The *xia2* manual

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Contents

1 Background

Users of macromolecular crystallography (MX) are well served in terms of data reduction software, with packages such as HKL2000, Mosflm[1], XDS[2] and d*TREK generally available and commonly used. In the main, however, these programs require tha the user makes sensible decisions about the data analysis to ensure that a useful result is reached. This manual describes a package, *xia2*, which makes use of some of the aforementioned software to reduce diffraction data automatically from images to scaled intensities and structure factor amplitudes, with no user input.

In 2005, when the *xia2* project was initiated as part of the UK BBSRC e-Science project e-HTPX, multi-core machines were just becoming common, detectors were getting faster and synchrotron beamlines were becoming brighter. Against this background the downstream analysis (e.g. structure solution and refinement) was streamlined and the level of expertise needed to use MX as a technique was reducing. At the same time mature software packages such as Mosflm, Scala[3], CCP4[4] and XDS were available and a new synchrotron facility was being built in the UK. The ground was therefore fertile for for the development of automated data reduction tools. Most crucially, however, the author was told that this was impossible and a waste of time - sufficient motivation for anyone.

2 Acknowledgements

Without the trusted and capable packes Mosflm, CCP4, Scala and XDS it would clearly be impossible to develop *xia2*. The author would therefore like to thank Andrew Leslie, Harry Powell, Phil Evans, Wolfgang Kabsch and Kay Diederichs for their assistance in using their programs and modifications

[1] A.G.W. Leslie, Acta Cryst. (2006) D62, 48-57
[2] W. Kabsch, Acta Cryst. (2010) D66, 125-132
[3] P. Evans, Acta Cryst. (2006) D62, 72-82
[4] CCP4, Acta Cryst. (1994) D50, 760-763

they have made. In addition, more recent developments such as Labelit [5], Pointless[6] and CCTBX[7] have made the development of *xia2* much more straightforward and the end product. The author would therefore like to additionally thank Nick Sauter and Ralf Grosee-Kunstleve for their help.

Development of a package such as this is impossible without test data, for which the author would like to thank numerous users, particularly the Joint Centre for Structural Genomics, for publishing the majority of their raw diffraction data.

During the course of *xia2* development the project has been supported by the UK BBSRC through the e-HTPX project, the EU Framework 6 through the BioXHit project and most recently by Diamond Light Source. The software itself is open source, distributed under a BSD licence, but relies on the user having correctly configured and licenced the necessary data analysis software, the details of which will be discussed shortly.

3 Introduction

In a nutshell, *xia2* is an expert system to perform X-ray diffraction data processing on *your* behalf, using *your* software with little or no input from *you*. It will correctly handle multi-pass, multi-wavelength data sets as described later but crucially it is not a data processing package. Specifically, if you use *xia2* in published work please include the references for the programs it has used, which are printed at the end of the output.

The system was initially written to support remote access to synchrotron facilities, however it may prove useful to anyone using MX, for example:

- assisting new or novice users,
- giving a second opinion to experts,
- assisting busy users to allow them to focus on problem cases, or
- providing reproducible processing.

The last of these may be most useful for users in a pharmacutical setting, or people wishing to test or benchmark equipment, for example beamline scientists. In all cases however the usage of the program is the same.

[5] N.K. Sauter et al. J. Appl. Cryst. (2004) 37, 399-409
[6] P. Evans, Acta Cryst. (2006) D62, 72-82
[7] R.W. Grosse-Kunstleve et al. J. Appl. Cryst. (2002) 35, 126-136

4 Using xia2

xia2 -2d /here/are/my/data

- or -

xia2 -3d /here/are/my/data

The program is used from the command-line, there is no GUI. In essence there are four command-line options which are useful on a daily basis:

- -atom X - tell xia2 to separate anomalous pairs i.e. $I(+) \neq I(-)$ in scaling
- -2d - tell xia2 to use MOSFLM and SCALA
- -3d - tell xia2 to use XDS and XSCALE
- -3dii - tell xia2 to use XDS and XSCALE, indexing with peaks found from all images

These specify in the broadest possible terms to the program the manner in which you would like the processing performed. The program will then read all of the image headers found in `/here/are/my/data` to organise the data, first into sweeps, then into wavelengths, before assigning all of these wavelengths to a crystal.

[FIXME FIGURE]

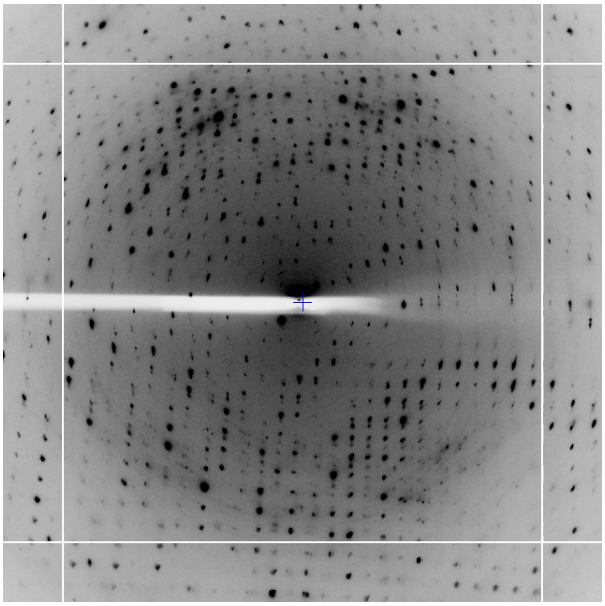
The data from the experiment is understood as follows. The SWEEP, which corresponds to one "scan," is the basic unit of indexing and integration. These are contained by WAVELENGTH objects which correspond to CCP4 MTZ datasets, and will ultimately have unique Miller indices. For example, a low and high dose pass will be merged together. A CRYSTAL however contains all of the data from the experiment and is the basic unit of data for scaling.

5 Introductory example

The most straightforward way to discuss the operation of the program is through demonstrations with real examples. The first of these is a DNA / ligand complex recorded at Diamond Light Source as part of ongoing research. The structure includes barium which may be used for phasing, and the data were recorded as a single sweep. As may be seen from [FIXME FIGURE] the quality of diffraction was not ideal, and radiation damage was an issue. Initially the data were processed with

```
BEGIN PROJECT AUTOMATIC
BEGIN CRYSTAL DEFAULT

BEGIN HA_INFO
ATOM Ba
END HA_INFO

BEGIN WAVELENGTH SAD
WAVELENGTH 0.979500
END WAVELENGTH SAD

BEGIN SWEEP SWEEP1
WAVELENGTH SAD
DIRECTORY /dls/i02/data/2011/mx1234-5
IMAGE K5_M1S3_3_001.img
START_END 1 450
END SWEEP SWEEP1

END CRYSTAL DEFAULT
END PROJECT AUTOMATIC
```

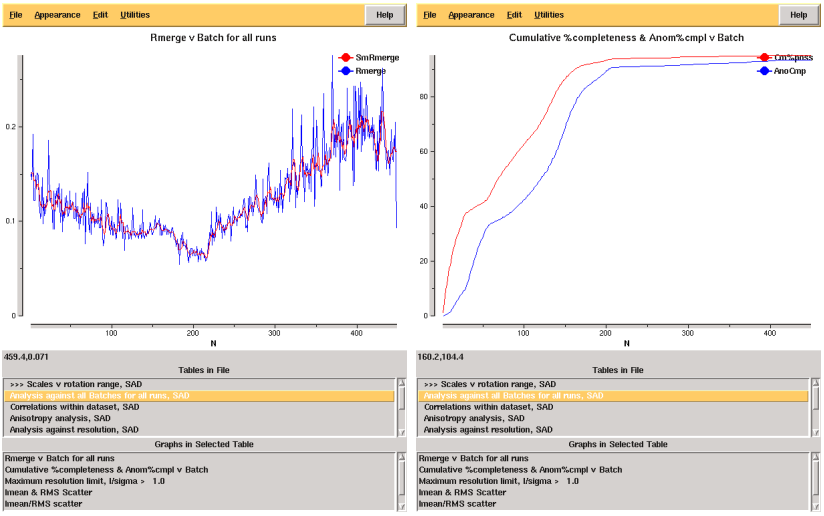
Figure 1: The input file to the program, which is generated automatically, shows how the input data are understood. This may be adjusted and the program rerun, which will be covered in more detail later in the manual.



```
xia2 -3d -atom Ba /here/are/my/data
```

giving the merging statistics shown in [FIXME TABLE]. From these it is clear that there is something wrong: it is very unusual to have near atomic resolution diffraction with $\sim$ 10% R_{merge} in the low resolution bin. The most likely reasons are incorrect assignment of the pointgroup and radiation damage - the latter of which is clear from the analysis of R_{merge} as a function of image number [FIXME FIGURE.] A development option is now available (-3da rather than -3d) which will run Aimless in the place of Scala for merging, and which gives the cumulative completeness as a function of frame number, as shown in FIXME FIGURE. From this it is clear that the data were essentially complete after approximately 200 frames.

High resolution limit	1.25	6.45	1.25
Low resolution limit	18.85	18.85	1.27
Completeness	95.2	60.1	70.2
Multiplicity	12.2	8.4	4.8
I/sigma	12.3	18.5	2.6
Rmerge	0.113	0.096	0.564
Rmeas(I)	0.129	0.118	0.633
Rmeas(I+/-)	0.121	0.105	0.679
Rpim(I)	0.034	0.038	0.267
Rpim(I+/-)	0.043	0.041	0.368
Wilson B factor	12.131		
Anomalous completeness	93.3	52.6	58.0
Anomalous multiplicity	6.4	5.0	2.0
Anomalous correlation	0.544	0.791	-0.297
Anomalous slope	1.085	0.000	0.000
Total observations	118588	529	1634
Total unique	9749	63	337



5.1 Modifying input

From the example it would seem sensible to investigate processing only the first 200 of the 450 images. While it is usual to limit the batch range in scaling when processing the data manually, *xia2* is not set up to work like this as decisions made for the full data set (e.g. scaling model to use) may differ from those for the subset - we therefore need to rerun the whole *xia2* job after modifying the input. All that is necessary is to adjust the image range (START_END) to get the modified input file shown in FIXME FIGURE and rerun as

```
xia2 -3d -xinfo modified.xinfo
```

giving the results shown in FIXME TABLE. These are clearly much more internally consistent and give nice results from experimental phasing. At the same time we may wish to adjust the resolution limits to give more complete data in the outer shell, which may be achieved by adding a RESOLUTION instruction to either the SWEEP or WAVELENGTH block.

```
BEGIN PROJECT AUTOMATIC
BEGIN CRYSTAL DEFAULT

BEGIN HA_INFO
ATOM Ba
END HA_INFO

BEGIN WAVELENGTH SAD
WAVELENGTH 0.979500
END WAVELENGTH SAD

BEGIN SWEEP SWEEP1
WAVELENGTH SAD
DIRECTORY /dls/i02/data/2011/mx1234-5
IMAGE K5_M1S3_3_001.img
START_END 1 200 ! THIS WAS 450
END SWEEP SWEEP1

END CRYSTAL DEFAULT
END PROJECT AUTOMATIC
```

High resolution limit	1.22	6.34	1.22
Low resolution limit	19.62	19.62	1.24
Completeness	86.9	49.1	37.8
Multiplicity	5.3	4.9	1.7
I/sigma	20.1	37.0	2.3
Rmerge	0.036	0.020	0.355
Rmeas(I)	0.060	0.038	0.448
Rmeas(I+/-)	0.043	0.023	0.491
Rpim(I)	0.023	0.014	0.297
Rpim(I+/-)	0.022	0.011	0.339
Wilson B factor	10.70		
Anomalous completeness	77.7	41.0	18.3
Anomalous multiplicity	2.7	3.5	0.5
Anomalous correlation	0.779	0.931	0.000
Anomalous slope	1.553	0.000	0.000
Total observations	50875	272	342
Total unique	9552	55	199

6 Program Output

As the program runs the key results are written to the screen and recorded in the file `xia2.txt`. This includes everything you should read and includes appropriate citations for the programs that *xia2* has used on your behalf. There is also a file `xia2-debug.txt` which should be send to xia2.support@gmail.com in the event of program failure. There are also two sensibly named directories, `LogFiles` and `DataFiles`, which will be discussed shortly.

6.1 xia2.txt

In intention of the program output from *xia2* is that it includes only the information which is critical to read, as will be shown for a 450 image Pilatus 2M data set recorded from a Thaumatin crystal. Tthe results from indexing are displayed as lattice / unit cell:

```
------------------- Autoindexing SWEEP1 --------------------
All possible indexing solutions:
tP  57.60  57.60 149.51  90.00  90.00  90.00
oC  81.45  81.46 149.51  90.00  90.00  90.00
oP  57.59  57.60 149.50  90.00  90.00  90.00
mC  81.46  81.45 149.50  90.00  89.95  90.00
mP  57.60  57.59 149.53  90.00  89.93  90.00
aP  57.59  57.61 149.52  89.93  89.99  89.99
Indexing solution:
tP  57.60  57.60 149.51  90.00  90.00  90.00
```

where in each case the solution with the lowest penalty is displayed. The results of integration are displayed as one character per image - which allows the overall behaviour of the data to be understood at a glance. While mostly 'o' is usually a good indication of satisfactory processing, '%' are not unusual, along with '.' for weaker data. If the output consists of mostly 'O' then it may be helpful to record a low dose data set. The output looks like:

```
------------------- Integrating SWEEP1 --------------------
Processed batches 1 to 450
Weighted RMSD: 0.26 (0.09)
Integration status per image (60/record):
oooooooooooooooooooooooooooooooooo.oooooooooooooooooooooo
oooooooooooooooooooooo.ooooooooooooooooooo.ooooooo.oooooooo
ooo.o.ooooooo.ooooooooooooooooooooooooooooooooooooooooooooo
ooooooooooooooooooooooooooooooooooooooooooooooooooooooooo
oooooooooooooooooooo.oooooooooooooooooooooooooooooooooooo
ooooooooooooooooooooooooooooooooooooooooooooooooooo.oooooooo
oooooooo.oooooooooo..ooo.oooooooooooooooooo.ooooooooooooo
ooooooooooooooooooo.oooooooo.
"o" => good        "%" => ok        "!" => bad rmsd
"O" => overloaded  "#" => many bad  "." => blank
```

```
"@" => abandoned
Mosaic spread: 0.140 < 0.189 < 0.290
```

and includes a convenient legend.

7 Commonly used program options

There are a number of program options which are used on a daily basis in *xia2*, which are:

- -atom X - tell xia2 to separate anomalous pairs i.e. $I(+) \neq I(-)$ in scaling
- -2d - tell xia2 to use MOSFLM and SCALA
- -3d - tell xia2 to use XDS and XSCALE
- -3dii - tell xia2 to use XDS and XSCALE, indexing with peaks found from all images
- -xinfo modified.xinfo - use specific input file
- -image /path/to/an/image.img - process specific scan
- -spacegroup spacegroup_name - set the spacegroup, e.g. P21
- -cell a,b,c,α,β,γ - set the cell constants
- -small_molecule - don't run things like TRUNCATE

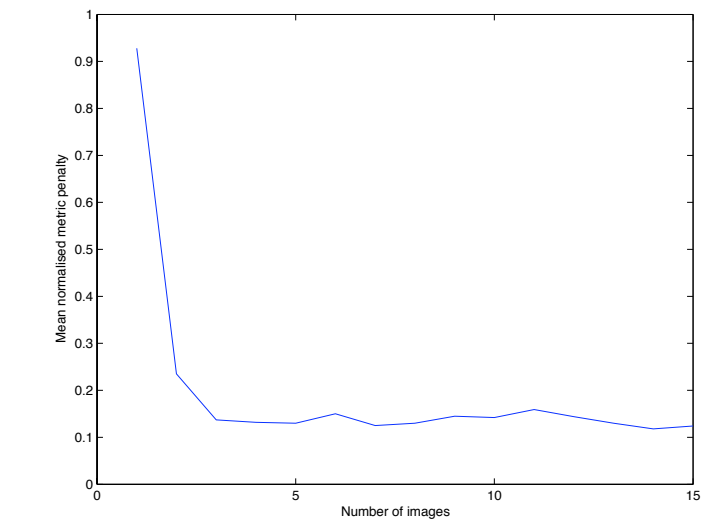
8 What did it do?

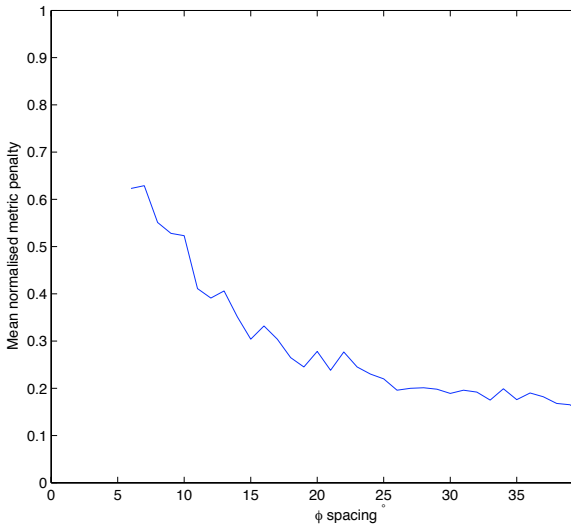
What did it do? and why?

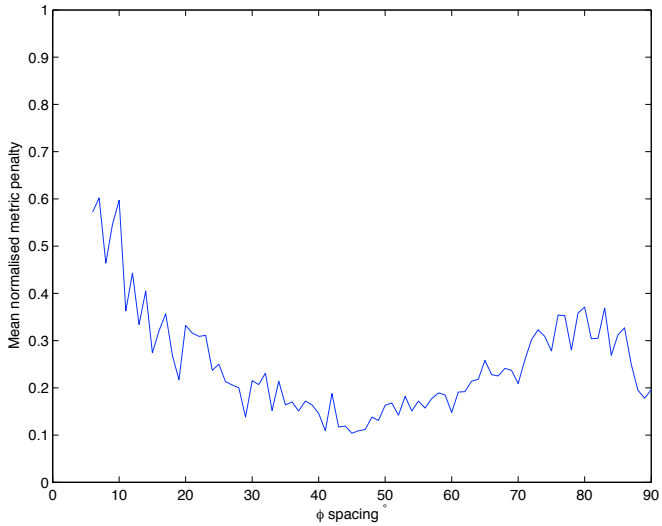
8.1 Indexing

- Initial indexing with LABELIT from 3 images[8]
- Refine results with XDS indexing
- Use data based on general analysis @ 0, 45, 90 degrees

[8] This is *not* good for small molecule data







8.2 Integration

- Integrate with lattice constraints applied
- Integrate to corners of detector
- If good reason, repeat integration e.g. with results of postrefinement
- Perform postrefinement in P1, assumed lattice - may reject lattice, feed back to indexing
- At the end of this we have LATTICE
- If XDS, includes iterative elimination of outliers in CORRECT step

8.3 Scaling

- Compare results of pointless with remaining allowed lattices:
  - If agree, proceed
  - If lattice not allowed, consider next solution
  - If solution lower symmetry than lattice, reject and return to indexing
- Ensure conclusions consistent
- Now have corrrect LAUE GROUP
- Ensure consistent setting / origin choice
- Place data into data collection order
- Analyse absences to decide likely SPACE GROUPs
- Decide scaling model[9]

8.4 Merging and analysis

- If using XDS for integration and XSCALE for scaling, data still merged with SCALA / AIMLESS
- Resolution limits calculated from the intensities, not program output
- "Downstream" analysis (e.g. TRUNCATE and SFCHECK) identical
- Working on scaling data direct from XDS with AIMLESS

9 What decisions were made?

9.1 Decisions: Indexing - LABELIT

Solution	Metric fit	rmsd	#spots	crystal_system	unit_cell			
:) 9	0.2097	dg 0.327	533	tetragonal tP	42.32	42.32	39.28	...
:) 8	0.2097	dg 0.364	541	orthorhombic oP	39.29	42.28	42.33	...
:) 7	0.2097	dg 0.300	519	monoclinic mP	39.26	42.32	42.32	...
:) 6	0.1950	dg 0.299	523	monoclinic mP	39.26	42.33	42.31	...
:) 5	0.1307	dg 0.411	523	orthorhombic oC	59.71	59.91	39.31	...
:) 4	0.1307	dg 0.412	524	monoclinic mC	59.91	59.71	39.31	...
:) 3	0.0937	dg 0.429	524	monoclinic mC	59.71	59.91	39.30	...
:) 2	0.1010	dg 0.298	512	monoclinic mP	42.27	39.31	42.32	...
:) 1	0.0000	dg 0.291	509	triclinic aP	39.31	42.26	42.32	...

[9]For XDS use not corrections in CORRECT, apply all corrections in XSCALE

9.2 Decisions: Indexing - IDXREF

```
 *  31        aP          0.0      39.1   42.1   42.1  90.0  90.0  89.9
 *  44        aP          0.1      39.1   42.1   42.1  90.0  90.0  90.1
 *  34        mP          0.7      39.1   42.1   42.1  90.0  90.1  90.0
 *  20        mC          0.7      59.6   59.6   39.1  90.1  90.1  90.0
 *  33        mP          0.8      39.1   42.1   42.1  90.0  90.1  90.0
 *  25        mC          0.9      59.6   59.6   39.1  89.9  90.1  90.0
 *  35        mP          1.7      42.1   39.1   42.1  90.0  90.0  90.1
 *  23        oC          1.7      59.6   59.6   39.1  89.9  90.1  90.0
 *  32        oP          1.8      39.1   42.1   42.1  90.0  90.0  90.1
 *  21        tP          1.9      42.1   42.1   39.1  90.0  90.1  90.0
    10        mC         79.5      57.5   57.4   42.1  90.0  90.0  94.2
    13        oC         79.9      57.4   57.5   42.1  90.0  90.0  85.8
    14        mC         79.9      57.4   57.5   42.1  90.0  90.0  85.8
```

9.3 Decisions: Testing lattice choice

- Perform postrefinement (MOSFLM and XDS) in P1 and putative lattice
- Compare R.M.S. deviation of observed / predicted centres
- Results comparable → lattice probably OK
- Results worse with lattice constraints → lattice probably wrong

9.4 Decisions: Testing lattice choice 1

```
 REFINED PARAMETERS:  DISTANCE BEAM ORIENTATION CELL AXIS
 USING   27389 INDEXED SPOTS
 STANDARD DEVIATION OF SPOT    POSITION (PIXELS)     1.28
 STANDARD DEVIATION OF SPINDLE POSITION (DEGREES)    0.23
...
 UNIT CELL PARAMETERS     42.180    42.183    39.236  90.002  89.989  89.986
 E.S.D. OF CELL PARAMETERS  1.8E-02 4.3E-02 1.5E-02 1.4E-02 1.0E-02 2.9E-02
 SPACE GROUP NUMBER      1
```

9.5 Decisions: Testing lattice choice 2

```
 REFINED PARAMETERS:  DISTANCE BEAM ORIENTATION CELL AXIS
 USING   27378 INDEXED SPOTS
 STANDARD DEVIATION OF SPOT    POSITION (PIXELS)     1.29
 STANDARD DEVIATION OF SPINDLE POSITION (DEGREES)    0.23
...
 UNIT CELL PARAMETERS     42.187    42.187    39.242  90.000  90.000  90.000
 E.S.D. OF CELL PARAMETERS  1.6E-02 1.6E-02 1.2E-02 0.0E+00 0.0E+00 0.0E+00
 SPACE GROUP NUMBER     75
```

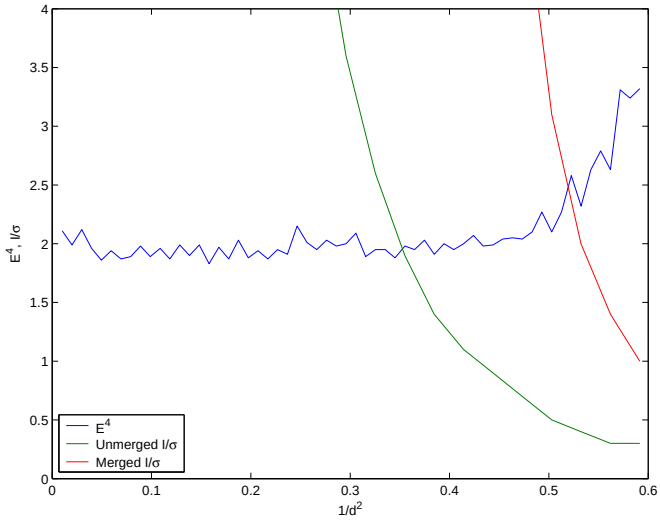
9.6 Decisions: Lattice observations

- Selecting lattice from indexing safe, as tested and challenged
- However strong argument for performing all processing in P1:
  - Processing only performed once
  - Incorrect constraints cannot break things
  - Results generally comparable
- This is on the to-do list...

9.7 Resolution limits - default criteria

- Merged $\frac{I}{\sigma_I} > 2$
- Unmerged $\frac{I}{\sigma_I} > 1$
- Control with -misigma, -isigma

9.8 Resolution limits - why unmerged $\frac{I}{\sigma_I} > 1$?



[10] Though resolution limits to need to be revisited...

10 Comments

10.1 Which options work best?

- It depends ...

[10] 90-fold multiplicity data from Ed Mitchell @ ESRF

- ... try for yourself!
- Sometimes -2d (MOSFLM / SCALA) works better, sometimes -3d (XDS etc.)
- Run both - compare results, make up your own mind
- Hint for small molecule: -3dii -small_molecule
- -3d often works better for very fine ϕ sliced Pilatus data

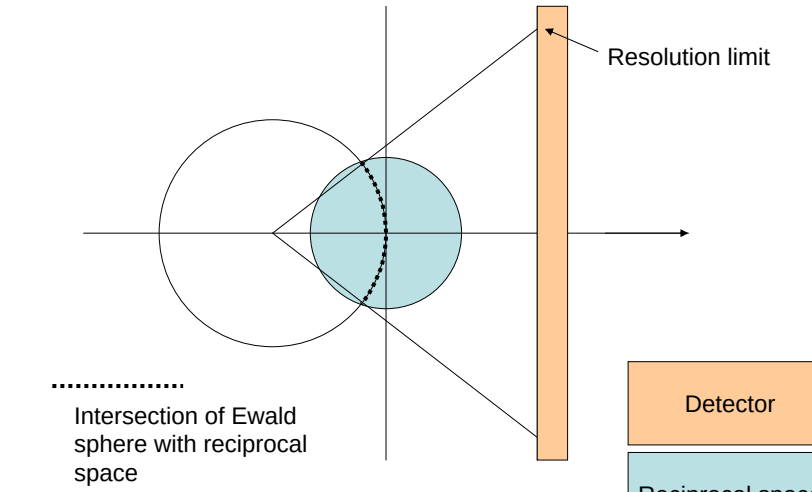
11 Conclusions

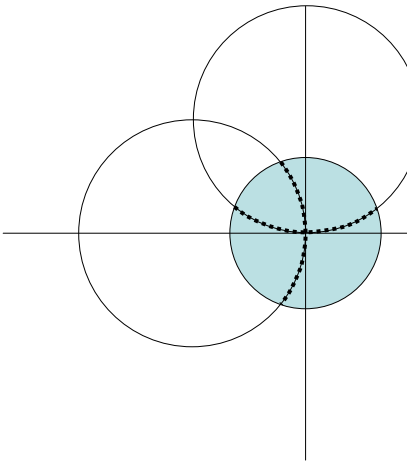
11.1 Conclusions

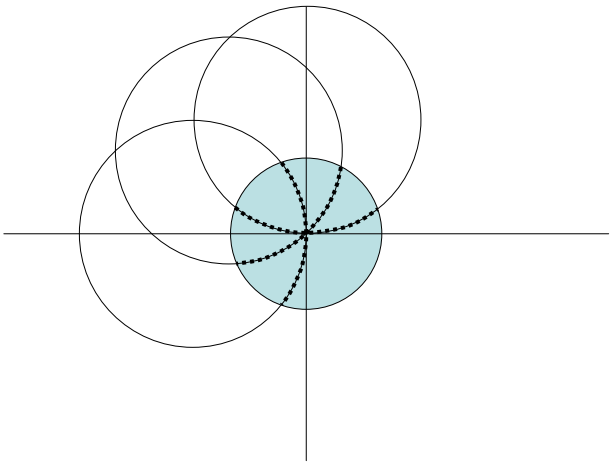
- System available which can reduce your data on your behalf
- Relies on your software: MOSFLM / LABELIT / CCP4 / XDS
- Handles complex strategies so use them
- Works on Windows / OS X / Linux / laptop / workstation / cluster
- Best way to learn data reduction is to teach it
- Computer is very dim but diligent pupil
- Have a go yourself, or feel free to contribute to xia2

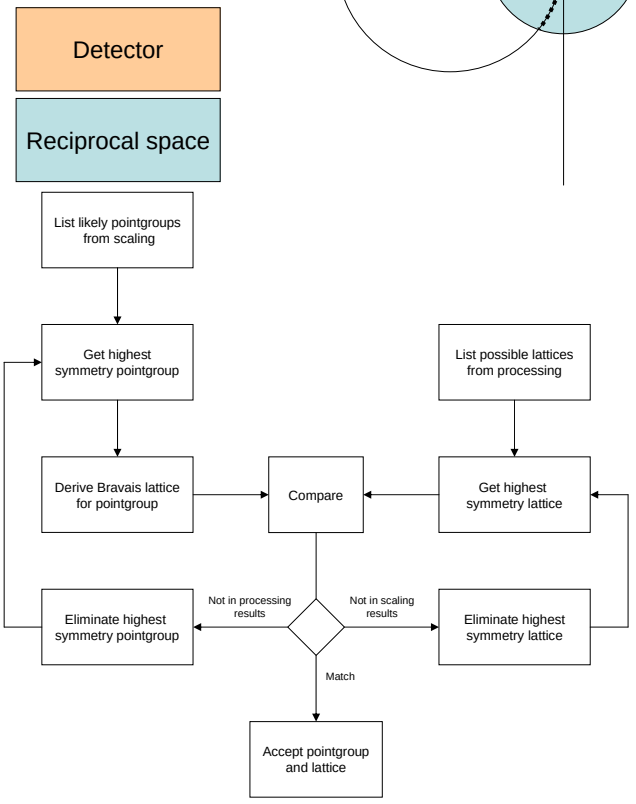
11.2 Getting xia2

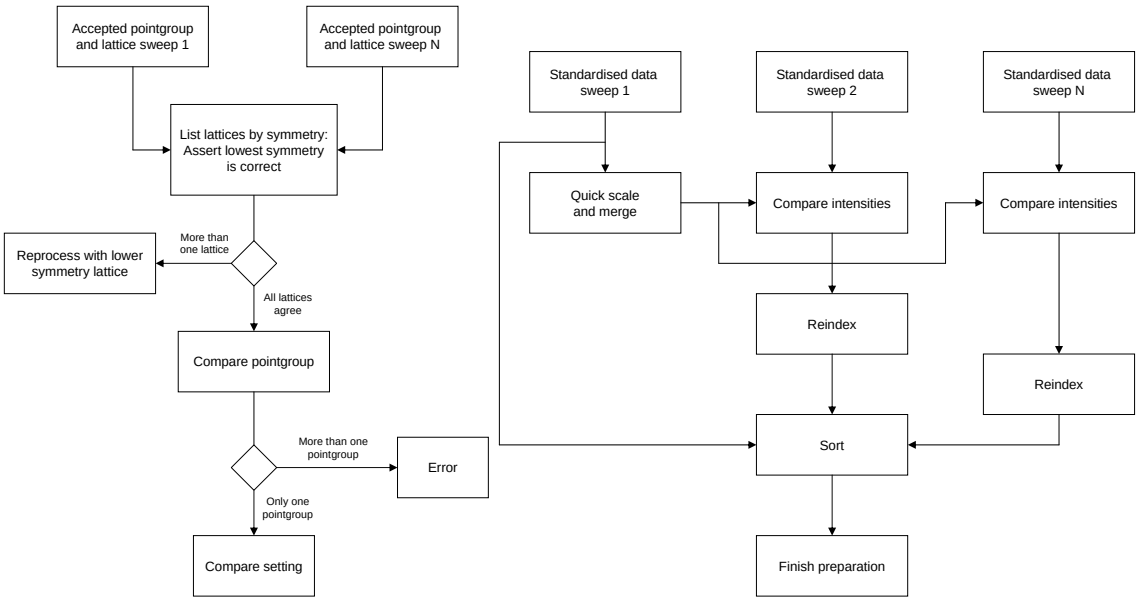
- Blog: xia2.blogspot.com
- Code: xia2.sf.net
- List: xia2-list@lists.sourceforge.net


Resolution limit
Intersection of Ewald sphere with reciprocal space






Detector
Reciprocal space
List likely pointgroups from scaling
Get highest symmetry pointgroup
List possible lattices from processing
Derive Bravais lattice for pointgroup
Compare
Get highest symmetry lattice
Not in processing results
Not in scaling results
Eliminate highest symmetry pointgroup
Eliminate highest symmetry lattice
Match
Accept pointgroup and lattice


Accepted pointgroup and lattice sweep 1
Accepted pointgroup and lattice sweep N
List lattices by symmetry: Assert lowest symmetry is correct
More than one lattice
Reprocess with lower symmetry lattice
All lattices agree
Compare pointgroup
More than one pointgroup
Error
Only one pointgroup
Compare setting
Standardised data sweep 1
Standardised data sweep 2
Standardised data sweep N
Quick scale and merge
Compare intensities
Compare intensities
Reindex
Reindex
Sort
Finish preparation