The *xia2* manual

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Contents

1 Quick Start Guide

If you don't like reading manuals and just want to get started, try:

```
xia2 -2d /here/are/my/images
```

or

```
xia2 -3d /here/are/my/images
```

(remembering of course -atom X if you want anomalous pairs separating in scaling.) If this appears to do something sensible then you may well be home and dry. Some critical options:

- -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.

If this doesn't hit the spot, you'll need to read the rest of the document.

2 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.

3 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 often available and commonly used. In the main, however, these programs require that 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

[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

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.

4 Acknowledgements

Without the trusted and capable packages 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 more reliable. The author would therefore like to additionally thank Nick Sauter and Ralf Grosse-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 Center 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.

[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

5 Using xia2

As mentioned in the quick start section, to get started simply run:

```
xia2 -2d /here/are/my/images
```

or

```
xia2 -3d /here/are/my/images
```

The program is used from the command-line; there is no GUI. The four most important command-line options are as follows:

Option	Usage
-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.

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. This description of the experiment is written automatically to an instruction file, an example of which is shown in Figure 1

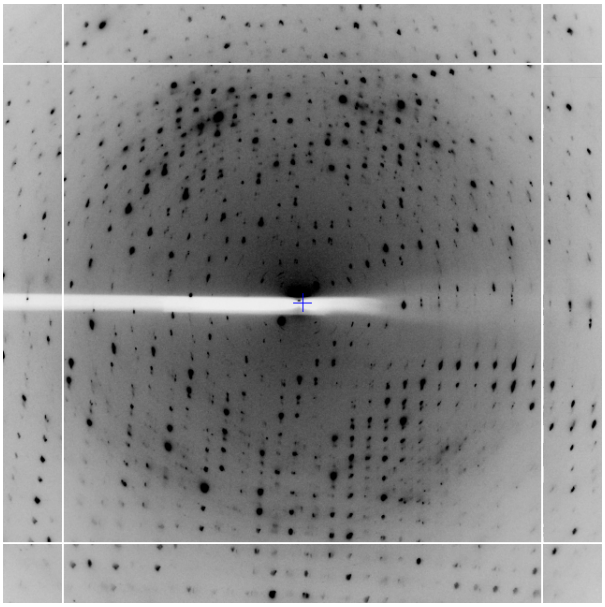
6 Introductory example

The most straightforward way to discuss the operation of the program is through demonstrations with real examples. The first of these is a dataset from 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 Figure 2, 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.

Figure 2: Illustration of the central region of a diffraction pattern from the example data set.



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

giving the merging statistics shown in Table 1. From these it is clear that there is something wrong: it is very unusual to have near atomic resolution diffraction with $\sim 10\%$ $R_{\rm merge}$ 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 $R_{\rm merge}$ as a function of image number (Figure 3 left.) 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 Figure 3 right. From this it is clear that the data were essentially complete after approximately 200 frames, though the low resolution completeness is poor.

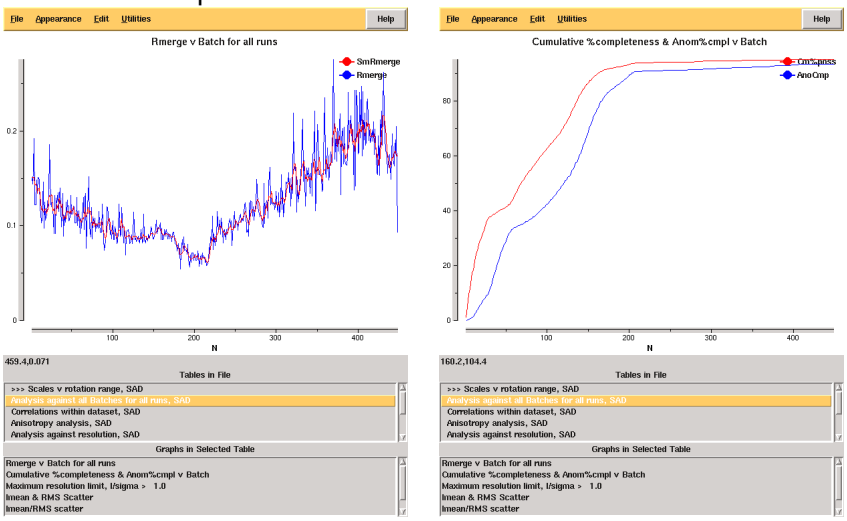
6.1 Modifying input

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

Table 1: Merging stats for processing of the full example data set.

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

Figure 3: Merging statistics and completeness as a function of frame number for the example data.



```
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
```

Figure 4: The modified input file to the program, showing the change to START_END.

range (START_END) to get the modified input file shown in Figure 4 and rerun as

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

giving the results shown in Table 2. These are clearly much more internally consistent and give nice results from experimental phasing though with very poor low resolution completeness. 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.

7 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.

Table 2: Merging stats for the first 200 frames of the example data set.

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

7.1 xia2.txt

By design, the program output from *xia2* includes only the information that is critical to read, as will be shown for a 450 image Pilatus 2M data set recorded from a thaumatin crystal. The 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 includes a convenient legend, and looks like the following:

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

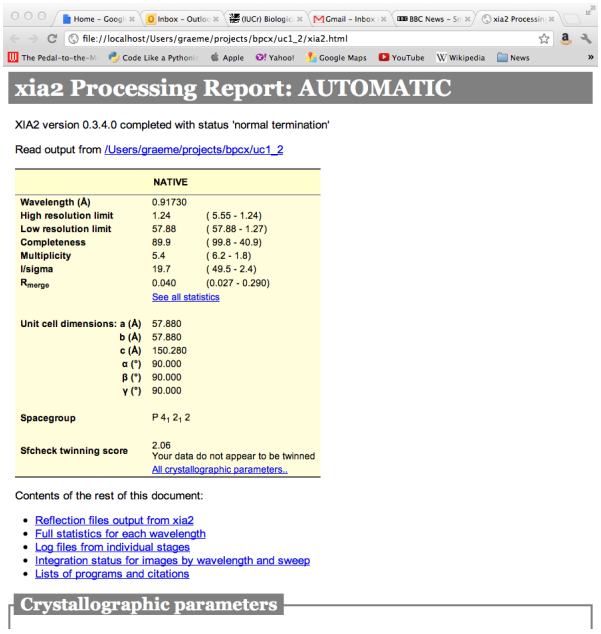
7.2 HTML pages

If `xia2html` has been run there is a nicely formatted html version of this report, which includes graphical representation of some of the log file output from e.g. Scala. Loading up `xia2.html` will give (hopefully self documenting) results as shown in Figure 5. If you have manually run xia2, immediately running `xia2html` in the same directory will generate this.

8 Commonly used program options

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

Figure 5: Illustration of xia2html output.



xia2 Processing Report: AUTOMATIC

XIA2 version 0.3.4.0 completed with status 'normal termination'

Read output from /Users/graeme/projects/bpcx/uc1_2

	NATIVE	
Wavelength (Å)	0.91730	
High resolution limit	1.24	(5.55 - 1.24)
Low resolution limit	57.88	(57.88 - 1.27)
Completeness	89.9	(99.8 - 40.9)
Multiplicity	5.4	(6.2 - 1.8)
I/sigma	19.7	(49.5 - 2.4)
R_{merge}	0.040	(0.027 - 0.290)
	See all statistics	
Unit cell dimensions: a (Å)	57.880	
b (Å)	57.880	
c (Å)	150.280	
α (°)	90.000	
β (°)	90.000	
γ (°)	90.000	
Spacegroup	$P 4_1 2_1 2$	
Sfcheck twinning score	2.06 Your data do not appear to be twinned	
	All crystallographic parameters..	

Contents of the rest of this document:

- Reflection files output from xia2
- Full statistics for each wavelength
- Log files from individual stages
- Integration status for images by wavelength and sweep
- Lists of programs and citations

Crystallographic parameters

-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
-2da	tell *xia2* to use MOSFLM and AIMLESS
-3da	tell *xia2* to use XDS and XSCALE, merging with AIMLESS
-3daii	tell *xia2* to use XDS and XSCALE, merging with AIMLESS, 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	process in manner more suited to small molecule data

Options running Aimless are able to cope with an extremely large number of images - i.e. many thousands, useful when trying to merge data from a number of crystals each with a large number of images, though time consuming!

8.1 Resolution limits

The subject of resolution limits is one often raised - by default in *xia2* they are:

- Merged $\frac{I}{\sigma_I} > 2$
- Unmerged $\frac{I}{\sigma_I} > 1$

However you can override these with -misigma, -isigma.

9 Installing xia2

xia2 depends critically on having CCP4 and CCTBX available. However to get access to the full functionality you will also need XDS and Phenix (which includes Labelit and CCTBX.) Therefore for a "standard" *xia2* installation I would recommend:

- Install CCP4 include updated versions of Pointless and Aimless from ftp://ftp.mrc-lmb.cam.ac.uk/pub/pre
- Download XDS from http://xds.mpimf-heidelberg.mpg.de/ and add this to your path[8]
- Download PHENIX from http://www.phenix-online.org and be sure to source the setup for this *after* CCP4
- Download *xia2* from http://xia2.sf.net and tweak the setup file to reflect where it's installed

By and large, if these instruction are followed you should end up with a happy xia2 installation. If you find any problems it's always worth checking the blog (http://xia2.blogspot.com) or sending an email to xia2.support@gmail.com.

[8] To use -xparallel you will need to fiddle with forkintegrate in the XDS distribution

10 Comments

A question often asked is “which options work best” to which the answer is always “it depends!” This is primarily because the outcome of the analysis depends more on the quality of the data than anything else. However I would always try for yourself and get a feel for how the program works for your data - running both -2d and -3d will simply require more computing time / disk space rather than more effort, so it is certainly worthwhile. For small molecule data though -3dii -small_molecule is a good mix. Also -3d often works better for very finely sliced data.

This manual may be cited freely, however it is preferred that references to the use of *xia2* be made to Winter, G. Journal of Applied Crystallography (2010) 43, 186-190. Please also remember that the programs that *xia2* has used must be correctly cited.

11 Getting xia2

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

12 Full List of Command-Line Options

Option	Usage
-2d	Use Mosflm and Scala
-2da	Use Mosflm and Aimless
-2dai	Use Mosflm (inc. for indexing) and Aimless
-2di	Use Mosflm (inc. for indexing) and Scala
-2dia	Pun of -2dai
-3d	Use XDS and XSCALE, Scala to merge
-3da	Use XDS and XSCALE, Aimless to merge
-3dai	Use XDS and XSCALE, Aimless to merge, XDS to index
-3daii	Use XDS and XSCALE, Aimless to merge, XDS to index (all frames)
-3di	Use XDS and XSCALE, Scala to merge, XDS to index
-3dii	Use XDS and XSCALE, Scala to merge, XDS to index (all frames)
-3diia	Pun for -3daii

Option	Usage
-atom X	Switch on separating anomalous pairs
-batch_scale	Use batch rather than smooth scaling
-beam	Beam coordinates, in Mosflm convention
-blend	Save files for BLEND
-cell	Set the input cell constants (needs symmetry)
-spacegroup	Set the spacegroup
-crystal	Assign the crystal ID
-project	Assign the project ID
-debug	Switch on debugging output (a bad idea - this goes to xia2-debug.txt anyway)
-executable	Override location of executable (deprecated)
-failover	Continue to process if one sweep fails
-image image	Process only sweep containing image
-start_end	Assign start and end of sweep, needs -image
-free_fraction	Fraction of reflections to use in free set
-free_total	Total number of reflections in free set
-freer_file	Copy FreeR_flags from this file
-hdr_in	Input header information (developer tool)
-hdr_out	Output header information (developer tool)
-ice	Exclude ice ring regions

Option	Usage
-indexer	Assign indexer to use (developer tool)
-integrater	Assign integrater to use (developer tool)
-scaler	Assign scaler to use (development tool)
-interactive	Manual selection of images for indexing (developer tool)
-isigma	Unmerged limit for resolution limit calculations
-misigma	Merged limit for resolution limit calculations
-mask	Additional mask for detector e.g. for backstop.
-microcrystal	Options for microcrystal processing
-min_images	Min no. of images to make a sweep
-no_lattice_test	Switch off Bravais lattice tests
-parallel N	Number of cores to use
-phil	Pass in Phil override file (developer tool)
-quick	Cut corners to go quicker
-reference_reflection_file ref.mtz	Assign reference reflection file
-resolution	Set the resolution limit
-reversephi	Warn of backwards phi axis
-scale_model decay,absorption,...	Manually set the scale model
-serial	Non-parallel (-parallel 1)
-small_molecule	Settings for processing small molecule data
-trust_timestamps	If no timestamp in header, trust file timestamps
-xinfo	Pass xinfo file in
-xparallel N	(XDS) use forkintegrate with N machines
-xparm GXPARM.XDS	Pass in refined coordinate description from calibration set
-xparm_ub GXPARM.XDS	Also use UB matrix from ... (developer tool)
-zero_dose	Switch on zero-dose extrapolation