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 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.

[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
[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

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.

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 Old stuff

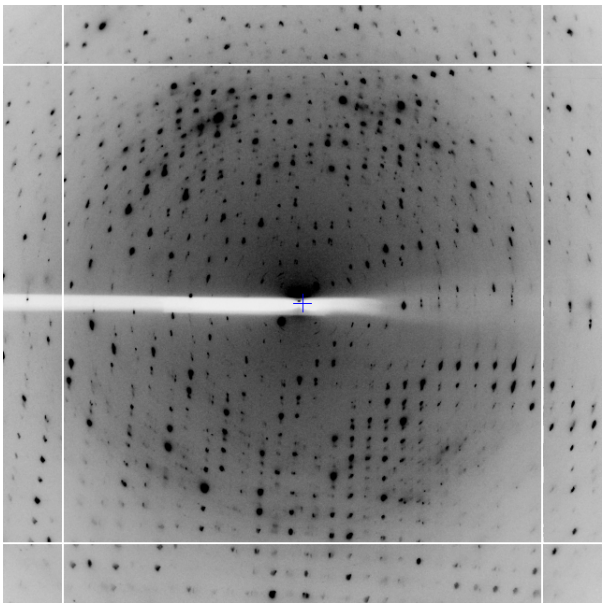
5.1 Example: 3QRN

- J.P. Hall et al., Proc. Natl. Acad. Sci. USA 2011 108 (43) 17573-17574
- Data recorded at Diamond I02
- DNA / ligand complex
- Demonstrates:
  - Radiation damage
  - Heavy atom
  - Resolution limits
- Better sample used for deposition

```
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.

5.2 Example: data



5.3 Example command line

xia2 -3d -atom Ba /dls/i02/data/...

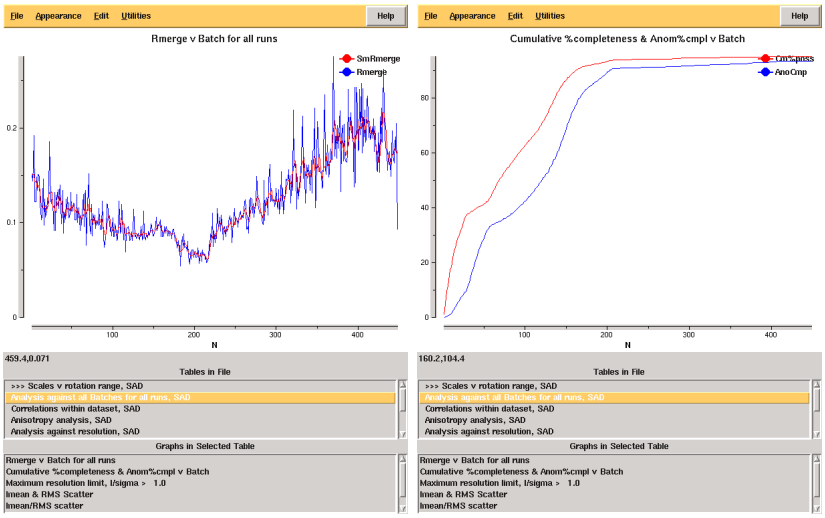
5.4 Example results

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

5.5 Development option - using AIMLESS

xia2 -3da ...

5.6 LogFiles/*aimless.log



5.7 What to do next?

- Edit automatic.xinfo
- Only process first 200 frames

5.8 Modify automatic.xinfo → modified.xinfo

```
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
```

5.9 Running again

xia2 -3d -xinfo modified.xinfo

5.10 Example results II

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

5.11 Resolution: much more in Lunchtime Bytes

- Data incomplete at high resolution
- Add RESOLUTION to xinfo file (in either SWEEP or WAVELENGTH
- Add -resolution to the command line

5.12 Output

- xia2.txt: everything you should read - including program citations
- xia2-debug.txt: everything you probably shouldn't
- LogFiles: you should look at these
- DataFiles: MTZ + erzatz scalepack

5.13 Output

```
------------------- 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
```

5.14 Output

```
-------------------- Integrating SWEEP1 --------------------
Processed batches 1 to 450
Weighted RMSD: 0.26 (0.09)
Integration status per image (60/record):
oooooooooooooooooooooooooooooooo.oooooooooooooooooooooo
oooooooooooooooooooo.ooooooooooooooooo.ooooooo.oooooooo
ooo.o.ooooooo.ooooooooooooooooooooooooooooooooooooooooo
oooooooooooooooooooooooooooooooooooooooooooooooooooooo
ooooooooooooooooooo.oooooooooooooooooooooooooooooooooo
ooooooooooooooooooooooooooooooooooooooooooooooooo.oooooooo
oooooooooo.ooooooooooo..ooo.oooooooooooooooooooo.ooooooooooooo
ooooooooooooooooooo.oooooooo.
"o" => good        "%" => ok        "!" => bad rmsd
"O" => overloaded  "#" => many bad  "." => blank
"@" => abandoned
Mosaic spread: 0.140 < 0.189 < 0.290
```

5.15 Options (2)

- -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

6 What did it do?

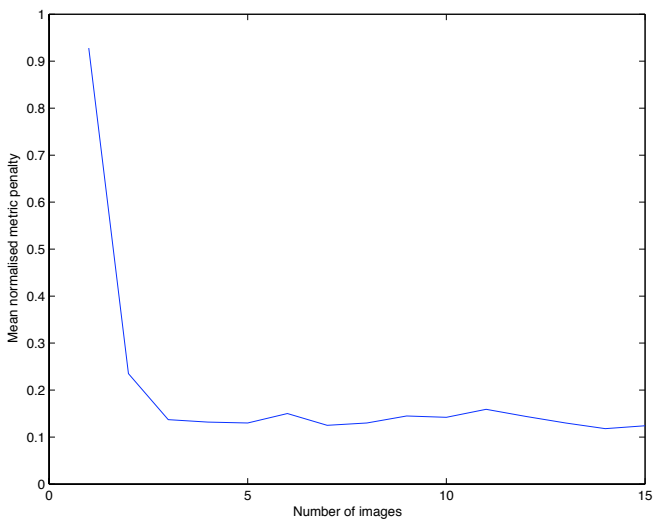
What did it do? and why?

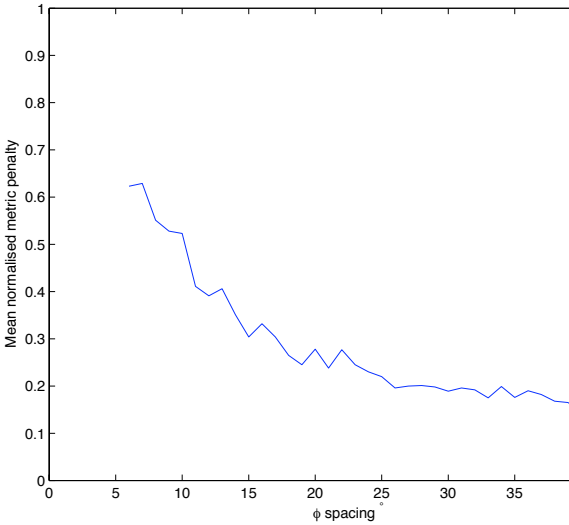
6.1 Indexing

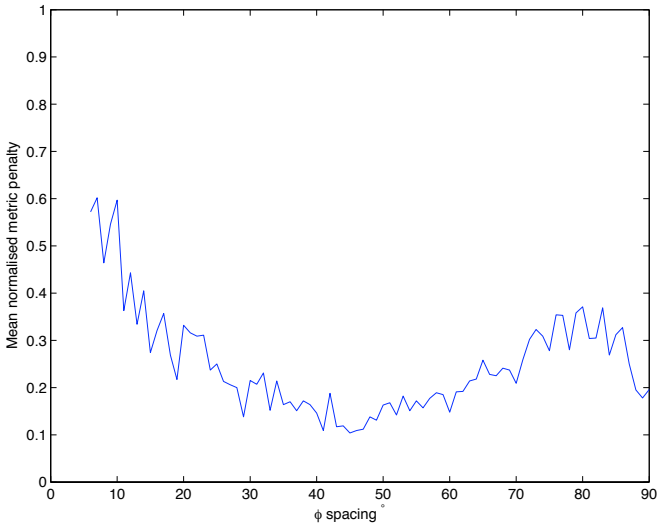
- Initial indexing with LABELIT from 3 images[8]

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

- Refine results with XDS indexing
- Use data based on general analysis @ 0, 45, 90 degrees







6.2 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

6.3 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]

6.4 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

7 What decisions were made?

7.1 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

7.2 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
```

7.3 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

7.4 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
```

7.5 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
```

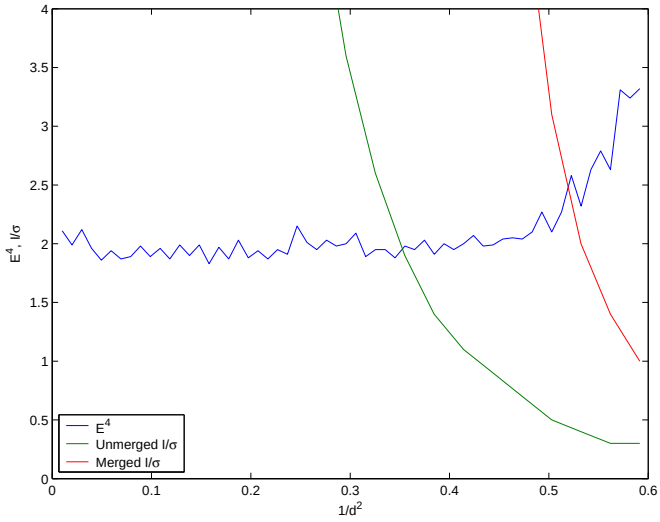
7.6 Decisions: Lattice observations

- 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...

7.7 Resolution limits - default criteria

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

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



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

8 Comments

8.1 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

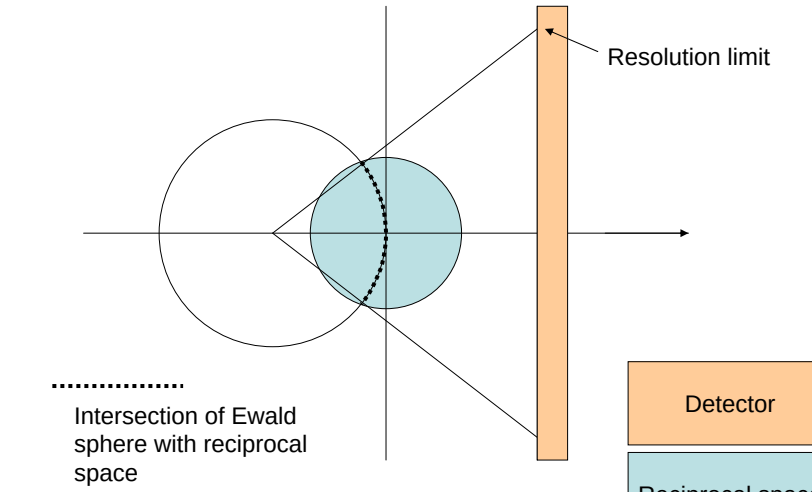
9 Conclusions

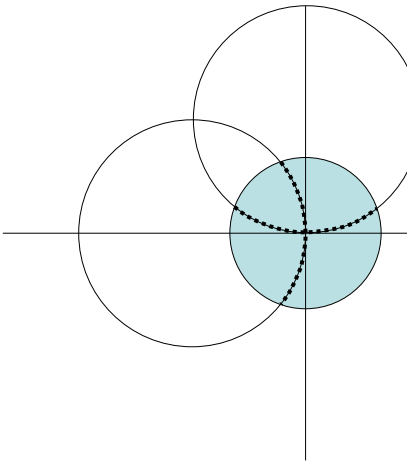
9.1 Conclusions

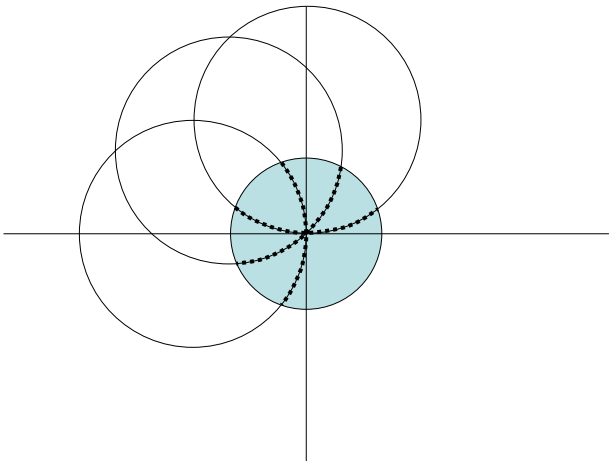
- System available which can reduce your data on your behalf
- Relies on your software: MOSFLM / LABELIT / CCP4 / XDS
- Handles complex strategies so use them
- Works on Windows / OS X / Linux / laptop / workstation / cluster
- Best way to learn data reduction is to teach it
- Computer is very dim but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

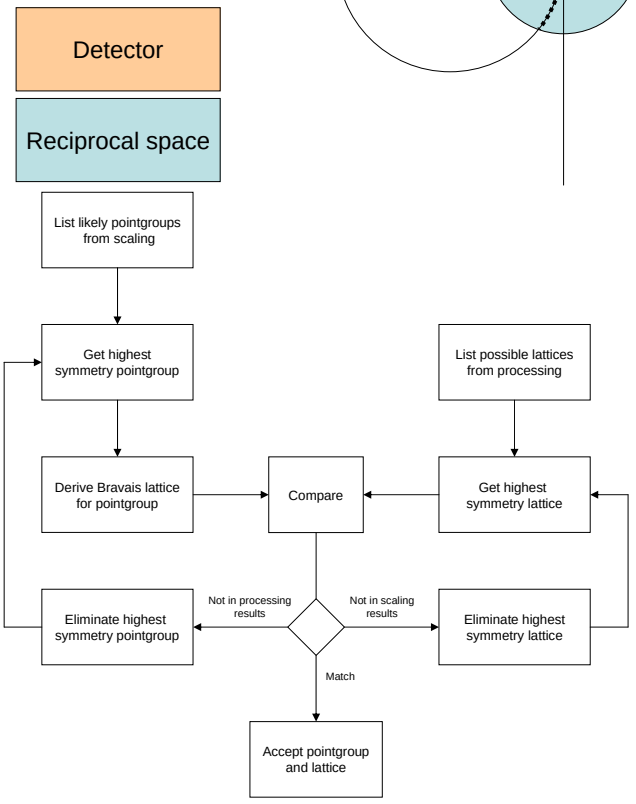
9.2 Getting xia2

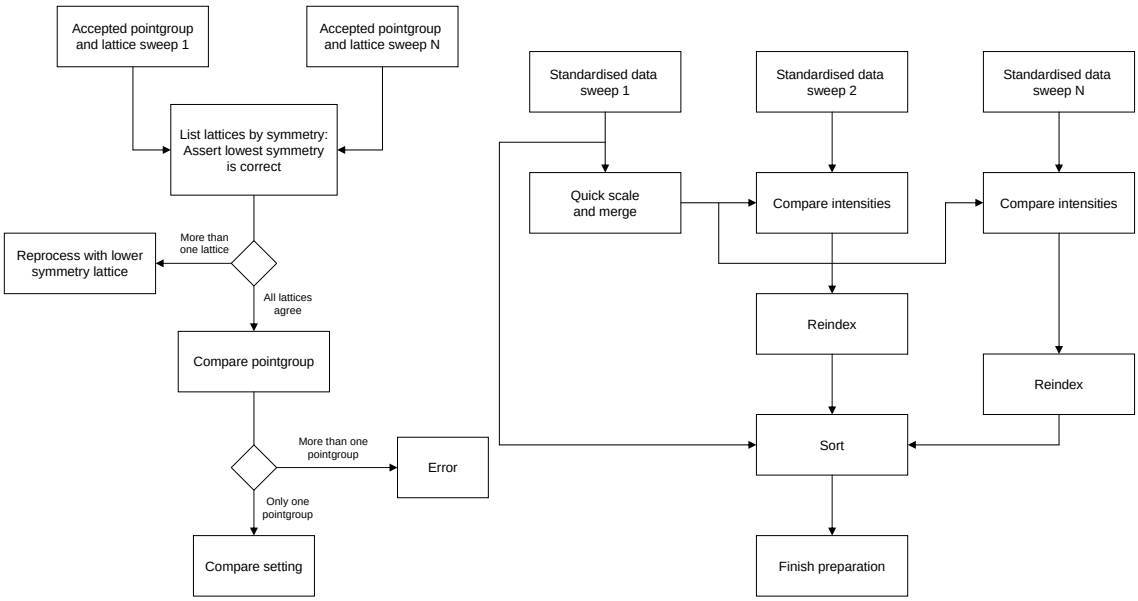
- Blog: xia2.blogspot.com
- Code: xia2.sf.net
- List: xia2-list@lists.sourceforge.net


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