3/2h-1/2k, -1/2h+1/2k, -l]
10		P -3		0.000	1.04	4.75	3.71	0.48	0.37	0.40	0.45	2.5	[-1/2h-1/2k, -1/2h+1/2k, -l]
11	C	m m m		0.000	2.13	5.23	3.10	0.52	0.31	0.32	0.52	2.5	[-1/2h-1/2k, -3/2h+1/2k, -l]
12	C	1 2/m 1		0.000	1.64	5.25	3.61	0.53	0.36	0.32	0.47	2.5	[-1/2h-1/2k, -3/2h+1/2k, -l]
13	C	1 2/m 1		0.000	1.67	5.27	3.60	0.53	0.36	0.32	0.47	2.5	[-3/2h+1/2k, 1/2h+1/2k, -l]
14	P	-3 1 m		0.000	0.12	4.00	3.87	0.40	0.39	0.44	0.44	2.5	[-1/2h-1/2k, -1/2h+1/2k, -l]
15	P	-3 m 1		0.000	0.14	4.00	3.86	0.40	0.39	0.44	0.44	2.5	[-1/2h-1/2k, -1/2h+1/2k, -l]
16	P	6/m m m		0.000	3.93	3.93	0.00	0.39	0.00	0.44	0.00	2.5	[-1/2h-1/2k, -1/2h+1/2k, -l]

Stage 3: space group from axial systematic absences

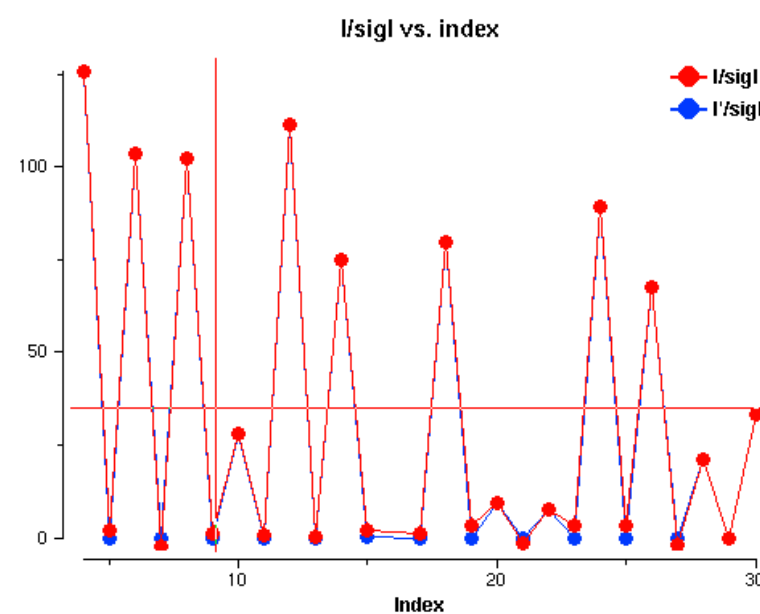
Zone	Number	PeakHeight	SD	Probability	ReflectionCondition
Zones for Laue group $P\ m\ m\ m$					
1 screw axis $2\{1\} [a]$	3	1.000	0.296	** 0.889	$h00: h=2n$
2 screw axis $2\{1\} [b]$	26	1.000	0.142	*** 0.971	$0k0: k=2n$
3 screw axis $2\{1\} [c]$	46	0.997	0.097	*** 0.986	$00l: l=2n$

Fourier analysis of $I/\sigma(I)$

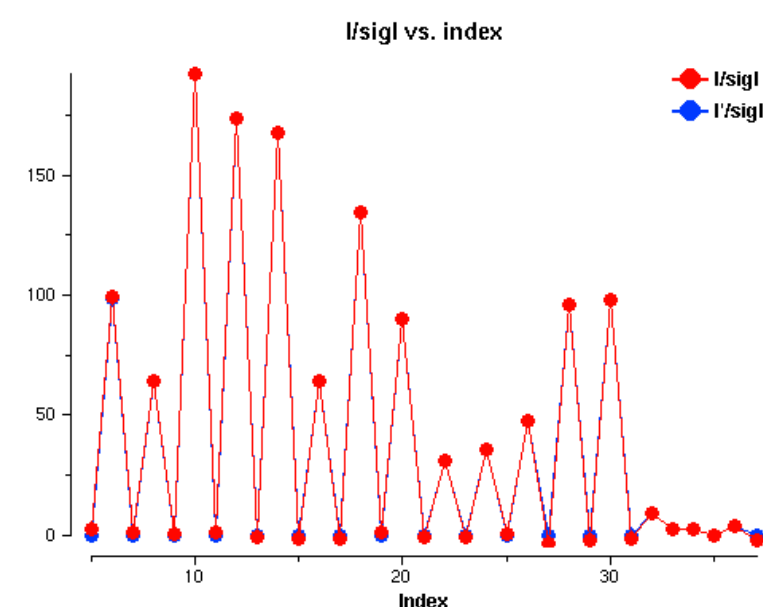
There are indications of 2_1 screw symmetry along all principle axes (though note there are only 3 observations on the a axis ($h00$ reflections))



Possible 2_1 axis along a



Clear 2_1 axis along b



Clear 2_1 axis along c

... BUT “confidence” in space group may be low due to sparse or missing information
Always check the space group later in the structure solution!

Possible spacegroups:

Indistinguishable space groups are grouped together on successive lines

'Reindex' is the operator to convert from the input hklin frame to the standard spacegroup frame.

'TotProb' is a total probability estimate (unnormalised)

'SysAbsProb' is an estimate of the probability of the space group based on the observed systematic absences.

'Conditions' are the reflection conditions (absences)

Spacegroup	TotProb	SysAbsProb	Reindex	Conditions
<P 21 21 21> (19)	0.838	0.851		h00: h=2n, 0k0: k=2n, 00l: l=2n (zones 1,2,3)
<P 2 21 21> (18)	0.104	0.106		0k0: k=2n, 00l: l=2n (zones 2,3)
<P 21 2 21> (18)	0.025	0.026		h00: h=2n, 00l: l=2n (zones 1,3)
<P 21 21 2> (18)	0.012	0.012		h00: h=2n, 0k0: k=2n (zones 1,2)

Best Solution space group P 21 21 21

Reindex operator:	[h,k,l]
Laue group probability:	0.985
Systematic absence probability:	0.851
Total probability:	0.838
Space group confidence:	0.784
Laue group confidence	0.982

Note high confidence in Laue group, but lower confidence in space group

Unit cell: 34.16 54.8 68 90 90 90

17.00 to 1.78 - Resolution range used for Laue group search

17.00 to 1.78 - Resolution range in file, used for systematic absence check

Number of batches in file: 100

What can go wrong?

Pseudo-symmetry or twinning (often connected) can suggest a point group symmetry which is too high. Careful examination of the scores for individual symmetry operators may indicate the truth (the program is not foolproof!)

POINTLESS works (usually) with unscaled data (hence use of correlation coefficients), so data with a large range of scales, including a dead crystal, may give a too-low symmetry. In bad cases either just use the first part of the data, or scale in P1 and run POINTLESS on the scaled unmerged data (*this **ought** to be done automatically* 😞)

Potential axial systematic absences may be absent or few, so it may not be possible to determine the space group. In that case the output file is labelled with the “space group” with no screw axes, eg P2, P222, P622 etc, and the space group will have to be determined later

NOTE that the space group is only a **hypothesis** until the structure has been determined and satisfactorily refined

What can go wrong?

Pseudo symmetry example

Monoclinic, pseudo-orthorhombic (from NCS), $\beta \approx 90^\circ$

Unit cell 107.99 270.51 155.96 90.00 **90.36** 90.00

NeImt	Lklhd	Z-cc	CC	N	Rmeas	Symmetry & operator (in Lattice Cell)
1	0.925	9.13	0.91	14115	0.126	identity
2	0.928	9.16	0.92	6811	0.176	*** 2-fold l (0 0 1) {-h,-k,l}, along original k
3	0.659	7.96	0.80	31850	0.252	* 2-fold k (0 1 0) {-h,k,-l}, along original l
4	0.678	8.02	0.80	6841	0.245	* 2-fold h (1 0 0) {h,-k,-l}, along original h

one 2-fold is stronger than the other two, but not enough to give the right answer

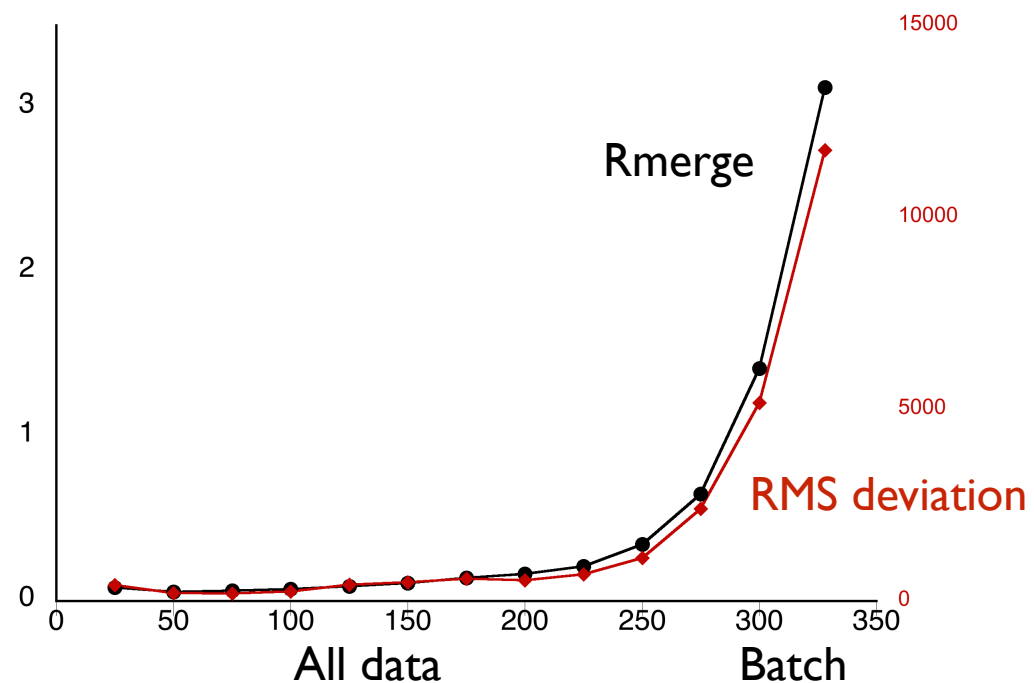
Laue Group	Lklhd	NetZc	Zc+	Zc-	CC	CC-	Rmeas	R-	Delta	ReindexOperator
> 1 P m m m **	0.745	8.33	8.33	0.00	0.83	0.00	0.20	0.00	0.4	[h,l,-k]
= 2 P 1 2/m 1	0.183	1.20	9.14	7.94	0.91	0.79	0.14	0.25	0.0	[h,k,l]
3 P 1 2/m 1	0.030	0.59	8.75	8.16	0.88	0.82	0.16	0.24	0.4	[-l,-h,k]
4 P 1 2/m 1	0.028	-0.32	8.27	8.59	0.83	0.86	0.20	0.21	0.4	[h,l,-k]
5 P -1	0.014	1.01	9.13	8.12	0.91	0.81	0.13	0.24	0.0	[-h,-l,-k]

Best Solution: point group P 2 2 2

Reindex operator: [h,l,-k]
 Laue group probability: 0.745
 Systematic absence probability: 0.832
 Total probability: 0.620
 Space group confidence: 0.000
 Laue group confidence: 0.647

Note low confidence in Laue (point) group

What can go wrong?

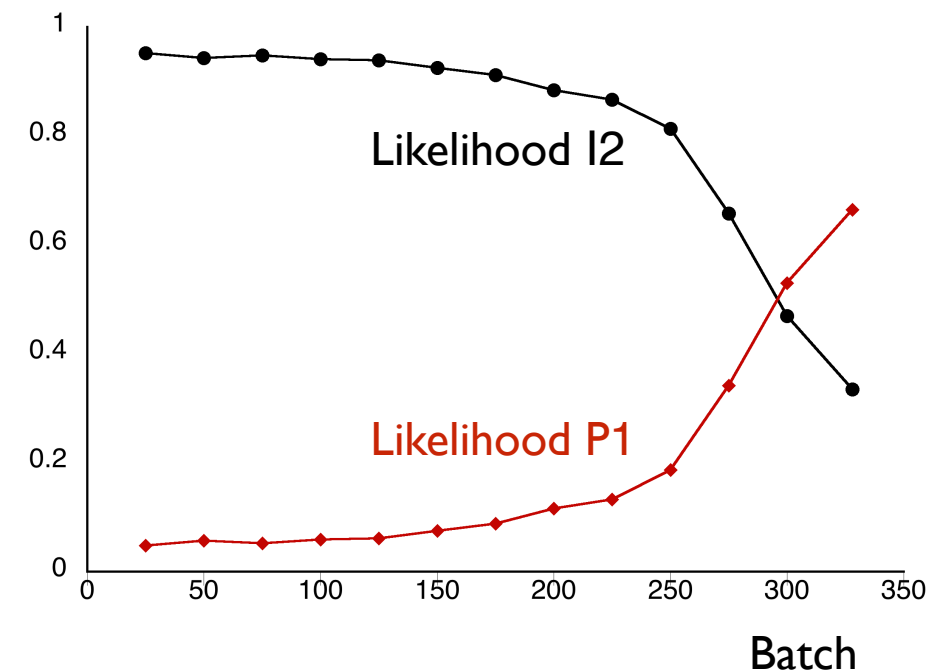
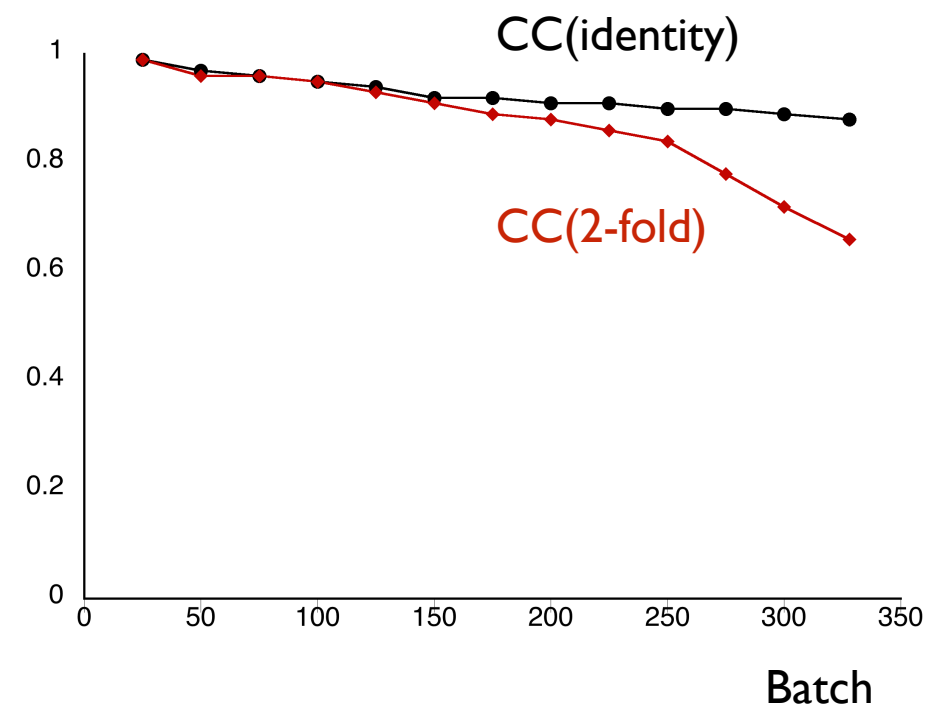


Severe radiation damage after image 250

True space group is I2 (== C2),
i.e. it has a crystallographic dyad
(2-fold rotation)

Radiation damage obscures the
dyad, giving the wrong lower
symmetry P1

Radiation damage example



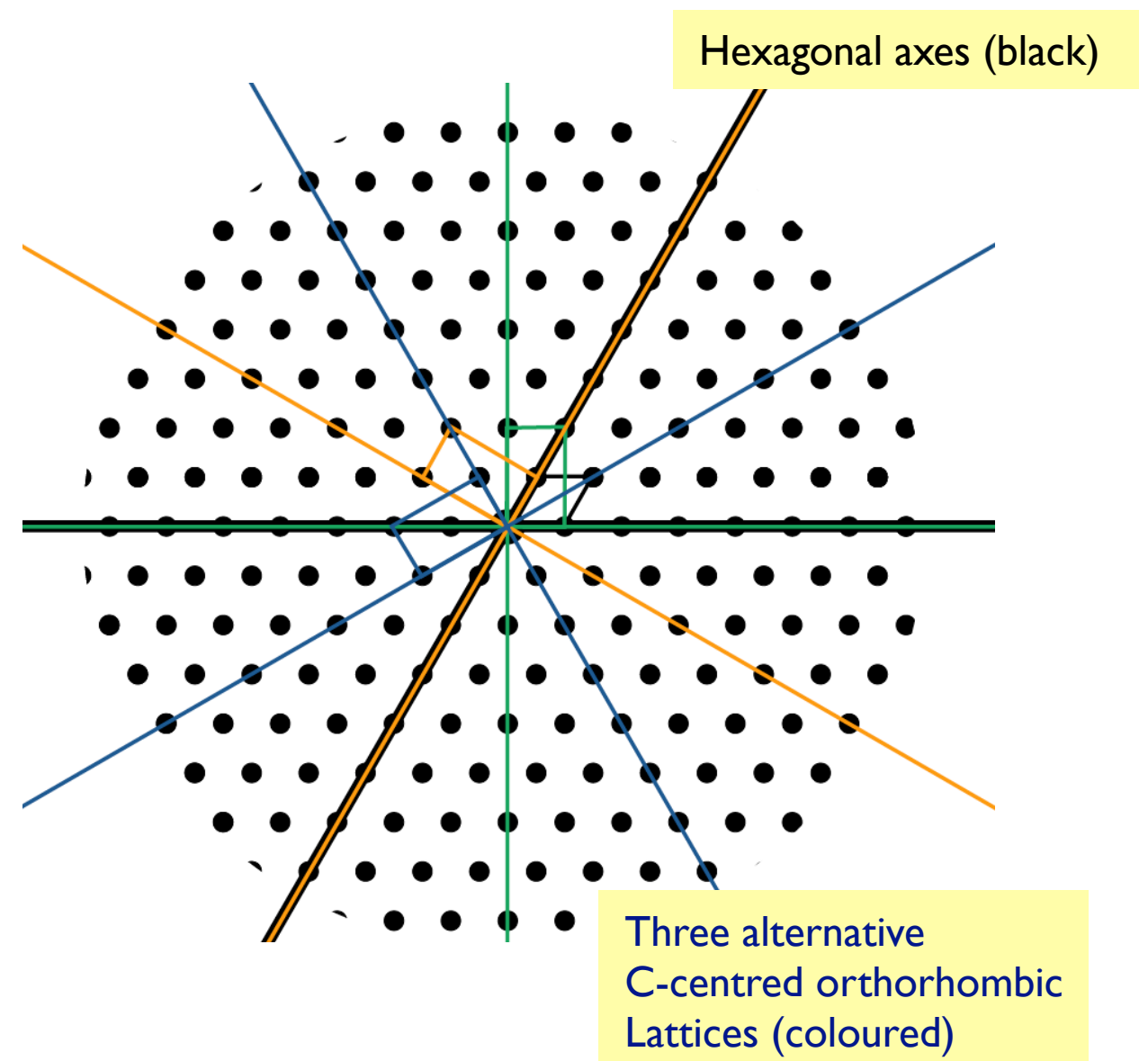
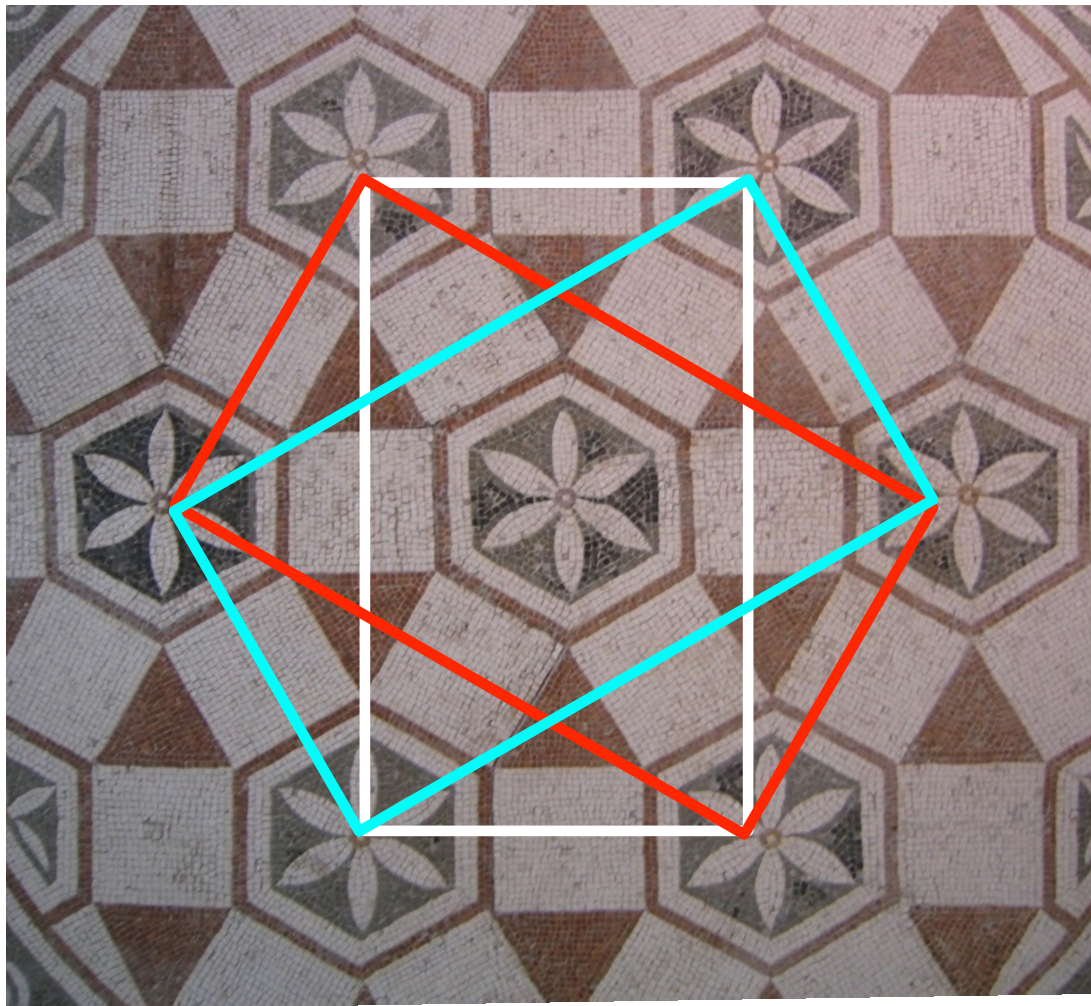
Scores from cumulative batch groups from the start,
i.e. 1-25, 1-50, 1-75, ... etc

A confusing case in C222:

Unit cell 74.72 129.22 184.25 90 90 90

This has $b \approx \sqrt{3} a$ so can also be indexed on a hexagonal lattice, lattice point group P622 (P6/mmm), with the reindex operator: $h/2+k/2, h/2-k/2, -l$

Conversely, a hexagonal lattice may be indexed as C222 in three distinct ways, so there is a 2 in 3 chance of the indexing program choosing the wrong one



Score each symmetry operator in P622

“Likelihood” Correlation coefficient on E² Rfactor (multiplicity weighted)

 Z-score(CC)

 ↓

Nelmt Lklhd Z-cc CC N Rmeas Symmetry & operator (in Lattice Cell)

1	0.808	5.94	0.89	9313	0.115		identity		
2	0.828	6.05	0.91	14088	0.141	***	2-fold l	(0 0 1)	{-h,-k,+l}
3	0.000	0.06	0.01	16864	0.527		2-fold	(1-1 0)	{-k,-h,-l}
4	0.871	6.33	0.95	10418	0.100	***	2-fold	(2-1 0)	{+h,-h-k,-l}
5	0.000	0.53	0.08	12639	0.559		2-fold h	(1 0 0)	{+h+k,-k,-l}
6	0.000	0.06	0.01	16015	0.562		2-fold	(1 1 0)	{+k,+h,-l}
7	0.870	6.32	0.95	2187	0.087	***	2-fold k	(0 1 0)	{-h,+h+k,-l}
8	0.000	0.55	0.08	7552	0.540		2-fold	(-1 2 0)	{-h-k,+k,-l}
9	0.000	-0.12	-0.02	11978	0.598		3-fold l	(0 0 1)	{-h-k,+h,+l} {+k,-h-k,+l}
10	0.000	-0.06	-0.01	17036	0.582		6-fold l	(0 0 1)	{-k,+h+k,+l} {+h+k,-h,+l}

Only the orthorhombic symmetry operators are present

Alternative indexing

If the true point group is lower symmetry than the lattice group, alternative valid but non-equivalent indexing schemes are possible, related by symmetry operators present in lattice group but not in point group (*note that these are also the cases where merohedral twinning is possible*)

eg if in space group $P3$ (or $P3_1$) there are 4 different schemes (h,k,l) or $(-h,-k,l)$ or $(k,h,-l)$ or $(-k,-h,-l)$

For the first crystal, you can choose any scheme

For subsequent crystals, the autoindexing will randomly choose one setting, and we need to make it consistent: *POINTLESS* will do this for you by comparing the unmerged test data to a reference dataset (merged or unmerged, or coordinates)

Note that the space group from the reference will be assumed to be correct

The screenshot shows the POINTLESS software interface for selecting unmerged data files. Key settings are highlighted with red circles:

- Crystal name:** AmpNT
- dataset name:** IP6_2
- Batches in file:** 1001 - 1238
- Resolution range (Å):** (empty) to (empty) Maximum resolution in files: 3.50Å
- Options for symmetry determination:** Match index to reference data
- Reference data are:** Reflection list
- Reflections:** amph1_P3121_scala1: se1_peak imported by job 36
- Free R set:** ..is not used

Combining multiple files

Multiple “sweeps” or datasets (eg MAD)

Peak, 3 files

Inflection, 1 file

Remote, 1 file

Use the
dataset names

Filename	Crystal	Dataset	Exclude batches
pk_1_001.mtz	Brap	pk	
pk_2_001.mtz	Brap	pk	
pk_180_1_001.mtz	Brap	pk	
ip_1_001.mtz	Brap	lp	
rm_1_001.mtz	Brap	Rm	

Unmerged reflections loaded from pk_180_1_001.mtz by job 35

Crystal name dataset name OR same dataset as

Batches in file: 5001 - 5360

Exclude batches from calculations and output

or assign files to
the same dataset

▼ Alternative index scores

Possible reindex operators: [h,k,l], [-k,h,l], [l,k,-h], [-h,l,k], [l,h,k], [k,l,h]

Reindex operator	Likelihood	CC
[h,k,l]	0.664	0.64
[-k,h,l]	0.236	0.50
[l,k,-h]	0.029	0.17
[-h,l,k]	0.024	0.11
[l,h,k]	0.024	0.11
[k,l,h]	0.023	0.11

Reindex operator	Likelihood	CC
[h,k,l]	0.873	0.74
[-k,h,l]	0.067	0.43
[l,k,-h]	0.018	0.13
[-h,l,k]	0.015	0.07
[k,l,h]	0.014	0.05
[l,h,k]	0.014	0.04

Reindex operator	Likelihood	CC
[h,k,l]	0.755	0.65
[-k,h,l]	0.153	0.45
[l,k,-h]	0.026	0.12
[-h,l,k]	0.022	0.07
[l,h,k]	0.022	0.07
[k,l,h]	0.021	0.06

Reindex operator	Likelihood	CC
[h,k,l]	0.757	0.65
[-k,h,l]	0.153	0.45
[l,k,-h]	0.026	0.12
[l,h,k]	0.022	0.06
[-h,l,k]	0.021	0.06
[k,l,h]	0.021	0.06

Because of an indexing ambiguity (pseudo-cubic orthorhombic), we must check for consistent indexing between files

Note also some ambiguity with the operator [-k,h,l] due to pseudo-merohedral twinning

Scaling, merging and Data Quality

Put observations on a common scale

Analyse to:-

- estimate resolution

- check for radiation damage

- reject outliers

- improve error estimates

Why are reflections on different scales?

- (a) Factors related to incident beam and the camera
incident beam intensity; illuminated volume; primary beam absorption
- (b) Factors related to the crystal and the diffracted beam
absorption; radiation damage (worse at high resolution)
- (c) Factors related to the detector
miscalibration; corners of fibre-optic tapers for CCDs
Beam-stop shadow etc (Important)

Scaling tries to make symmetry-related and duplicate measurements of a reflection equal, by modelling the diffraction experiment, principally as a function of the incident and diffracted beam directions in the crystal. This makes the data **internally consistent** (not necessarily correct)

$$\text{Minimize } \Phi = \sum_{hl} w_{hl} (I_{hl} - g_{hl} \langle I_h \rangle)^2$$

I_{hl} l'th intensity observation of reflection h k_{hl} scale factor for I_{hl}
 $\langle I_h \rangle$ current estimate of I_h

$g_{hl} = 1/k_{hl}$ is a function of the parameters of the **scaling model**

$g_{hl} = g(\varphi \text{ rotation/image number}) \cdot g(\text{time}) \cdot g(s) \quad \dots \text{ other factors}$
Primary beam s_0 B-factor Absorption

The scale model should reflect the data collection strategy

Data collection strategy should be designed to get good scaling and analysis

high multiplicity (low dose) gives:-

- good scaling
- good outlier rejection
- the opportunity to reject radiation damaged parts of the data without losing completeness

For example, in the extreme case of serial crystallography, with small rotation (or zero) range per crystal and many crystals, use one scale & B-factor / crystal

*Average radiation damage
(scales up high resolution observations)*

$$g_{hl} = g(\varphi \text{ rotation/image number}) \cdot g(\text{time}) \cdot g(s^2) \quad \dots \text{other factors}$$

*Primary beam s_0
Illuminated volume etc*

B-factor Absorption

*Important with big crystals
at long wavelength*

$$\exp(-2B(\sin \theta/\lambda)^2)$$

Viewing the output statistics (job report from ccp4i2)

1. key summary

▼ Key summary

Selecting space group P 21 21 21
as there is a single space group with the highest score

Solution probability: 0.853, Confidence 0.835 (high resolution limit for symmetry testing 3.252Å)

NOTE: the final selected symmetry and cell have alternative indexing schemes, but no reference data has been given
Possible alternative indexing operators (with cell differences in Å): [h,k,l] (0.00), [-h,l,k] (0.71), [-k,h,l] (0.73), [l,h,k] (1.25), [k,l,h] (1.25), [l,k,-h] (1.44)

If you already have a matching dataset, you should choose it as a reference set to get consistent indexing

Key statistics for Dataset: I2demo/Brp/pk

Resolution of input data: 2.79Å, resolution estimate 2.87Å

Rmeas: overall 0.151, inner bin 0.069

In outer bin: Mean(I/sdI) 0.8 CC(1/2) 0.266

Anomalous CC(1/2) in inner bin 0.753

Significant anomalous signal extends to a resolution of 3.74Å (above CCanom threshold 0.15)

Warning: Possible twinning, twin fraction estimates from Britton plot 0.20, from H-test 0.23

No evidence of possible translational non-crystallographic symmetry

Some anisotropy detected. This may have an effect on statistics.

Warning: Completeness test shows some issues.

No ice rings found.

Warnings:
red, bad;
orange, maybe
OK;
green, OK

Viewing the output statistics (job report)

“Table 1”

2. main summary

Space group determination

scores for individual symmetry elements may detect pseudo-symmetry ...

... or suggest twinning

Space group determination

WARNING: the L-test suggests that the data may be twinned, so the indicated Laue symmetry may be too high
 Rough estimated twin fraction: 0.096

Selecting space group P 21 21 21
 as there is a single space group with the highest score

Solution type: space group

Group name	P 21 21 21
Reindex	[h,k,l]
Space group confidence	0.835
Laue group confidence	0.938
Laue group probability	0.948
Systematic absence probability	0.900

Scores for each symmetry element

Lattice group name P 4 3 2

Reindex operator from input to lattice: [h,k,l]

Likelihood	CC	R		Symmetry
0.913	0.88	0.097		identity
0.901	0.87	0.109	***	2-fold l (0 0 1) { -h, -k, l }
0.917	0.88	0.090	***	2-fold k (0 1 0) { -h, k, -l }
0.913	0.88	0.103	***	2-fold h (1 0 0) { h, -k, -l }
0.214	0.56	0.191		2-fold (1 -1 0) { -k, -h, -l }
0.051	0.06	0.588		2-fold (0 1 -1) { -h, -l, -k }
0.052	0.13	0.494		2-fold (1 0 -1) { -l, -k, -h }
0.223	0.57	0.193		2-fold (1 1 0) { k, h, -l }
0.051	0.11	0.512		2-fold (1 0 1) { l, -k, h }
0.051	0.06	0.562		2-fold (0 1 1) { -h, l, k }
0.053	0.04	0.720		3-fold (1 -1 -1) { -k, l, -h }
0.054	0.04	0.712		3-fold (1 1 -1) { -l, h, -k }
0.053	0.04	0.701		3-fold (1 -1 1) { l, -h, -k }
0.053	0.04	0.704		3-fold (1 1 1) { k, l, h }
0.203	0.55	0.198		4-fold l (0 0 1) { -k, h, l }
0.050	0.10	0.509		4-fold k (0 1 0) { l, k, -h }
0.052	0.05	0.565		4-fold h (1 0 0) { h, l, -k }

Data internal consistency statistics

Summary of merging statistics for dataset
I2demo/Brp/pk

	Overall	Inner	Outer
Low resolution limit	57.82	57.82	2.94
High resolution limit	2.79	8.81	2.79
Rmerge(within I+ /I-)	0.140	0.064	2.085
Rmerge(all I+ and I-)	0.154	0.076	2.218
Rmeas (within I+ /I-)	0.151	0.069	2.336
Rmeas (all I+ & I-)	0.161	0.080	2.352
Rpim (within I+ /I-)	0.057	0.026	1.013
Rpim (all I+ & I-)	0.045	0.023	0.754
Rmerge in top intensity bin	0.063		
Number of observations	178940	5691	18047
Number unique	14045	501	1955
Mean(I)/sd(I)	9.7	33.5	0.8
Half-set correlation CC(1/2)	0.998	0.997	0.266
Completeness %	99.5	97.6	96.9
Multiplicity	12.7	11.4	9.2
Anomalous completeness %	98.5	98.1	90.8
Anomalous multiplicity	6.4	6.9	4.7
DelAnom CC(1/2)	0.664	0.753	-0.026
Mid-Slope of Anom Probability	1.020		

Download

Download as CSV file

ers to be a significant anomalous signal so
anomalous flag was switched ON

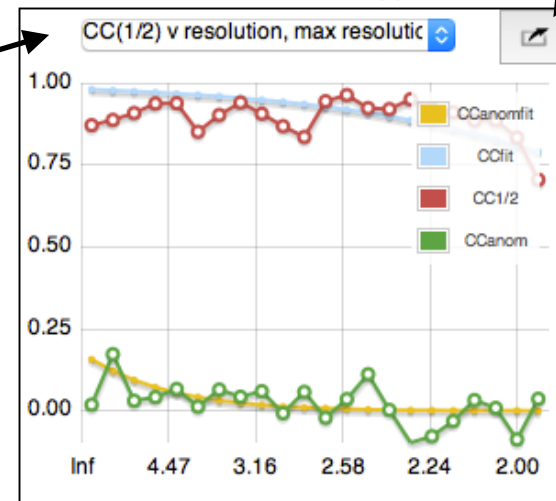
Viewing the output statistics (job report)

pop out
separate
graph viewer

3. The most important graphs

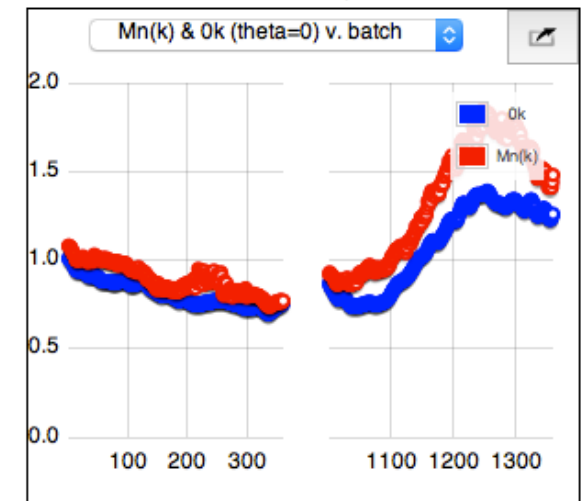
pull-down to change graph

Analysis as a function of resolution
Plot of CC(1/2) vs. resolution may indicate a suitable resolution cutoff, and indicate presence of an anomalous signal (but check anisotropy)



Analyses by resolution

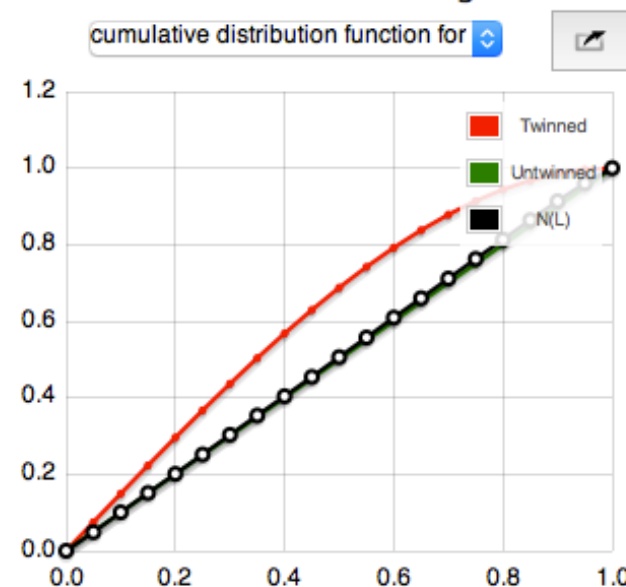
Analysis as a function of batch
Analyses against Batch may show radiation damage, and which parts of the data should be removed (but consider completeness)



Analyses by batch

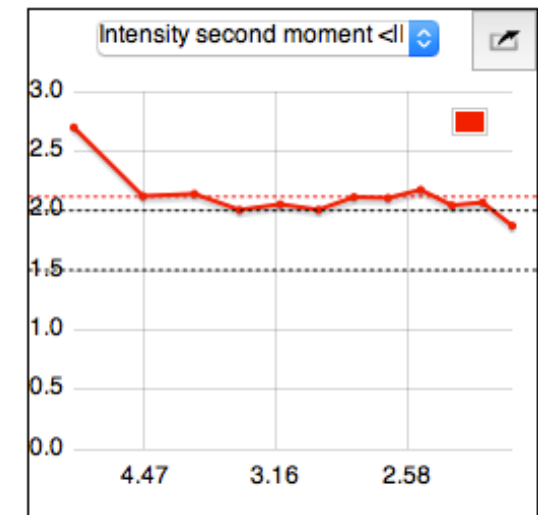
ing etc, more
This dataset is probably NOT twinned

L-test for twinning



Analyses for twinning

Acentric intensity moments



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

Operator	Value	Untwinned	Perfect twin
$\langle I^2 \rangle / \langle I \rangle^2$	2.117	2.000	1.500
$\langle I^3 \rangle / \langle I \rangle^3$	7.082	6.000	3.000
$\langle I^4 \rangle / \langle I \rangle^4$	32.680	24.000	7.500

4. more details in folders, closed by default

- Details of space group determination
- Other merging graphs
- Details of merging
- Intensity statistics: twinning tNCS etc

What should you look at? What are the questions?

Are there some parts of the data which much worse than the best parts? Maybe these should be omitted (subject to completeness)

Should you apply a resolution cutoff?

Measures of quality:

Signal/noise estimates

$$\langle I/\sigma(I) \rangle$$

$$\text{note } \neq \langle I \rangle / \langle \sigma(I) \rangle$$

but $\sigma(I)$ estimates are not perfect

Measures of internal consistency:

(1) R-factors

$$R_{\text{merge}} = \sum |I_{hl} - \langle I_h \rangle| / \sum \langle I_h \rangle$$

a.k.a R_{sym} or R_{int}

traditional overall measures of quality, but increases with multiplicity although the data improves

$$R_{\text{meas}} = R_{\text{r.i.m.}} = \sum \sqrt{(n/n-1)} |I_{hl} - \langle I_h \rangle| / \sum \langle I_h \rangle$$

multiplicity-weighted, better (but larger)

$$R_{\text{p.i.m.}} = \sum \sqrt{(1/n-1)} |I_{hl} - \langle I_h \rangle| / \sum \langle I_h \rangle$$

“Precision-indicating R-factor” gets better (smaller) with increasing multiplicity, ie it estimates the precision of the merged $\langle I \rangle$

(2) correlation coefficients

Half-dataset correlation coefficient $CC_{1/2}$:

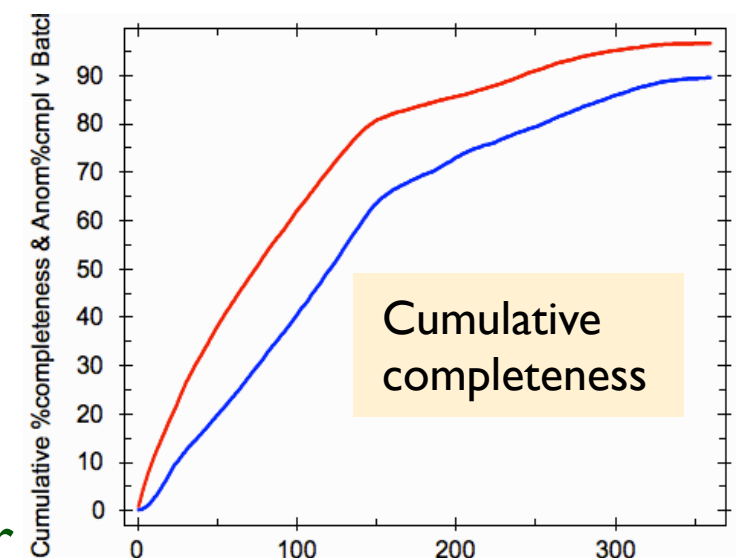
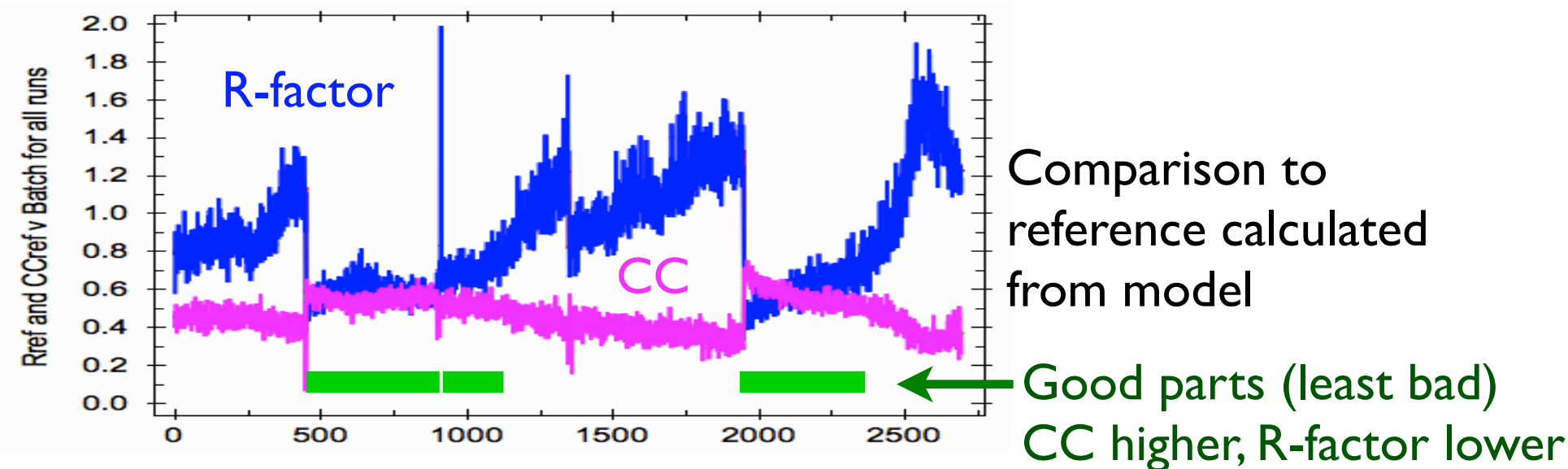
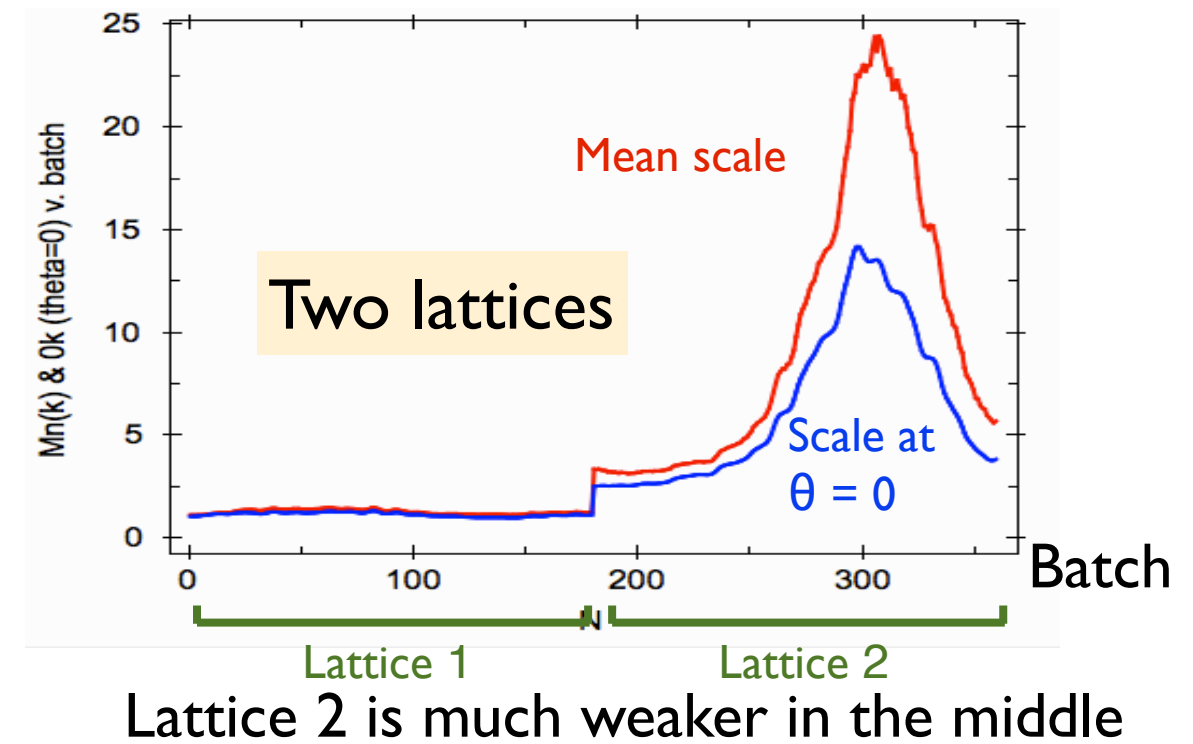
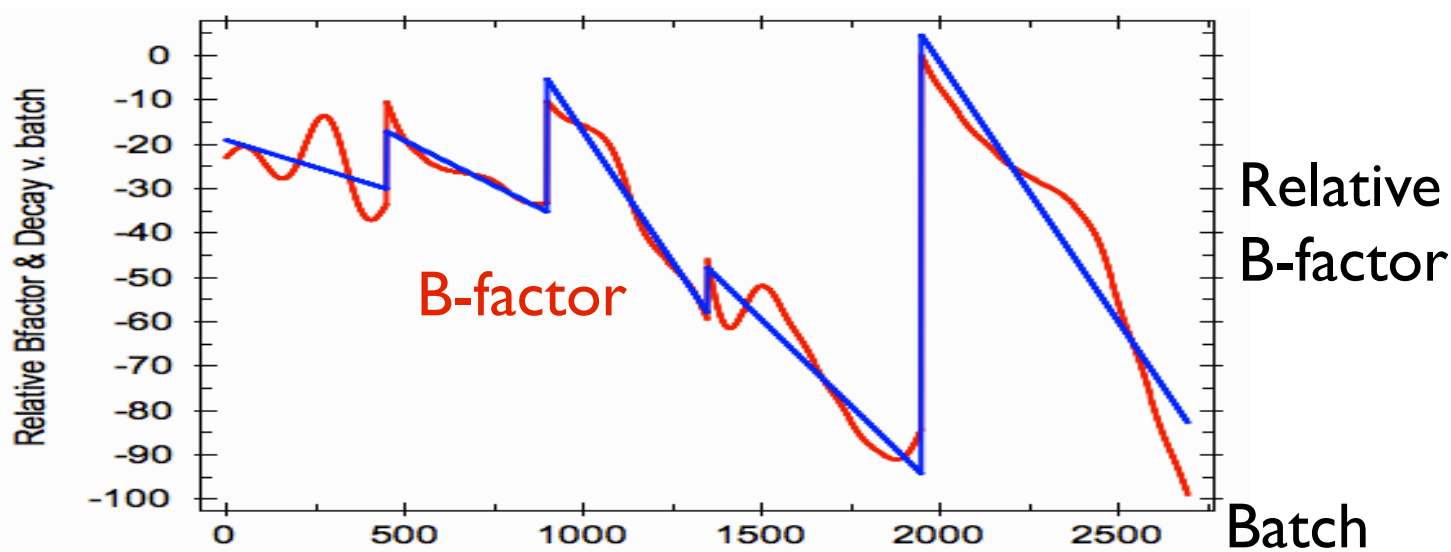
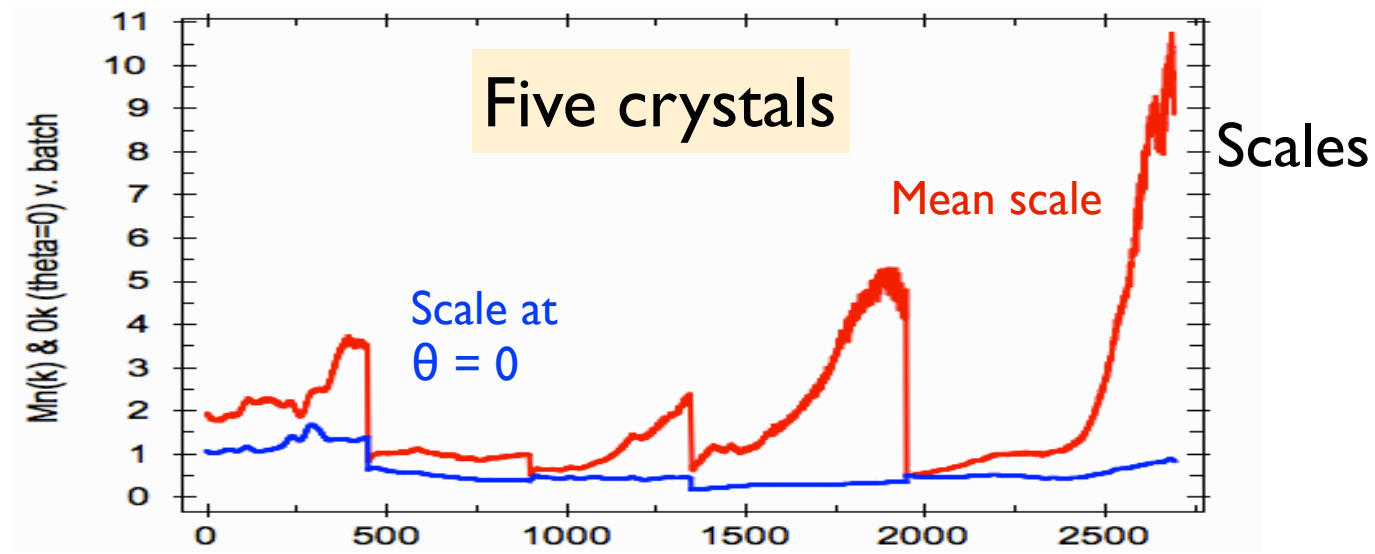
Split observations for each reflection data randomly into 2 halves, and calculate the correlation coefficient between them (essentially comparing the dispersion of individual observations with the dispersion of the data)

What should you look at?

Analyses as a function of “batch” (ie image number)

Look at :

- scales
- relative B-factor (overall radiation damage)
- cumulative completeness
- maybe comparison to reference



Analyses as a function of resolution

We can plot various statistics against resolution to determine where we should cut the data, allowing for anisotropy.

What do we mean by the “resolution” of the data? We want to determine the point at which adding another shell of data does not add any “significant” information, but how do we measure this?

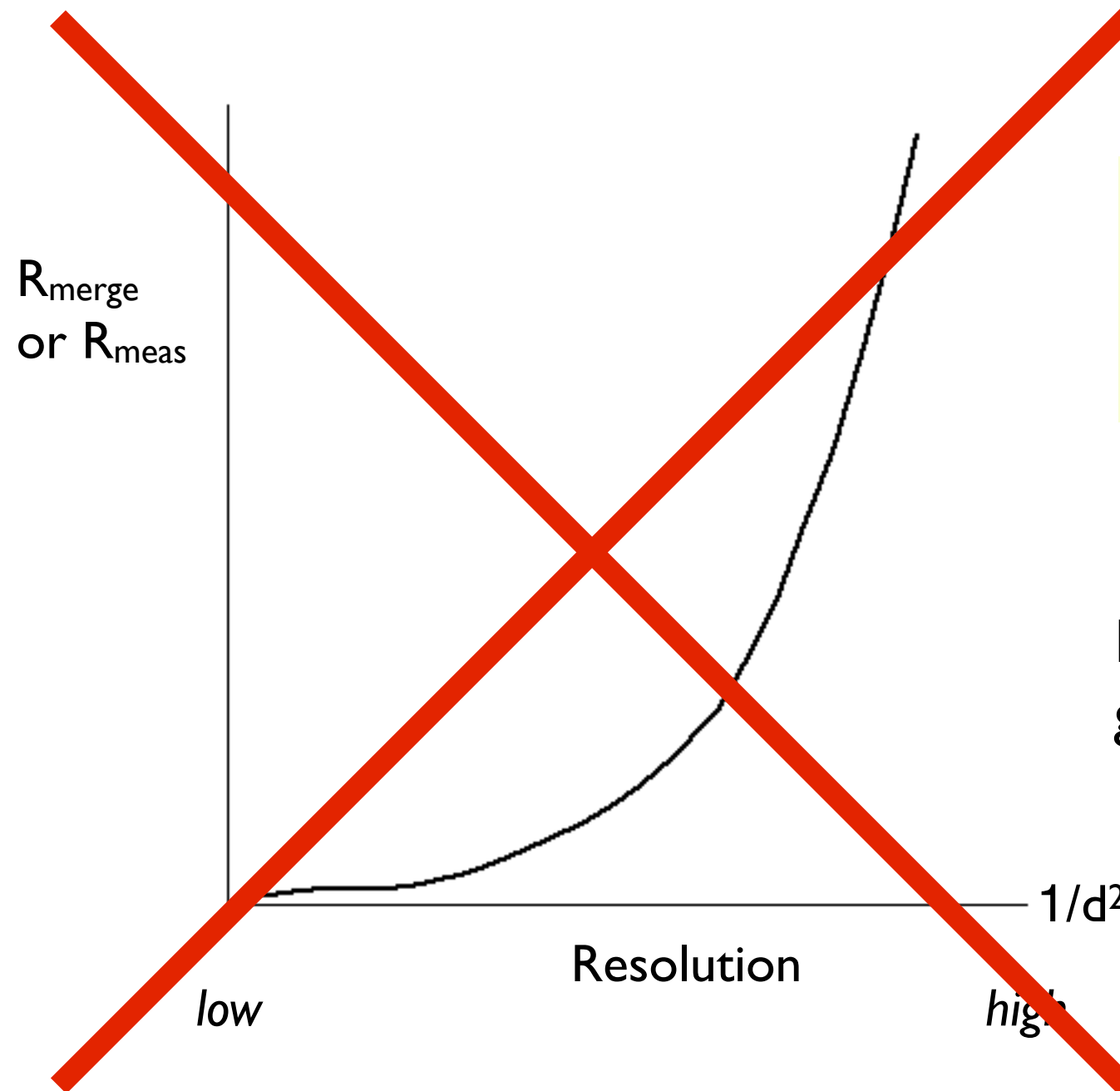
Resolution is a contentious issue, often with referees, eg:

“The crystallographic R_{merge} and R_{meas} values are not acceptable, although, surprisingly the I/σ and R -factors for these shells are OK. **I cannot ever recall seeing such high values of R_{merge} - 148% !** The discrepancy between the poor R_{merge} values and vs. the other statistics is highly unusual- generally a high R_{merge} corresponds a low I/σ -I would have expected a value closer to 1.0 than 2.0 here - and no explanation is offered. The authors may want to decrease the claimed resolution such that **acceptable** values are obtained for all metrics.”

“The crystallographic structure determination is reported to be at 2.7Å resolution. However, the data statistics presented in table S1 show $\langle I \rangle / \langle \sigma \rangle = 1.2$ in the highest resolution shell and thus represent marginally significant reliability. **Consequently, the resolution may be overstated.**”

What scores can we use?

What about R-factors?



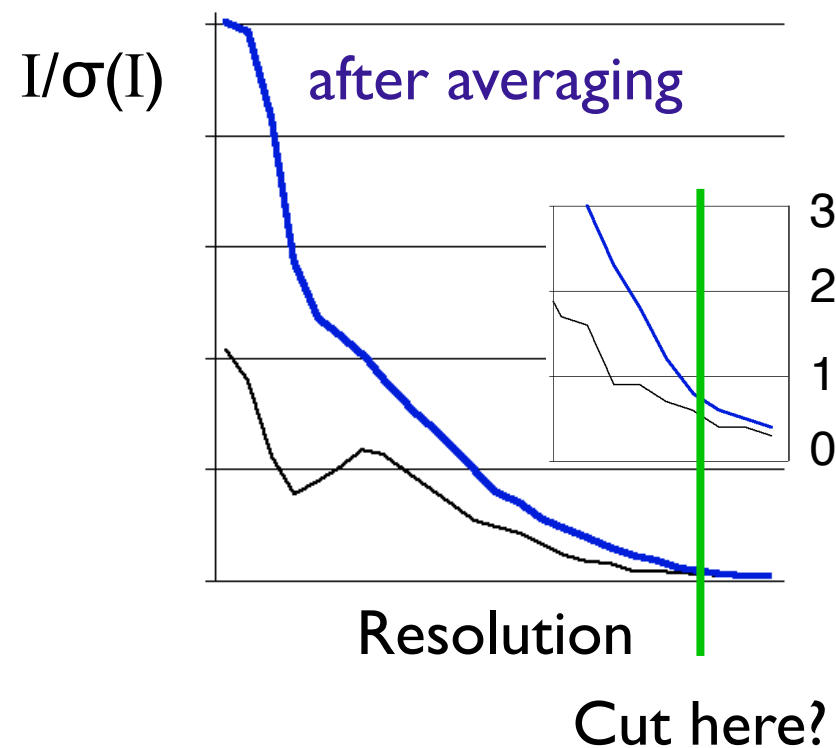
Note that R_{merge} and R_{meas} are useful for other purposes, but not for deciding the resolution cutoff

R_{merge} tends to infinity as data gets weaker

Where is the cut-off point?

Note that the crystallographic R-factor behaves quite differently: at higher resolution as the data become noisier, R_{cryst} tends to a constant value, not to infinity

1. $\langle I/\sigma(I) \rangle \approx \langle \text{signal/noise} \rangle$



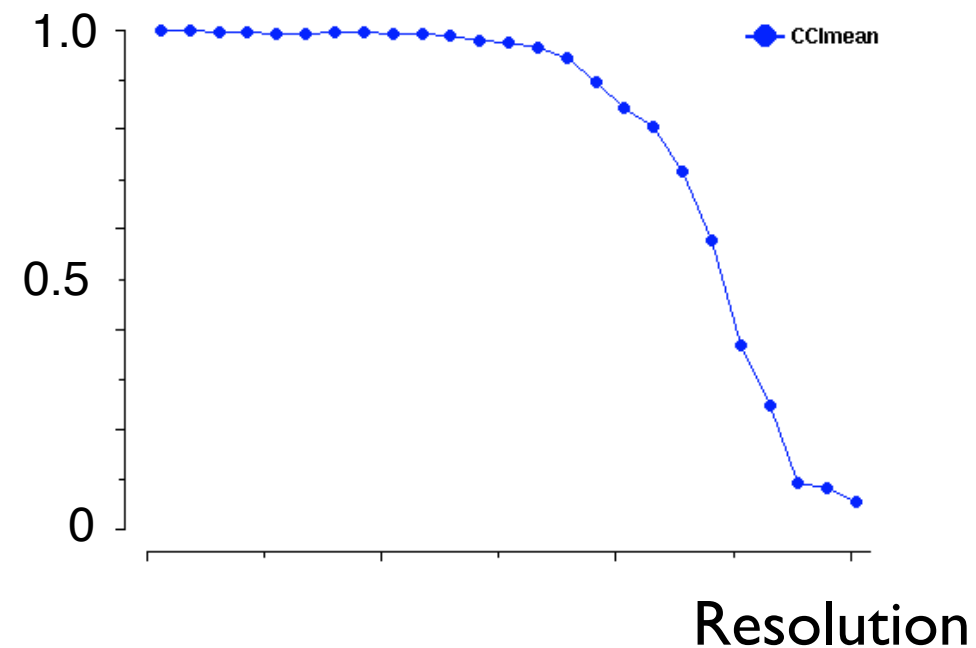
A reasonably good criterion, but it relies on $\sigma(I)$, which is not entirely reliable

Cut resolution at
 $\langle I/\sigma(I) \rangle$ after averaging
 $M_n(I/sd) = 1 - 2$

2. $CC_{1/2}$

Half-dataset correlation coefficient:

Split observations for each reflection randomly into 2 halves, and calculate the correlation coefficient between them (or equivalent calculation)



Advantages:

- Clear meaning to values (1.0 is perfect, 0 is no correlation), known statistical properties
- Independent of $\sigma(I)$

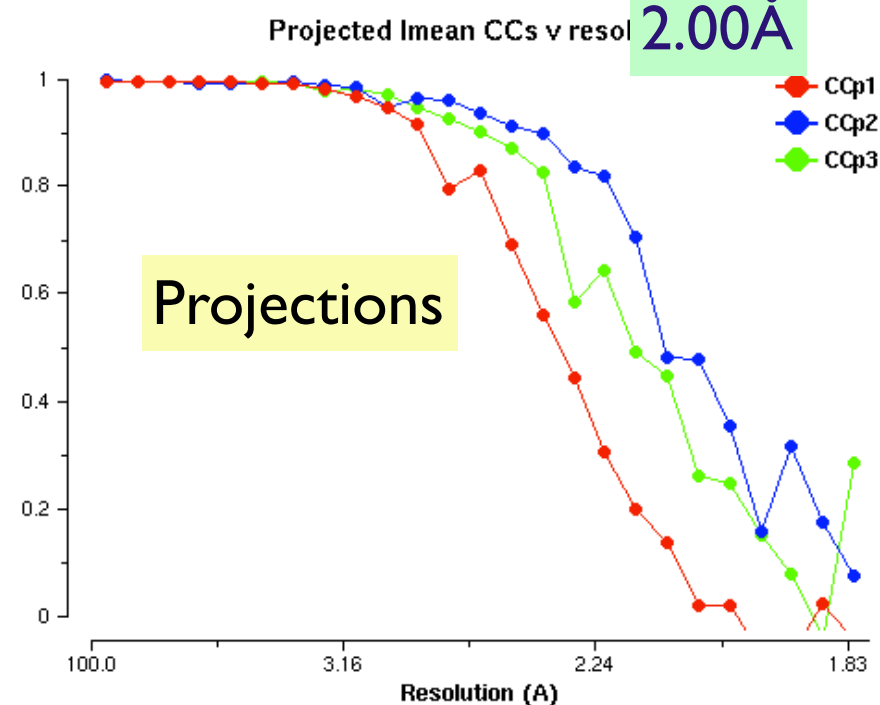
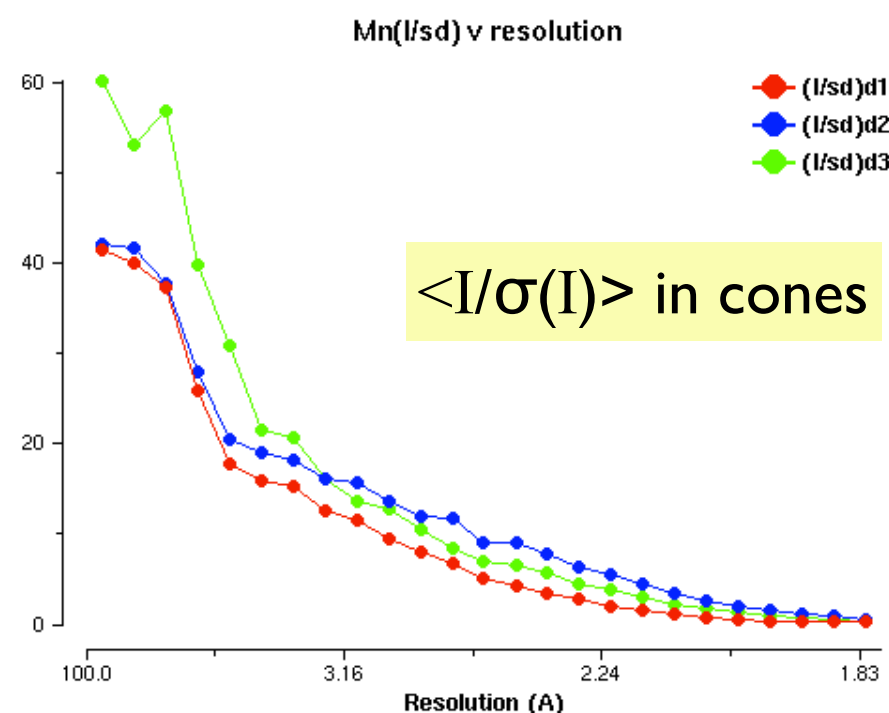
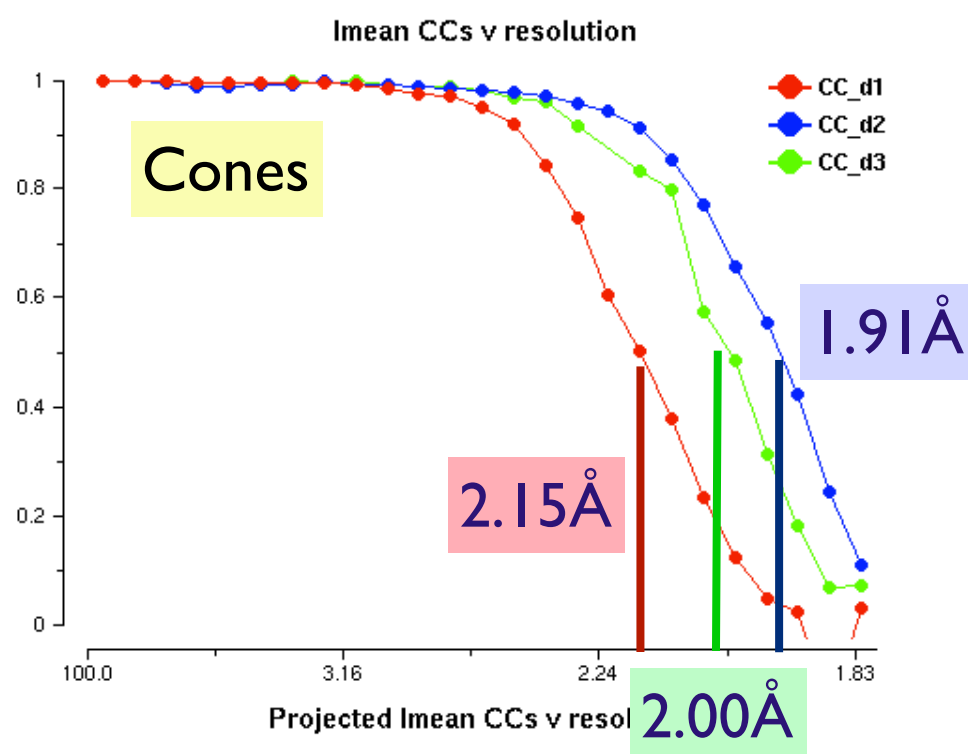
cut resolution at $CC \approx 0.3 - 0.5$

Anisotropy

Many (perhaps most) datasets are *anisotropic*

The principal directions of anisotropy are defined by symmetry (axes or planes), except in the monoclinic and triclinic systems, in which we can calculate the orthogonal principle directions

We can then analyse half-dataset CCs or $\langle I/\sigma(I) \rangle$ in cones around the principle axes, or as projections on to the axes



Anisotropic cutoffs are probably a Bad Thing, since it leads to strange series termination errors and problem with intensity statistics

So where should we cut the data?
Maybe at some compromise point

How should we decide the resolution of a dataset?

I don't know, but ...

Look at $CC1/2$, $\langle I/\sigma(I) \rangle$, and anisotropy

“Best” resolution is different for different purposes, so don't cut it too soon

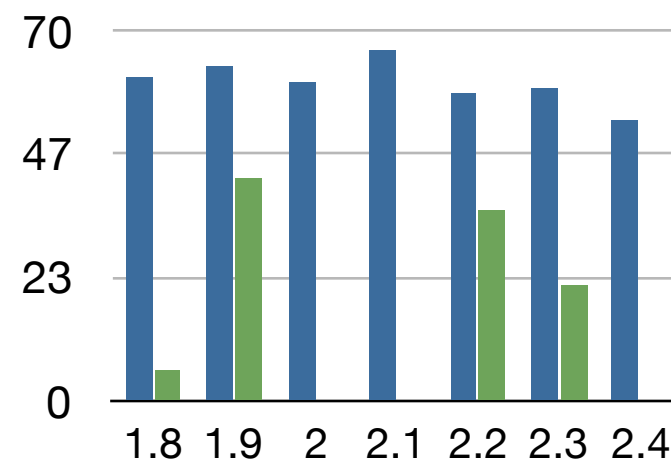
- Experimental phasing
 - substructure location is generally unweighted, so cut back conservatively to data with high signal/noise ratio
 - for phasing, use all “reasonable” data
- Molecular replacement: Phaser uses likelihood weighting, but there is probably no gain in using the very weak high resolution data
- Model building and refinement: if everything is perfectly weighted (perfect error models!), then extending the data should do no harm and may do good

There is no reason to suppose that cutting back the resolution to satisfy referees will improve your model!

Future developments may improve treatment of weak noisy data

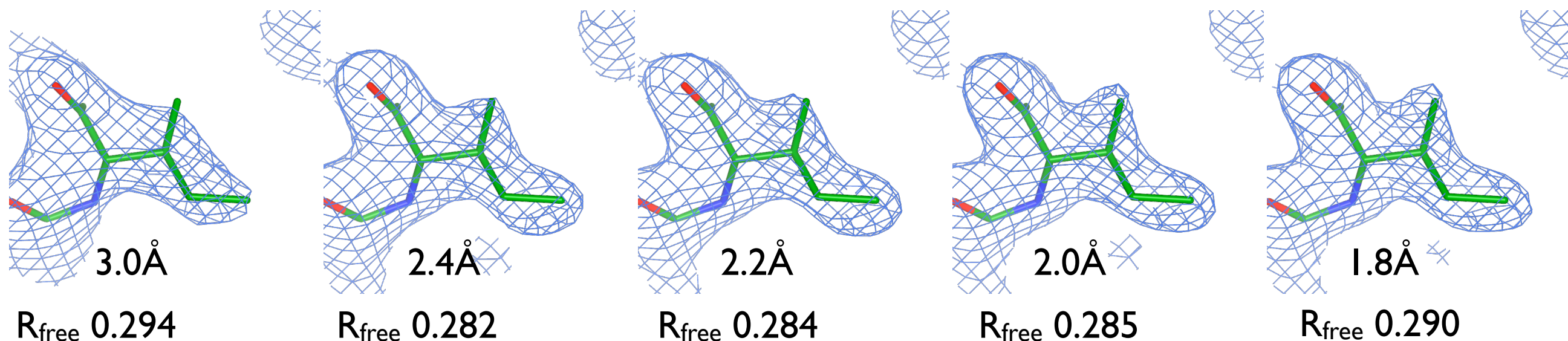
Example:

3 molecules/asu, omit 22/276 residues from each molecule, model build with Arp/warp at different resolutions

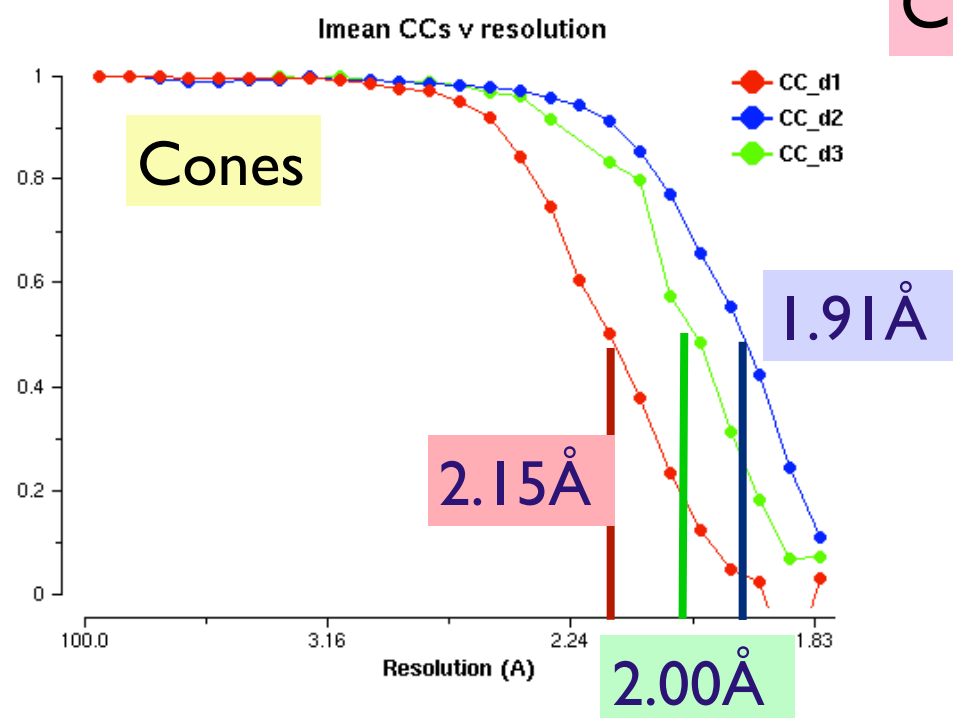


Number of residues **built** and **sequenced**

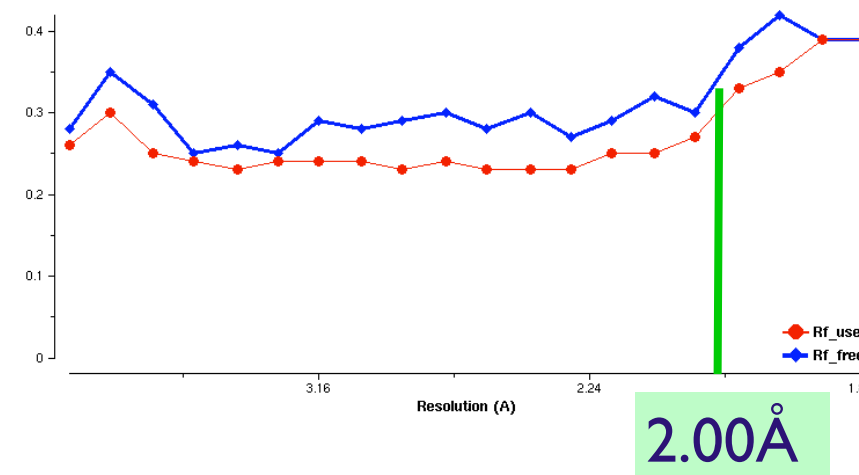
figures made with ccp4mg



Conclusion: there is not a huge difference



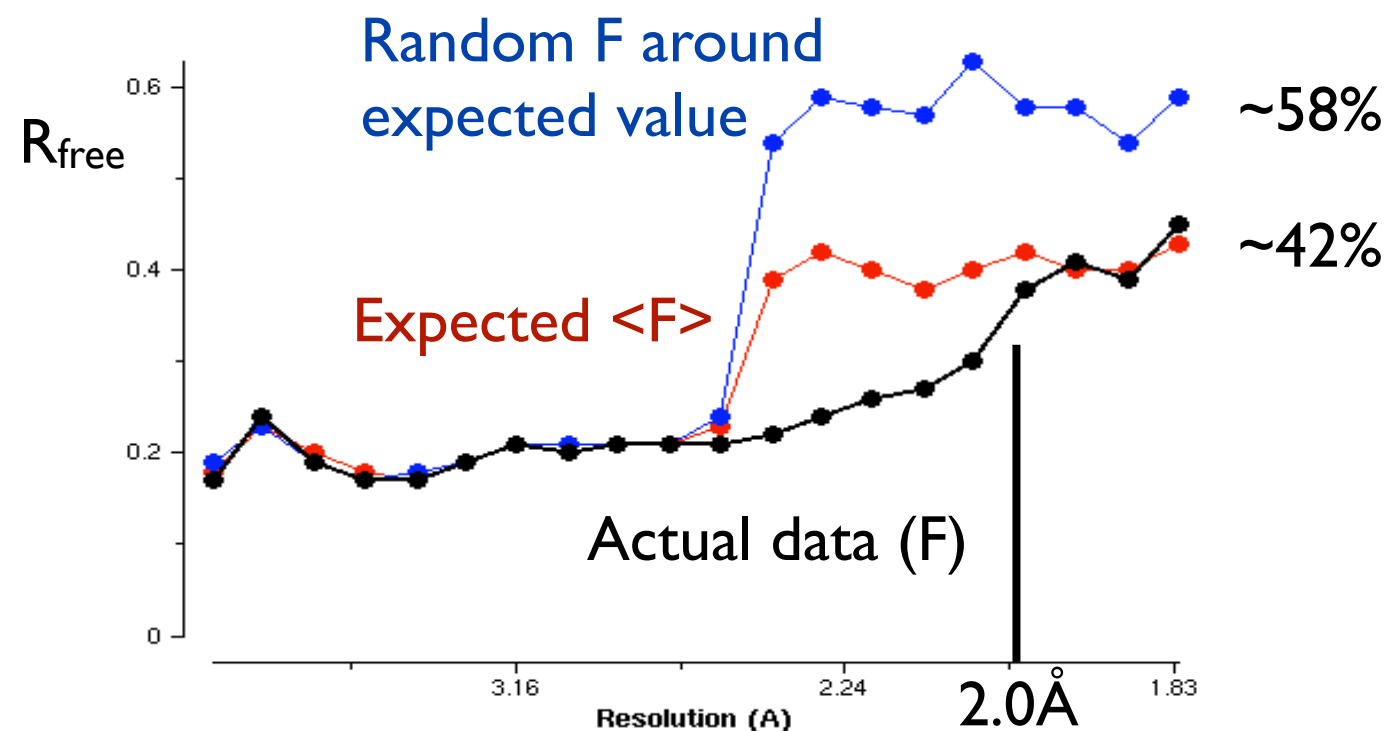
R & R_{free} after initial refinement



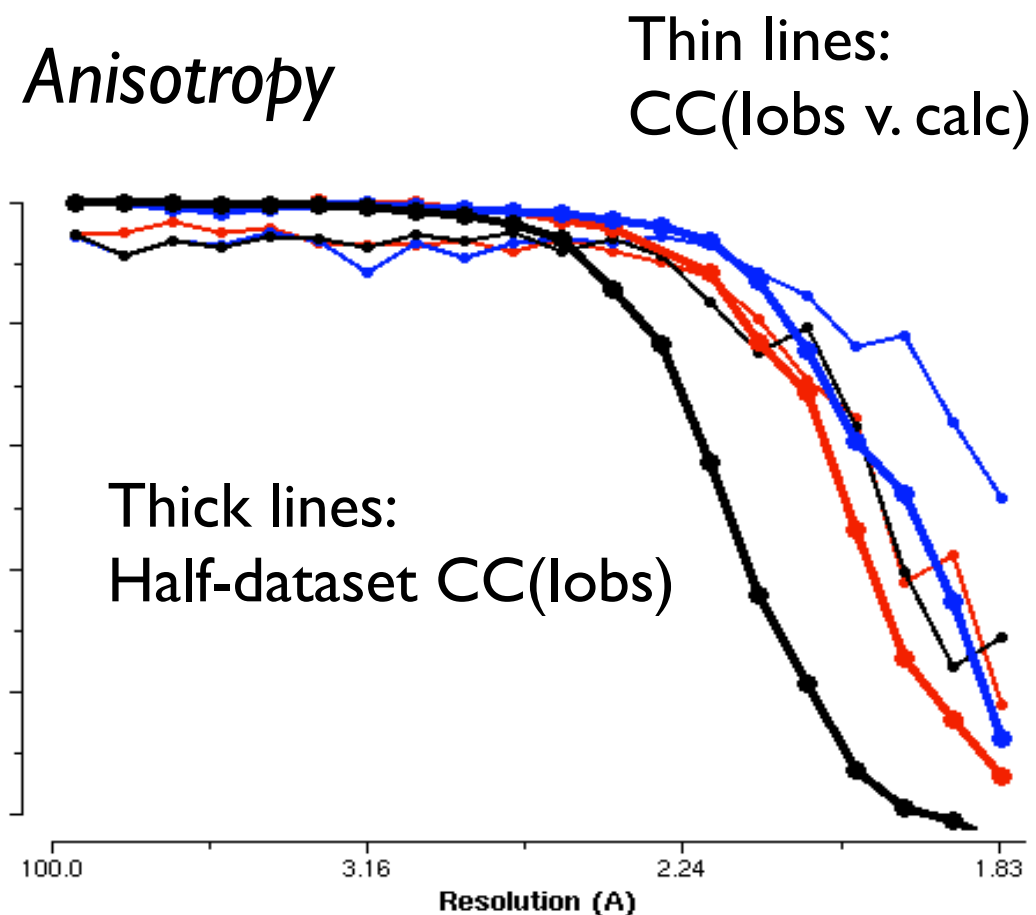
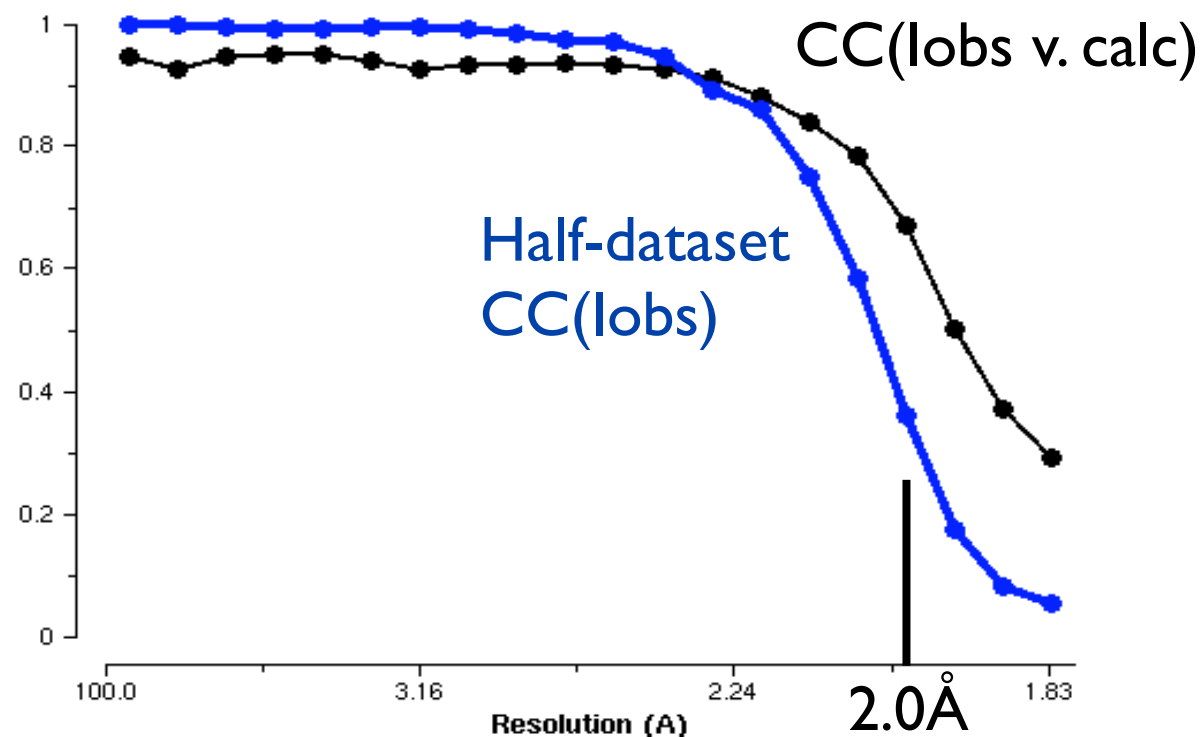
Example continued: refinement against real data or simulated data



thanks to Garib Murshudov



All these indicators are roughly consistent that a suitable resolution cutoff is around 2.0 Å, but that anything between 1.9 Å and 2.1 Å can be justified, **with current technologies**



Improved estimate of $\sigma(I)$

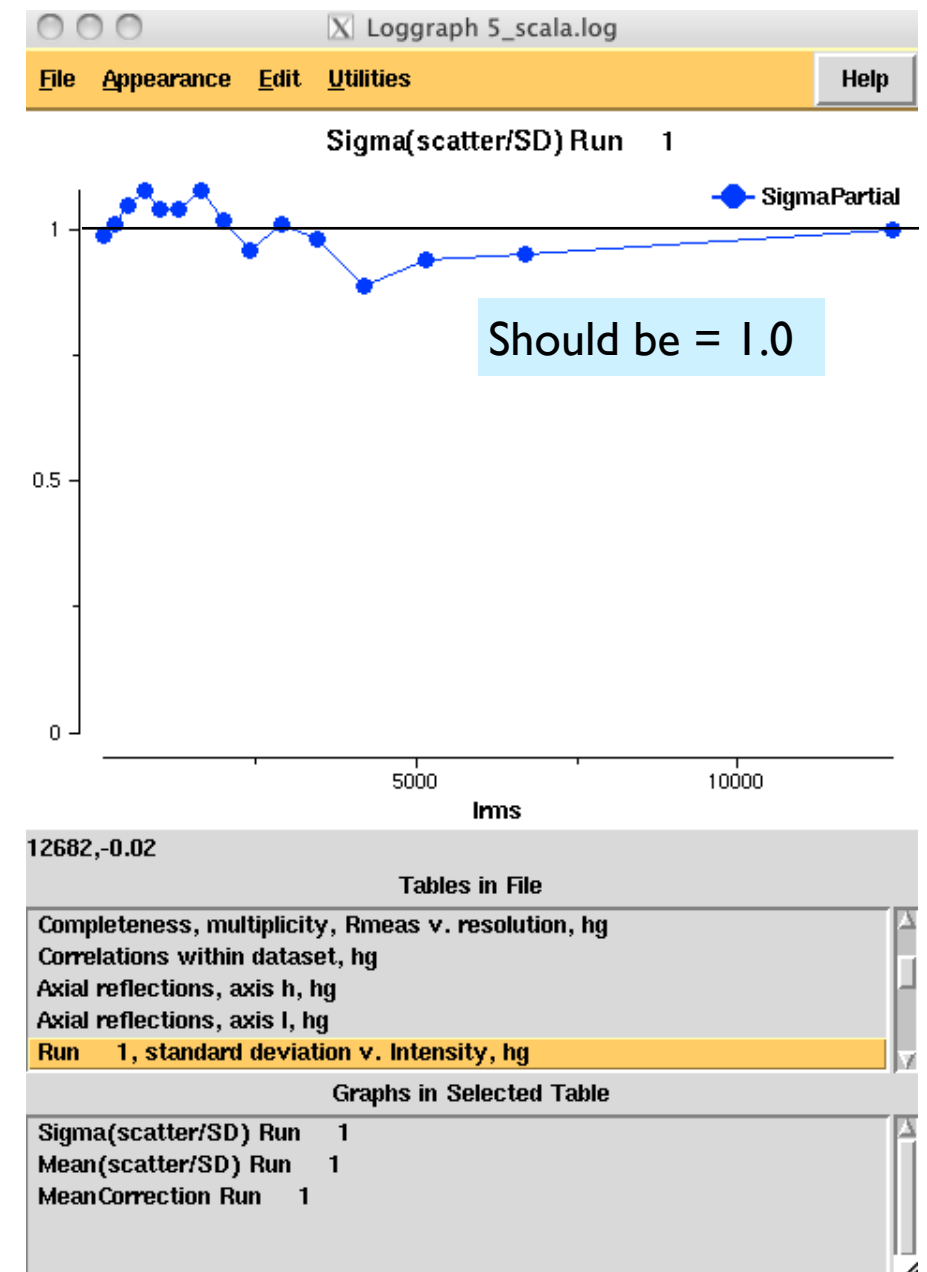
The error estimate $\sigma(I)$ from the integration program is too small particularly for large intensities. A “corrected” value may be estimated by increasing it for large intensities such that the mean scatter of scaled observations on average equals $\sigma'(I)$, in all intensity ranges

$$\text{Corrected } \sigma'(I)^2 = \text{SDfac}^2 [\sigma^2 + \text{SdB} \langle I_h \rangle + (\text{SdAdd} \langle I_h \rangle)^2]$$

SDfac, SdB and SdAdd are automatically adjusted parameters

$\text{Sigma}(\text{scatter}/\text{SD})$ and $\text{mean}(\chi^2)$ should ≈ 1.0

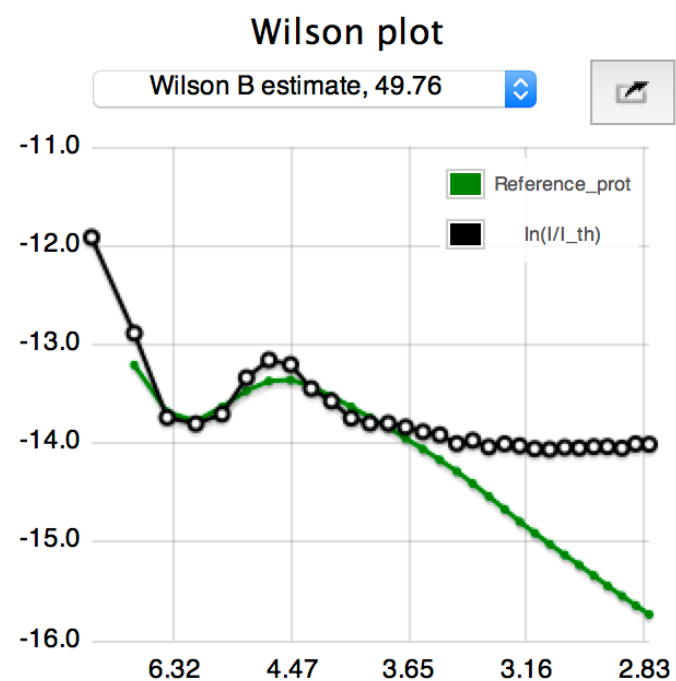
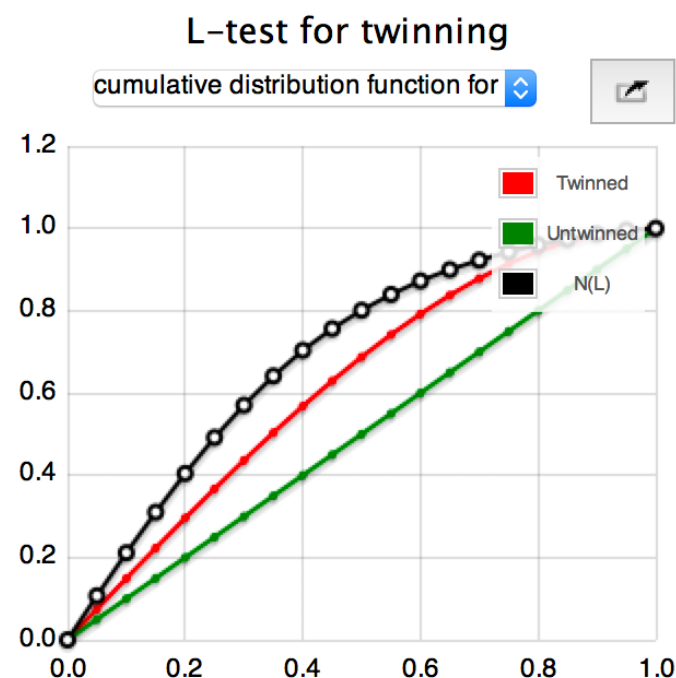
... but error estimation is difficult



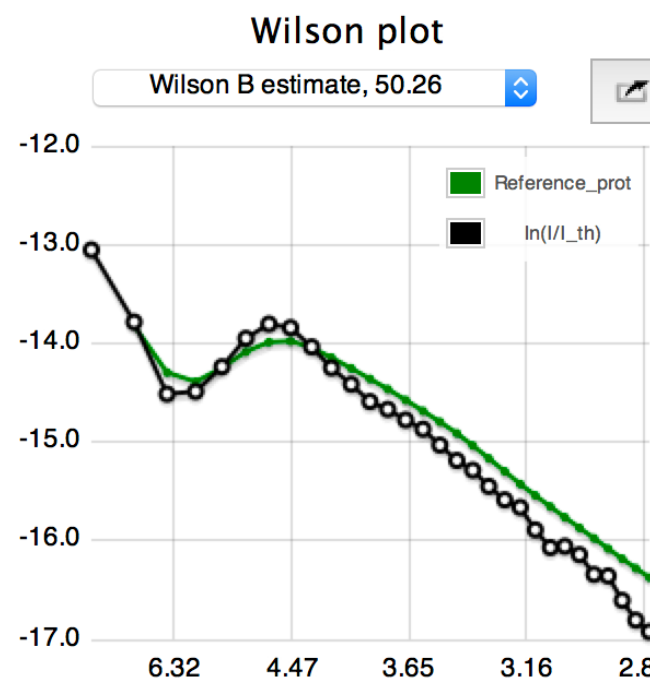
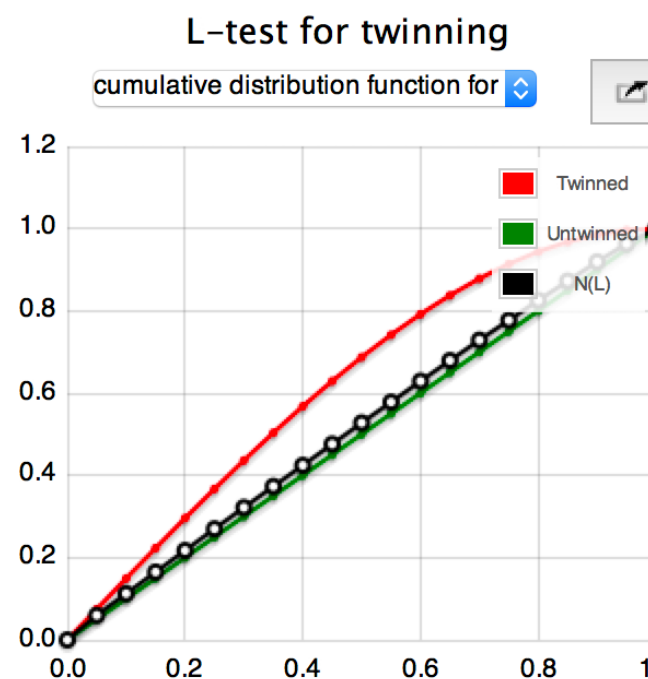
Wilson plot as a quality indicator

A dataset with tNCS (2x2x2): lots of weak data

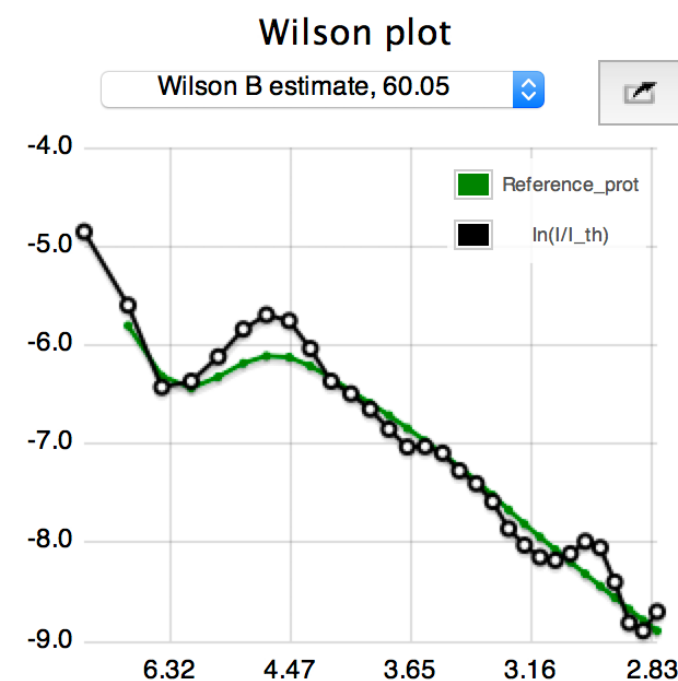
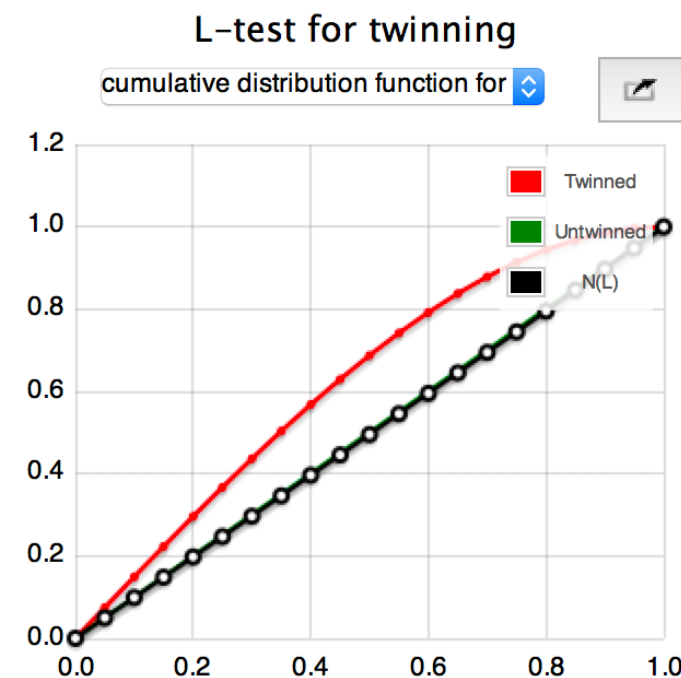
DIALS 9



DIALS 10



XDS



Outliers

Detection of outliers is easiest if the multiplicity is high

Removal of spots behind the backstop shadow does not work well at present: usually it rejects all the good ones, so tell integration program (eg Mosflm) where the backstop shadow is.

Reasons for outliers

- outside reliable area of detector (eg behind shadow)

specify backstop shadow, calibrate detector

- ice spots

do not get ice on your crystal!

- multiple lattices

find single crystal

- zingers

- bad prediction (spot not there)

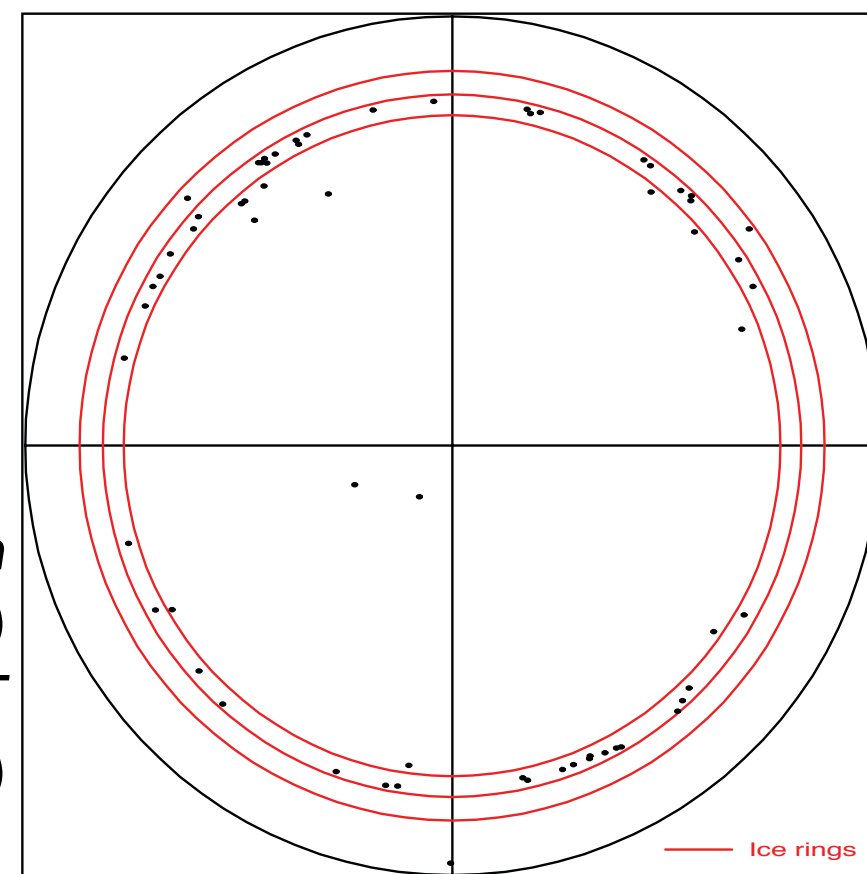
improve prediction

- spot overlap

lower mosaicity, smaller slice, move detector back

deconvolute overlaps

*Rejects lie on
ice rings (red)
(ROGUEPLOT
in Scala)*

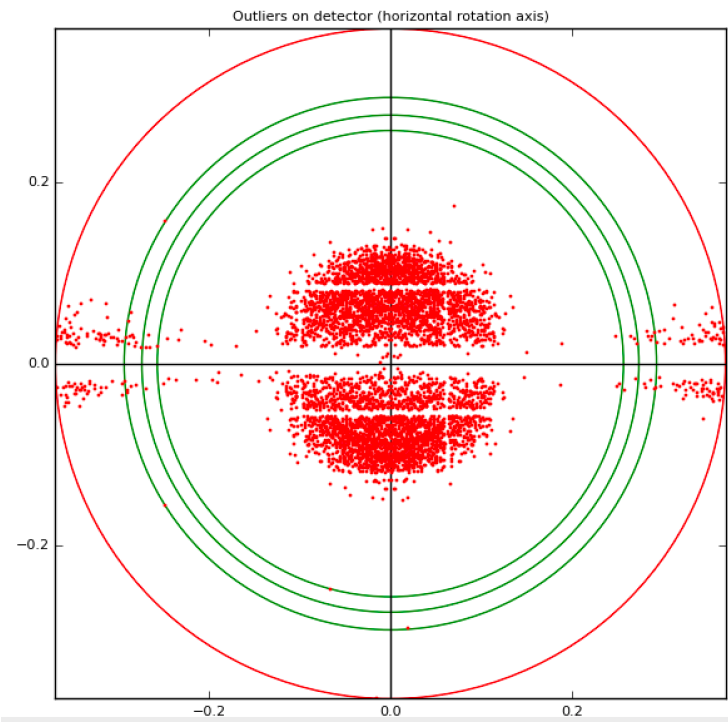
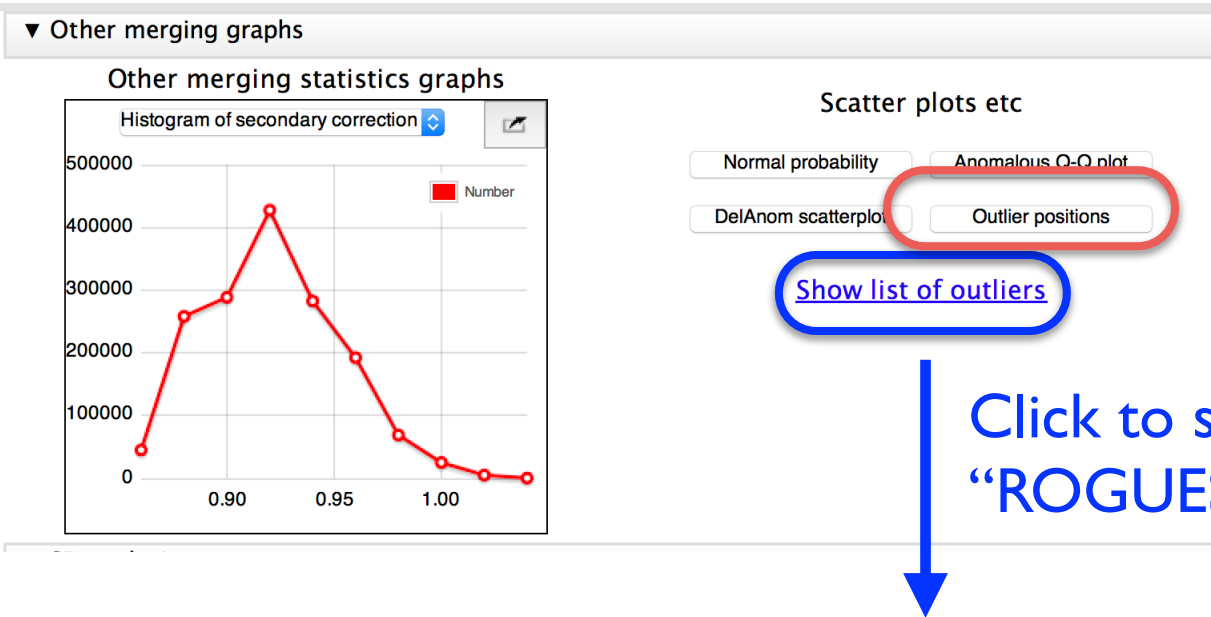


Position of rejects on detector

Two tests: Outliers in Aimless

- (1) deviations of observation from mean I for each reflection
- (2) too large to be reasonable, ie much larger than the average at that resolution

This is problematic with translation NCS, which messes up the statistics



The ROGUES file contains all monitored outliers, including unrejected outliers measured twice
Rej = '*' , '@' for I+- rejects, '#' for Emax rejects, 'x' for accepted flagged observation, 'd' for deviant but kept
Flag is taken from the Mosflm FLAG column or XDS MISFIT
TotFrc = total fraction, fulls (f) or partials (p), Bijv I+ or I- for Bijvoet classes
DelI/sd = (Ihl - Mn(I)others)/sqrt[sd(Ihl)**2 + sd(Mn(I))**2]

Flagged observations kept are labelled as: B BGratio; P PKratio; N TooNeg; G BGgradient; O Overload; E Edge; X XDS MISFIT
Deviant reflections with two measurements are always listed. Policy for deviant reflections measured twice: KEEP

h	k	l	h	k	l	Batch	I	sigI	E	TotFrc	Bijv	Scale	DelI/sd	d(A)	Xdet	Ydet	Phi	LP	Rej	Flag
(measured)			(unique)																	
0	0	6	0	0	6	1023	107	10	19.57	0.0f	I+	1.146	0.0	49.61	1252.8	1169.5	214.5	0.0157	#	
0	0	-6	0	0	6	123	97	11	18.62	0.0f	I-	1.173	0.0	49.61	1326.1	1169.5	34.6	0.0157	#	
Weighted mean, sd							0	0	I+	0	0	I-	0	0	0	0				
0	0	8	0	0	8	115	0	0	-0.67	0.0f	I+	1.175	2.8	37.21	1241.5	1281.7	33.0	0.0210		
0	0	8	0	0	8	1024	0	0	-0.83	0.0f	I+	1.147	-0.7	37.21	1240.4	1154.1	214.7	0.0209		
0	0	-8	0	0	8	125	0	0	-0.89	0.0f	I-	1.173	-0.8	37.21	1338.4	1153.0	34.8	0.0210		
0	0	-8	0	0	8	1015	0	0	0.20	0.0f	I-	1.144	0.0	37.21	1338.8	1281.2	212.8	0.0209	*	
Weighted mean, sd							0	0	I+	0	0	I-	0	0	0	0				
0	0	12	0	0	12	113	7324	506	179.86	0.0f	I+	1.175	0.0	24.81	1216.9	1313.5	32.5	0.0314	#	
0	0	12	0	0	12	1027	6336	493	167.28	0.0f	I+	1.149	0.0	24.81	1216.4	1122.0	215.2	0.0314	#	
0	0	-12	0	0	12	127	7209	505	178.44	0.0f	I-	1.173	0.0	24.81	1362.5	1121.1	35.3	0.0314	#	
0	0	-12	0	0	12	1012	6774	492	172.98	0.0f	I-	1.143	0.0	24.81	1363.3	1313.1	212.4	0.0314	#	
Weighted mean, sd							0	0	I+	0	0	I-	0	0	0	0				

All observations of reflection rejected

▼ Details of merging

Resolution estimates

Estimates of limits from CC(1/2) (threshold 0.30)
and Mn(I/sd) (threshold 1.50)

	CC(1/2)	Mn(I/sd)
Overall	2.78*	2.91
- In h k plane	2.78*	2.98
- Along l axis	2.78*	2.84

estimates extending to the maximum resolution marked '*'

Dataset: CMuNathan/2309-08/2309-08

Unit cell: a,b,c	136.136	136.136	297.690
alpha,beta,gamma	90.000	90.000	120.000
Mosaicity	0.00		
Number of reflections	80974		
Number of observations	1595511		
Number of rejected outliers	277		
Number of Emax rejects	4939		

If you get too many rejects, then switch off the test

Input Data

Important Options

Additional Options

Options for symmetry determination in Pointless: -----

Choice of cell setting conventions:
Default: set primitive orthorhombic groups in order (a<b<c) & allow monoclinic I2 setting of C2

Options for scaling in Aimless: -----

Scale No scaling only merge

☒ override default parameters for outlier rejection

Reject outliers if > 6.0 from mean, or > 6.0 if 2 observations

Reject outliers between I+ and I- if > 8.0 , ☒ increase for large anomalous differences

☐ compare outliers within each datasets

Set maximum E to reject unreasonably large intensities 0

This is essentially a
bug in the current
version of Aimless

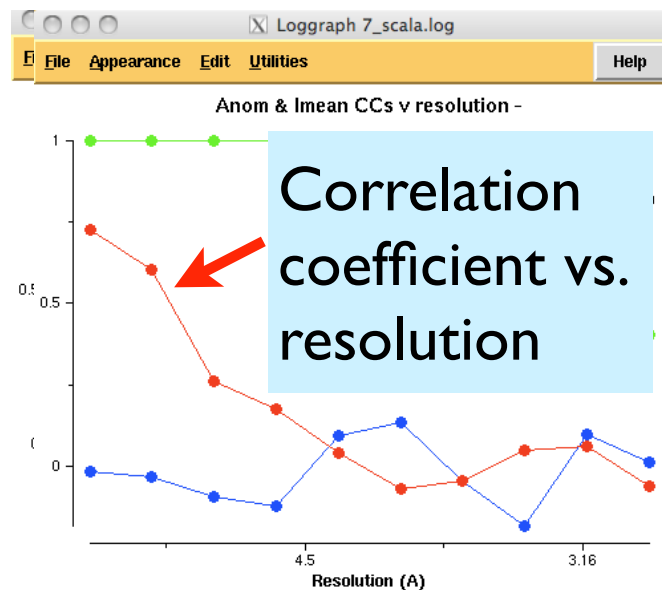


It will be improved!

Set limit to 0 to disable

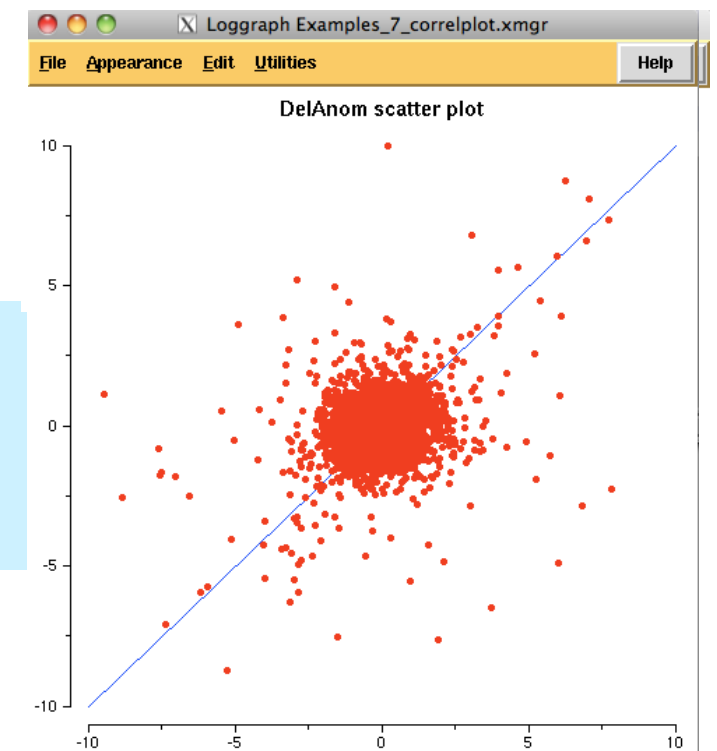
Detecting anomalous signals

The data contains both I^+ (hkl) and I^- (-h-k-l) observations and we can detect whether there is a significant difference between them.

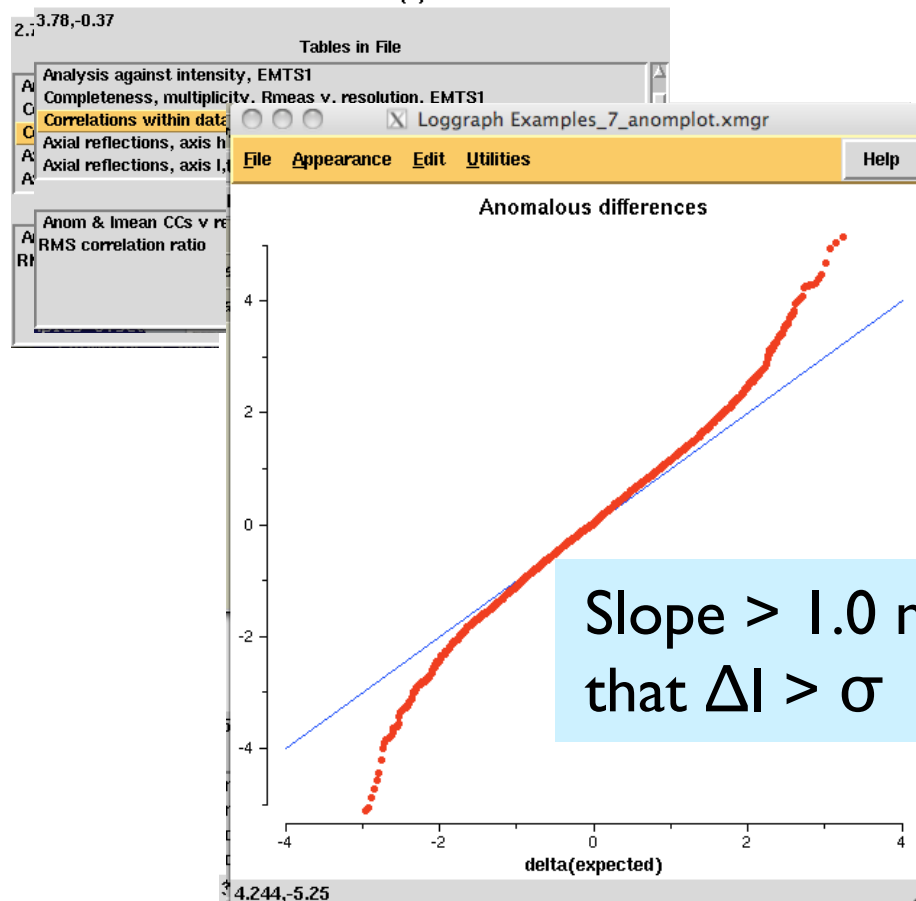


Split one dataset randomly into two halves, calculate correlation between the two halves or compare different wavelengths (MAD)

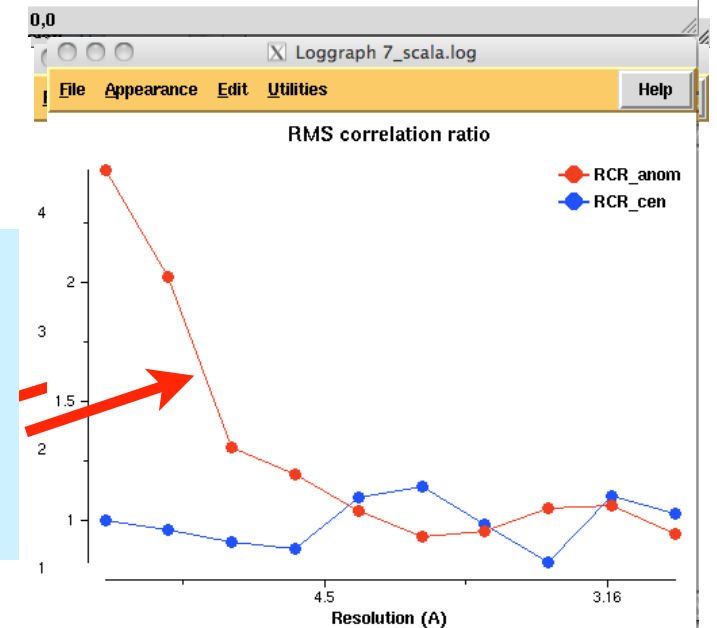
Plot ΔI_1 against ΔI_2 should be elongated along diagonal



Weak but useful anomalous signal



Ratio of width of distribution along diagonal to width across diagonal



“RMS correlation ratio”

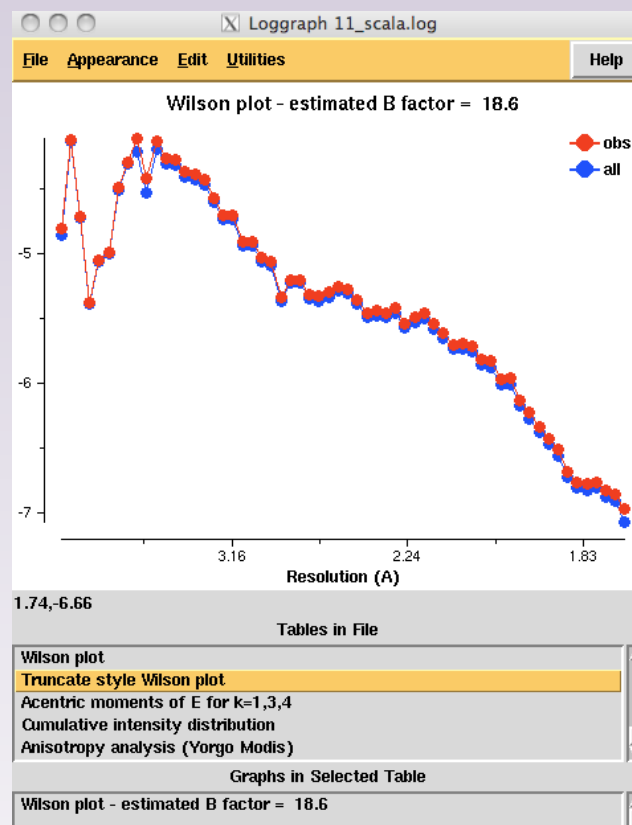
Intensity statistics

We need to look at the distribution of intensities to detect twinning

Assuming atoms are randomly placed in the unit cell, then

$$\langle I \rangle(s) = \langle F F^* \rangle(s) = \sum_j g(j, s)^2$$

where $g(j, s)$ is the scattering from atom j at $s = \sin\theta/\lambda$



Average intensity falls off with resolution, mainly because of atomic motions (B-factors)

For the purposes of looking for crystal pathologies, we are not interested in the variation with resolution, so we can use “normalised” intensities which are independent of resolution

$$\langle I \rangle(s) = C \exp(-2 B s^2)$$

Wilson plot: $\log(\langle I \rangle(s))$ vs s^2

This would be a straight line if all the atoms had the same B-factor

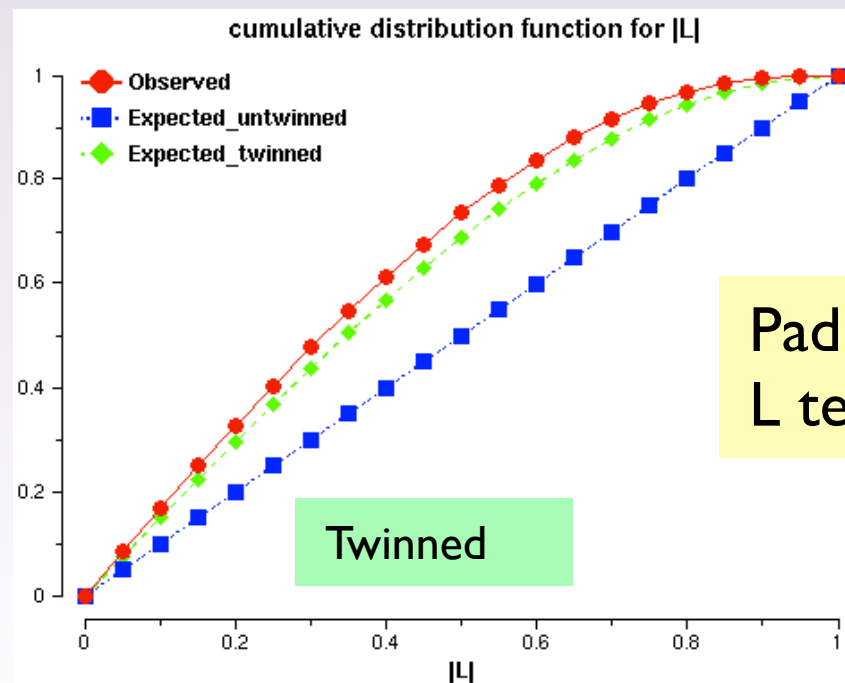
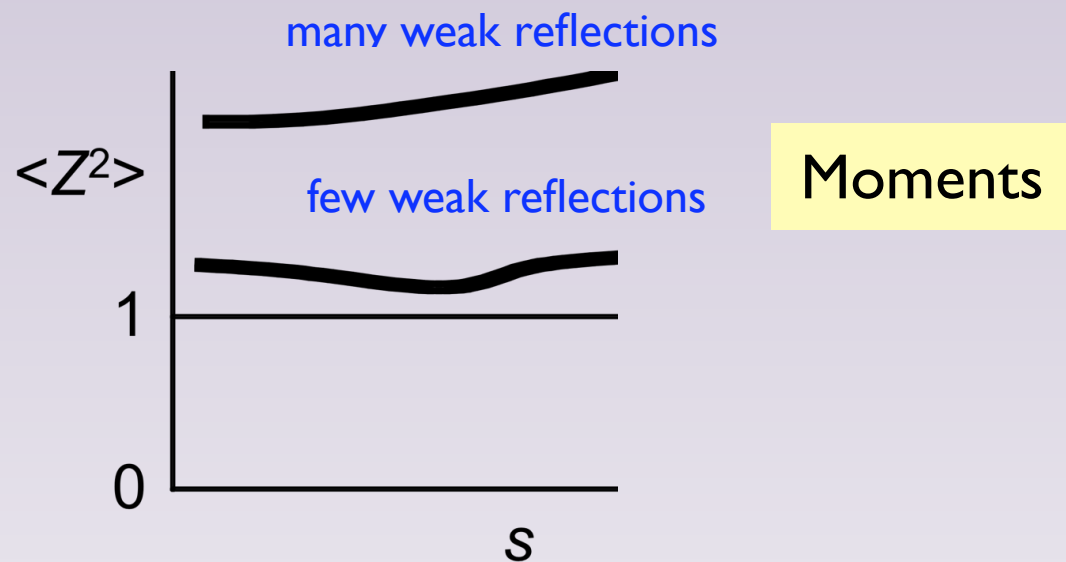
Normalised intensities: relative to average intensity at that resolution

$$Z(h) = I(h)/\langle I(s) \rangle \approx |E|^2$$

$$\langle Z(s) \rangle = 1.0 \text{ by definition}$$

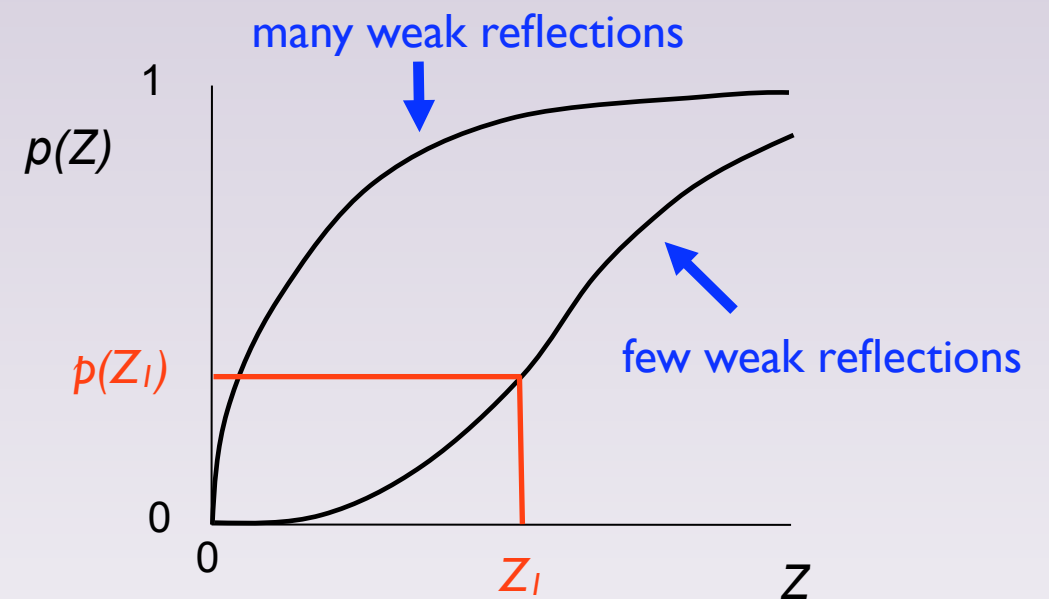
$$\langle Z^2(s) \rangle > 1.0 \text{ depending on the distribution}$$

$\langle Z^2(s) \rangle$ is larger if the distribution of intensities is wider: it is the 2nd moment
ie the *variance* (this is the 4th moment of E)



Padilla – Yeates
L test

Cumulative distribution of Z : $p(Z)$ vs. Z



$p(Z_I)$ is the proportion of
reflections with $Z < Z_I$

Other features of the intensity distribution which may obscure or mimic twinning

Translational non-crystallographic symmetry:

whole classes of reflections may be weak

eg h odd with a NCS translation of $\sim 1/2, 0, 0$

$\langle I \rangle$ over all reflections is misleading, so Z values are inappropriate

The reflection classes should be separated (not yet done)

Anisotropy: $\langle I \rangle$ is misleading so Z values are wrong

ctruncate applies an anisotropic scaling before analysis

Weak data: the ideal statistics are based on perfect data.

If the signal/noise ratio is small, then the statistics may falsely suggest twinning

Overlapping spots: a strong reflection can inflate the value of a weak neighbour, leading to too few weak reflections

this mimics the effect of twinning

Estimation of amplitude $|F|$ from intensity I

If we knew the true intensity J then we could just take the square root

$$|F| = \sqrt{J}$$

But measured intensities I have an error $\sigma(I)$ so a small intensity may be measured as negative.

The “best” estimate of $|F|$ larger than $\sqrt{I}$ for small intensities ($< \sim 3 \sigma(I)$) to allow for the fact that we know that $|F|$ must be positive

[c]truncate estimates $|F|$ from I and $\sigma(I)$ using the average intensity in the same resolution range: this give the prior probability $p(J)$

$$E(F ; I, \sigma(I)) = \int_0^{\infty} F p(I ; J, \sigma(I)) p(J) dJ$$

French & Wilson 1978

BUT best to use intensities I rather than amplitude F wherever possible

Summary: Questions & Decisions

- *Do look critically at the data processing statistics*
 - What is the point group (Laue group)?
 - What is the space group?
 - Was the crystal dead at the end?
 - Is the dataset complete?
 - Do you want to cut back the resolution?
 - Is this the best dataset so far for this project?
 - Should you merge data from multiple crystals?
 - Is there anomalous signal (if you expect one)?
 - Are the data twinned?

Try alternative processing strategies: different choices of cutoffs, merging crystals, etc

test with MR (log-likelihood gain) or refinement (R_{free} , map quality)

Data processing is not necessarily something you just do once

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