XDS usage - introduction and demonstration

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Outline

1) Introduction to XDS (XSCALE, XDSCC12)

2) Demo: Processing data with XDSGUI (and using XDSSTAT, XDSCC12, SPOT2PDB, COOT, POINTLESS)

The *XDS* program suite

- Original author: Wolfgang Kabsch (Max-Planck-Institute Heidelberg)
- Since ~1986
- I joined 2007

The *XDS*+ programs

- *XDS*: the main program - indexing, integrating, scaling, statistics
- *XSCALE*: scale several *XDS* intensity data sets together; zero-dose extrapolation; statistics
- *XDSCONV*: convert to MTZ / SHELX /... format (AIMLESS and CTRUNCATE are not needed!)

Programs independent from the *XDS* distribution:

- *XDS-Viewer*: inspect diagnostic images written by XDS, or (single) data frames (open source). *adxv* or *dials.image_viewer* may be used instead.
- *XDSSTAT*: additional statistics
- *XDSGUI*: graphical user interface for XDS, SHELX C/D/E, ARCIMBOLDO (open source)
- *XDSCC12*: (XDS) which frames are bad?
  (XSCALE) which data sets to re-index and merge?
- *XSCALE_ISOCLUSTER* multi-data-set: visualize relations and cluster

Sources of information

- *XDS* main website: https://xds.mr.mpg.de ; complete, accurate, up-to-date documentation; download: Linux, Intel- and Silicon-Mac; Windows - use WSL.
- XDSwiki: https://wiki.uni-konstanz.de/xds
  Installation; data sets; documentation; download; links to e.g. Matthew J. Whitley's excellent tutorial given at CSHL 2018
- CCP4 bulletin board
- SBGrid talk (May 2020) at https://www.youtube.com/watch?v=3WU9NrILECo
- Making a difference in multi-data-set crystallography: simple and deterministic data-scaling/selection methods. Assmann, G.M., Wang, M., Diederichs, K. (2020) Acta Cryst D76, 636 (serial crystallography, *XDSCC12*)

Automatic processing with *XDS*

- beamline software (provides XDS.INP)
- scripts: *xia2* (CCP4), *autoPROC* (Globalphasing), *generate_XDS.INP* (XDSwiki), *fast_dp* (Diamond), *xdsme* (Soleil), *autoxds* (SSRL), *autoprocess* (CMCF), ...
- CCP4: *pointless, xdsconv* (type CCP4_I+F, or CCP4, or CCP4_I, or CCP4_F)
- SHELX: *shelxc* reads XDS_ASCII.HKL

Principle of *XDS* processing

- There is one JOB= line in XDS.INP which specifies a list of tasks:

JOB= XYCORR INIT COLSPOT IDXREF DEFPIX INTEGRATE CORRECT

- data reduction is divided into tasks in a **modular** way
- information storage/exchange/flow between tasks by data files which may be inspected/analyzed
- each task needs the result from the previous tasks
- fine-tuning of a task does *not* require previous tasks to be repeated
- each task writes its output file <TASK>.LP

The tasks are ...

- XYCORR : write positional correction files (X-CORRECTIONS.cbf, Y-CORRECTIONS.cbf)
- INIT : find background pixels (defaults usually OK)
- COLSPOT: find reflection positions
- IDXREF : "index" reflections; user may supply/choose spacegroup
- XPLAN [not required] : strategy for data collection
- DEFPIX : mask shadows on detector (use *XDSGUI*!)
- INTEGRATE : evaluates intensities on all frames, writes INTEGRATE.HKL and FRAME.cbf
- CORRECT : scales, rejects outliers, statistics, writes scaled, unmerged XDS_ASCII.HKL (and other files)

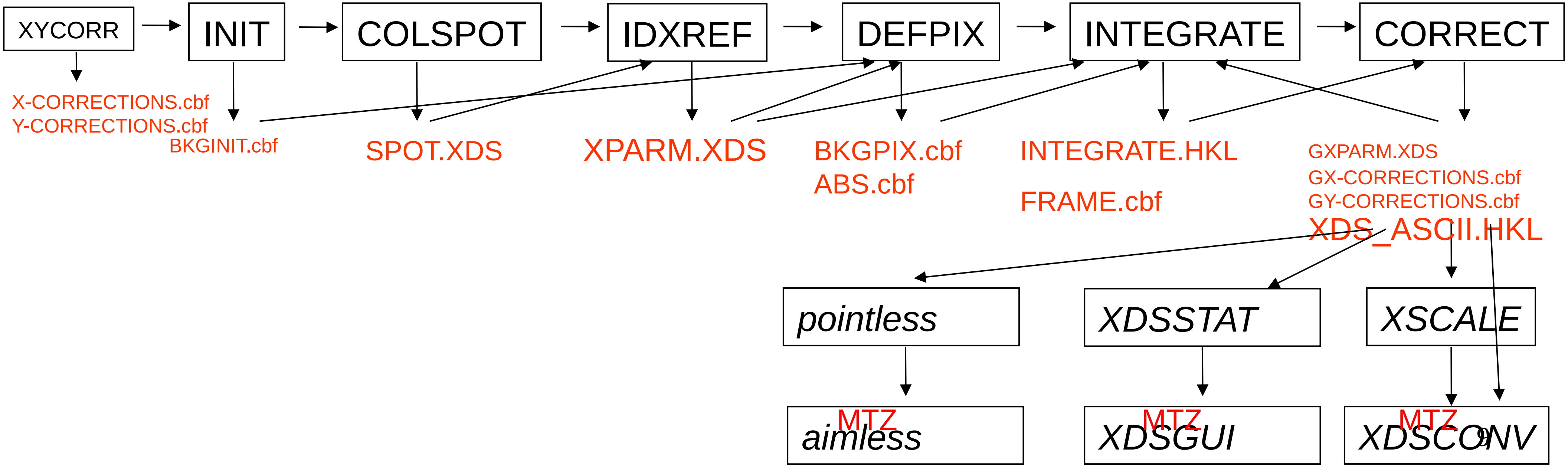
Information flow

NAME_TEMPLATE_OF_DATA_FRAMES
DETECTOR

OSCILLATION_RANGE
SEPMIN
STRONG_PIXEL

ORGX
ORGY
DETECTOR_DISTANCE
X_RAY_WAVELENGTH
SPACE_GROUP_NUMBER

DATA_RANGE



Example XDS.INP

```
JOB= XYCORR INIT COLSPOT IDXREF DEFPIX INTEGRATE CORRECT
ORGX=1546 ORGY=1552        !Detector origin (pixels); e.g. NX/2 NY/2
DETECTOR_DISTANCE=180      !(mm)
OSCILLATION_RANGE=0.50     !degrees (>0)
X-RAY_WAVELENGTH=0.980243 !Angstroem
NAME_TEMPLATE_OF_DATA_FRAMES=frms/wga2-27_1_???.img
DATA_RANGE=1 360           !Numbers of first and last data image collected
BACKGROUND_RANGE=1 10      !Numbers of first and last data image for background
SPACE_GROUP_NUMBER=  19    !0 for unknown crystals; cell constants are ignored.
UNIT_CELL_CONSTANTS= 44.4   86.4   104.5  90 90  90  ! not required if spgr=0
REFINE(IDXREF)=BEAM AXIS ORIENTATION CELL DISTANCE
REFINE(INTEGRATE)=DISTANCE BEAM ORIENTATION CELL ! AXIS
ROTATION_AXIS= 1.0 0.0 0.0
INCIDENT_BEAM_DIRECTION=0.0 0.0 1.0
FRACTION_OF_POLARIZATION=0.99                    ! SLS X06SA
POLARIZATION_PLANE_NORMAL= 0.0 1.0 0.0
DETECTOR=CCDCHESS        MINIMUM_VALID_PIXEL_VALUE=1     OVERLOAD=65000
DIRECTION_OF_DETECTOR_X-AXIS= 1.0 0.0 0.0
DIRECTION_OF_DETECTOR_Y-AXIS= 0.0 1.0 0.0
VALUE_RANGE_FOR_TRUSTED_DETECTOR_PIXELS= 7000 30000 !Used by DEFPIX
                                  !for excluding shaded parts of the detector.
INCLUDE_RESOLUTION_RANGE=50.0 1.3 !Angstroem; used by DEFPIX,INTEGRATE,CORRECT
```

!No blanks allowed!

Bold keyword/parameter pairs are required. Complete documentation at xds.mr.mpg.de/html_doc/xds_parameters.html

Example XSCALE.INP

```
!============ EXAMPLE 3: specific reindexing of input data sets
!
!       Use of specific reindexing of input data sets for resolving
!       indexing ambiguities in the scaled output data set. This
!       happens if the crystal's space group symmetry is lower than
!       its lattice symmetry.
!
RESOLUTION_SHELLS= 100 10 6 4 3 2 1.9
SPACE_GROUP_NUMBER=78
UNIT_CELL_CONSTANTS=57.39 57.39 106.9   90 90 90
OUTPUT_FILE=scaf8_all_merged.hkl
MERGE=TRUE FRIEDEL'S_LAW=FALSE
STRICT_ABSORPTION_CORRECTION=TRUE
INPUT_FILE= ../xds-1_2/XDS_ASCII.HKL
REIDX_ISET= -1  0  0  0  0  1  0  0  0  0 -1  0
INPUT_FILE= ../xds-2_1/XDS_ASCII.HKL
INPUT_FILE= ../xds-3_1/XDS_ASCII.HKL
INPUT_FILE= ../xds-1_4/XDS_ASCII.HKL
INPUT_FILE=*../xds-5_1/XDS_ASCII.HKL
```

Bold keyword/parameter pairs are required. Complete documentation at xds.mr.mpg.de/html_doc/xscale_parameters.html

Output file XSCALE.LP shows level of systematic error **ISa** before/after scaling.

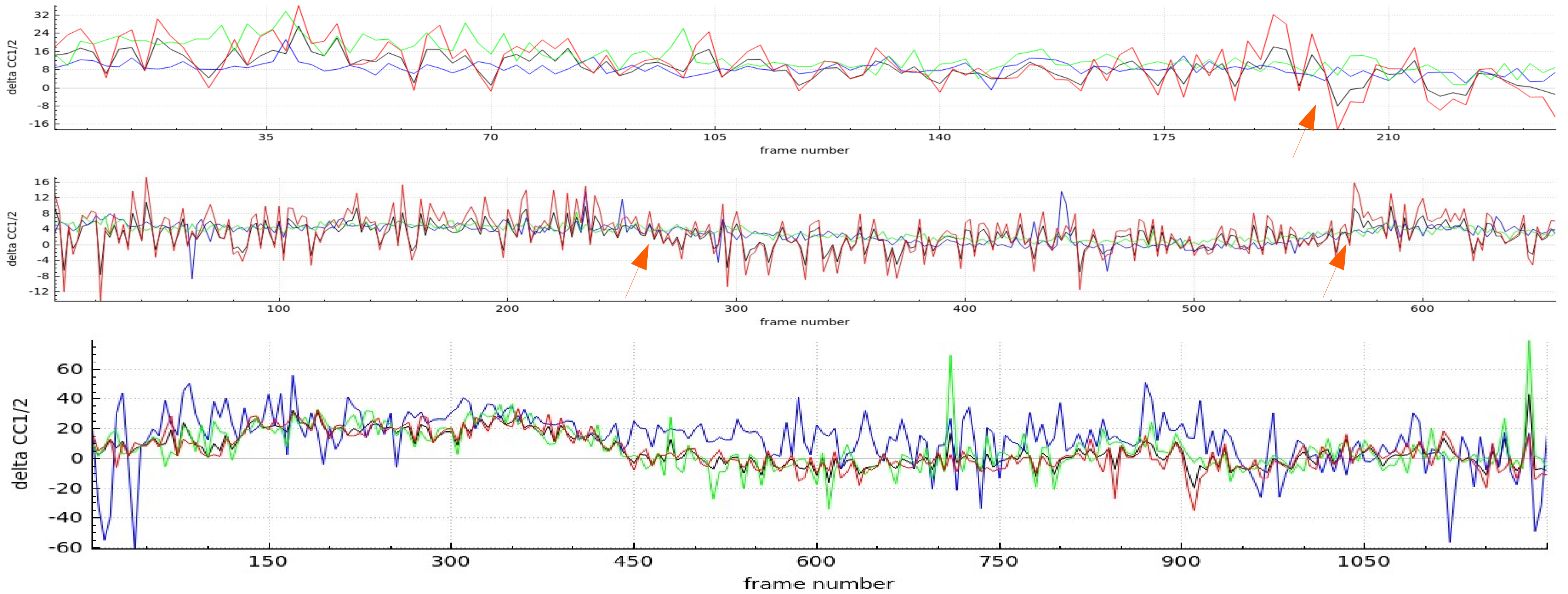
Example XDSCONV.INP

```
! UNIT_CELL_CONSTANTS= 10 20 30 90 90 90
! SPACE_GROUP_NUMBER= 96
! GENERATE_FRACTION_OF_TEST_REFLECTIONS=0.05

INPUT_FILE=XDS_ASCII.HKL
OUTPUT_FILE=temp.hkl CCP4_I+F ! or CCP4_I or CCP4_F or SHELX or CNS
FRIEDEL'S_LAW=FALSE ! store anomalous signal in output file even if weak
```

Bold keyword/parameter pairs are required. Complete documentation at xds.mr.mpg.de/html_doc/xdsconv_parameters.html

3 examples of single data sets (plots from *XDSGUI*)



XDSCC12: calculates $\Delta CC_{1/2,i} = CC_{1/2,with\_i} - CC_{1/2,without\_i}$

Three resolution ranges (blue=low green=medium red=high) - i refers to batches of width 1°

- find bad frame ranges
- radiation damage

xdscc12 for data from XSCALE

```
overall CC1/2:    99.784 nref=   29744 (but the overall CC1/2 is meaningless!)
<CC1/2>:    68.971 (frequency-weighted average of CC1/2 in resolution shells)
CC1/2 in resolution shells:
  99.8   99.6   99.6   99.3   97.8   92.9   81.3   52.7   33.4   12.5
CC* in resolution shells:
  99.9   99.9   99.9   99.8   99.4   98.1   94.7   83.1   70.7   47.1
frequency, i.e. number of unique reflections in resolution shells:
   970   1741   2257   2669   3005   3336   3604   3904   4104   4154

headings for lines starting with a,b,c:
a:  <CC1/2> of each dataset:
a:   reflections of this dataset only      reflections of all datasets
a: set   nref    with without   delta     nref    with without   delta
b: delta-CC1/2 in resolution shells
c: # reflections for delta-CC1/2
a      1  27910  66.807  60.517  10.560    28672  66.486  60.349  10.249
b 30.906 29.045 30.431 27.944 34.663 27.727 23.134 14.772 13.087 5.266
c    912   1654   2173   2527   2803   3169   3394   3679   3849   3750
a      2  25492  68.433  61.215  12.423    26174  70.905  63.097  14.130
b 27.445 18.627 5.799 14.503 25.465 32.858 31.704 23.753 13.638 -.254
c    858   1486   2064   2349   2517   2928   3153   3435   3317   3385
```

output for two datasets in P1 that scale quite well

xdscc12 writes a sorted XSCALE.INP.rename_me

```
SPACE_GROUP_NUMBER=    1
UNIT_CELL_CONSTANTS=     53.00     59.93     61.34  99.975 105.633 119.747
OUTPUT_FILE= temp.ahkl
PRINT_CORRELATIONS= FALSE
SAVE_CORRECTION_IMAGES= FALSE
FRIEDEL'S_LAW= FALSE
! median of delta-cc1/2 "only" values=    11.492
! noise (MAD) of these values=     0.932
! median of delta-cc1/2 "all" values=    12.189
! noise (MAD) of these values=     1.941
! median of delta-cc1/2-ano "only" values=    -2.201
! noise (MAD) of these values=     2.699
! median of delta-cc1/2-ano "all" values=    -2.010
! noise (MAD) of these values=     2.614
! input files sorted by deltacc12_only (highest first):
! deltacc12 only / all:   12.4231   14.1301 deltacc12-ano only /all:    -4.9002  -4.6243
INPUT_FILE=../xds_14_2/XDS_ASCII.HKL
! deltacc12 only / all:   10.5600   10.2488 deltacc12-ano only /all:     0.4976   0.6043
INPUT_FILE=../xds_14_3/XDS_ASCII.HKL
```

output for two datasets in P1 that scale quite well

For important data, do not rely on automatic data processing

There are no perfect criteria for the quality of a data set - “Table 1” does not tell the whole story. Synchrotrons typically run multiple pipelines, and the user has to choose ...

What can go wrong in automatic data processing?

- does not handle radiation damage (we need to discard frames towards the end of the data set, but where should we cut?)
- does not handle shadowed areas of detector
- does not handle indexing problems (multiple lattices, ice, …) flexibly
- does not optimize processing

Automatic processing with GlobalPhasing’s *autoPROC,* CCP4’s *xia2,* HZB’s *XDSAPP* is rather reliable for good data! Difficult data sets typically benefit from human insight.

Manual processing with *XDSGUI*

- problems in phasing and refinement
  often due to bad / wrong data processing
- visually inspect frames; mask shadows
- optimize parameters, frame range,
  resolution cutoff ..
- presents tables as plots
- interfaces to *XDS* through its files, e.g. XDS.INP
- user – extensible / modifiable commands

Brehm, Triviño, Krahn, Usón and Diederichs (2023) XDSGUI: a graphical user interface for XDS, SHELX and ARCIMBOLDO. *J. Appl. Cryst.* **56**, https://doi.org/10.1107/S1600576723007057

Manual checks are easily performed with *XDSGUI*

- before running *XDS*: check frames (*adxv*, *Albula*, *XDSGUI* ...)
- **Frame** tab: FRAME.cbf for match obs/calc, and masked areas
- **IDXREF** tab: for ice rings / indexed *vs* un-indexed reflections
- look at indexing in reciprocal space:
  tools tab/Further analyses/spot2pdb-coot command
  see XDSwiki:XDSGUI#tools
- **statistics** tab: *XDSCC12* and *XDSSTAT*

Optimize data processing

- XDSwiki:Optimisation#Re-INTEGRATEing_with_the_correct_spacegroup.2C_refined_geometry_and_fine-slicing_of_profiles

  XDSwiki:Optimisation#using_the_refined_values_for_beam_divergence_and_mosaicity_for_re-integration

- **tools** tab:
  * “Saving and comparing good results” and “Optimizing data quality”. After changing parameters, run “JOB=DEFPIX INTEGRATE CORRECT”, compare and save if better/restore old if worse.
  * “Further analysis”: Inspect indexed/unindexed spots in reciprocal space

- consider use of *StarAniso* if anisotropy is strong (i.e. visible)

… may make the difference between structure solved or not, interpretable or non-interpretable map, good or bad refinement, ...

Summary

Data processing is the crucial link between your experiment and your structure

* garbage in – garbage out!
* for important data, do not rely on automatic data processing alone
* manual checks are easily performed with *XDSGUI*
* try to optimize data processing – this converts noise to signal, and may enable structure solution, and/or improve refinement