The *xia2* manual

Graeme Winter

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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

[1] A.G.W. Leslie, Acta Cryst. (2006) D62, 48-57
[2] W. Kabsch, Acta Cryst. (2010) D66, 125-132

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.

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,

[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

- 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]

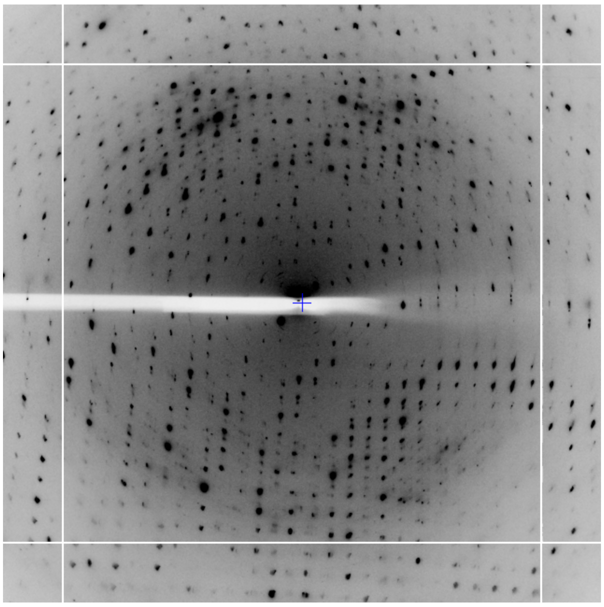
The data from the experiment is understood as follows. The SWEEP, which corresponds to one "scan," is the basic unit of indexing and integration. These are contained by WAVELENGTH objects which correspond to CCP4 MTZ datasets, and will ultimately have unique Miller indices. For example, a low and high 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.

```
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 Introductory example

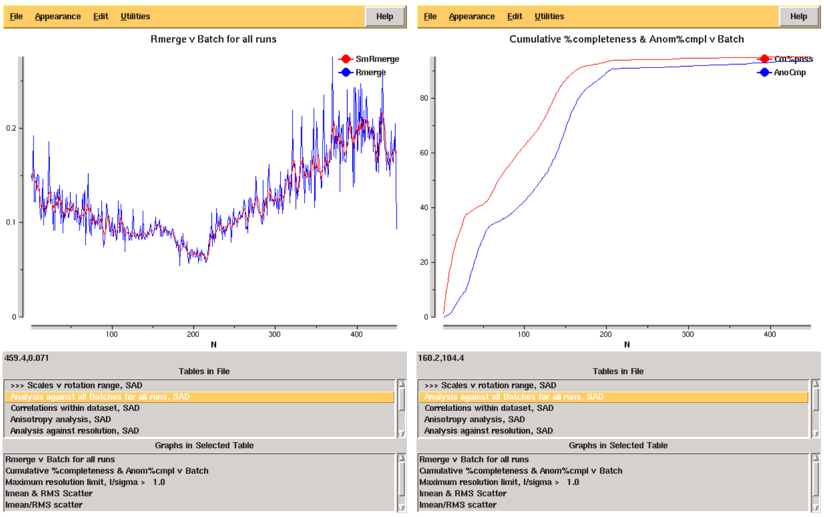
The most straightforward way to discuss the operation of the program is through demonstrations with real examples. The first of these is a DNA / ligand complex recorded at Diamond Light Source as part of ongoing research. The structure includes barium which may be used for phasing, and the data were 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



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):
ooooooooooooooooooooooooooooooooooo.oooooooooooooooooooooooo
ooooooooooooooooooooooo.ooooooooooooooooooo.ooooooo.oooooooo
ooo.o.ooooooo.oooooooooooooooooooooooooooooooooooooooooooooo
oooooooooooooooooooooooooooooooooooooooooooooooooooooooooooo
oooooooooooooooooooo.ooooooooooooooooooooooooooooooooooooooo
ooooooooooooooooooooooooooooooooooooooooooooooooooo.oooooooo
ooooooooo.oooooooooo..ooo.oooooooooooooooooooo.ooooooooooooo
oooooooooooooooooooo.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

7.1 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.

8 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://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.

9 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.

10 Getting xia2

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

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