Data Processing Using *XDS*

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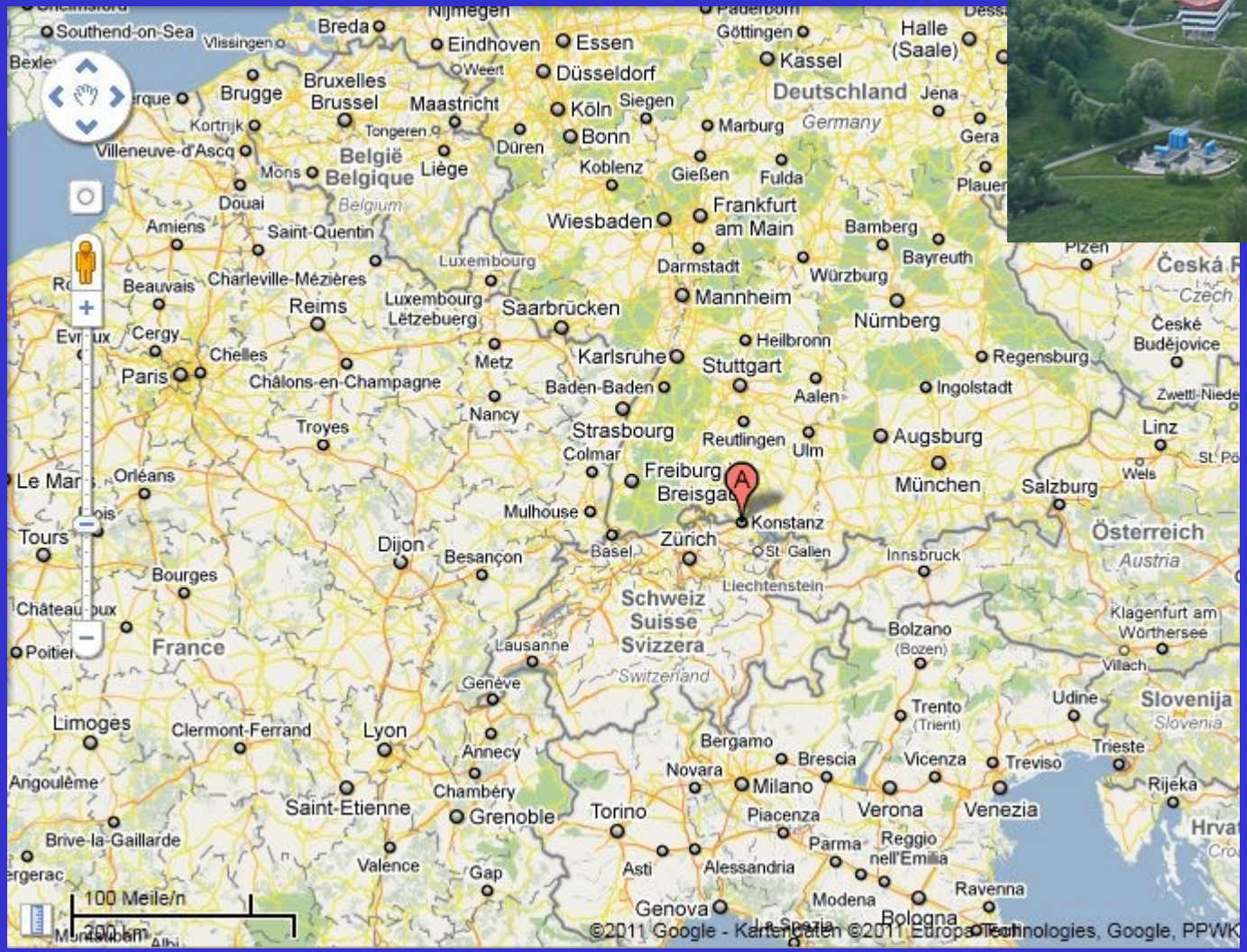
Konstanz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Outline

- „*XDS* is a data processing program for X-ray data collected by the oscillation method on area detectors“. Use: stand-alone or in „pipeline“ mode. No GUI, but visual feedback available
- How to get information about *XDS*
- Problems and their diagnostics
- Highlights of 3CSL, 2QVO, 1Y13, 2VB1, 1G1C-simulated

throughout this talk: *program*, file

The *XDS* program suite

Original author: Wolfgang Kabsch (Max-Planck-Institute Heidelberg); I joined ~ 4 yrs ago

Distribution for Linux & Mac: new version every ca. 6 months (latest: Apr-2011)

- *XDS*: the main program (indexing, integrating, scaling)
- *XSCALE*: scale several *XDS* intensity data sets together; zero-dose extrapolation; statistics
- *XDSCONV*: convert to other programs' formats

  (the following are independant of the main distribution:)
- *XDS-Viewer* - inspect diagnostic images written by *XDS*, or (single) data frames; alternatively, latest *adxv* may be used (sourcecode available from sourceforge.net)
- *XDSSTAT* - additional statistics (download and use: see XDSwiki; not part of main distribution)

Interfaces

- GUIs: *XDSi* (P. Kursula), *XDSi* (M. Krug)
- CCP4: *pointless*, *xdsconv* (type CCP4, or CCP4_I, or CCP4_F)
- *CNS / PHENIX / SHELX*: *xdsconv*
- pipelines: *xia2* (CCP4), *autoPROC* (Globalphasing), *autoxds* (SSRL), *autoprocess* (CMCF) ...

XDS algorithms

Features:

- 3D - profiles of reflections are transformed into their own coordinate systems which makes them highly similar
- Pixel-labelling method
- Smooth scaling
- Zero-dose extrapolation (*XSCALE*)
- Two levels of parallelization

Sources of information

- *XDS* main website: http://xds.mpimf-heidelberg.mpg.de - complete, accurate, up-to-date documentation; download
- XDSwiki: http://strucbio.biologie.uni-konstanz.de/xdswiki/index.php/Main_Page
- CCP4 bulletin board
- “XDS webinar” (at Rigaku website)
- Email to kay.diederichs@uni-konstanz.de

*XDS*wiki

- started Feb 2008; ~ 150 pages at

http://strucbio.biologie.uni-konstanz.de/xdswiki/index.php/Main_Page

- e.g. „Optimization“; explanations of task output
- „Tips and Tricks“, „FAQ“
- „Quality Control“ with datasets and results, and links to the five projects of this workshop
- anybody can contribute!
  (same holds for CCP4wiki: ~ 300 pages at
  http://strucbio.biologie.uni-konstanz.de/ccp4wiki/index.php/Main_Page)

Some typical questions ...

- “How to scale & merge different datasets from similar or same xtal(s), using XDS?”
- “What about twinning?”
- “Is it possible to integrate small molecule data as well?”
- …
- Questions and Answers in FAQ article of XDSwiki

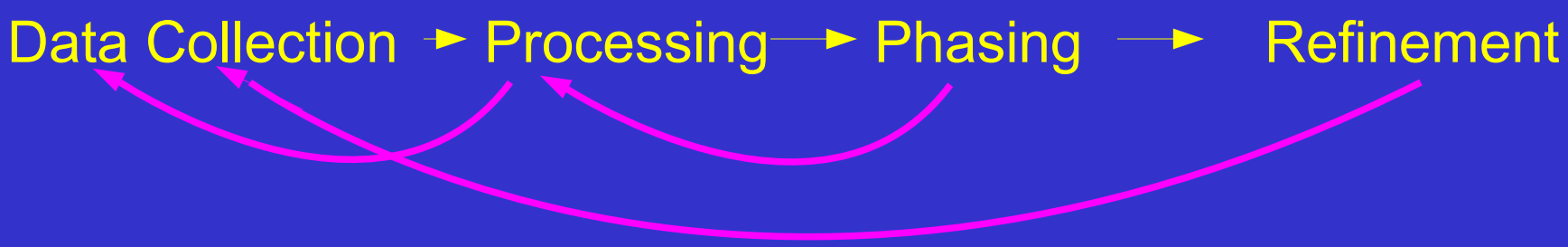
Easy vs. difficult

Data Collection → Processing → Phasing and Refinement

Easy:

1) *generate_XDS.INP "mydata_???.img"* (see XDSwiki)
2) *xds* (or rather *xds_par*)
3) *xdsconv* (or *pointless xdsin XDS_ASCII.HKL hklout mydata.mtz)*

Difficult



Information for making decisions?

What can go wrong?

Beamline: beam center wrong (90%) or unusual convention, rotation backwards; shutter jitter, vibrations ...

Experiment: crystal with split reflections; ice rings; radiation damage

Interpretation of data: twinning overlooked, or wrong spacegroup

Phasing and refinement: anomalous signal too weak, resolution too low, disorder, anisotropy

Which diagnostics to look at?

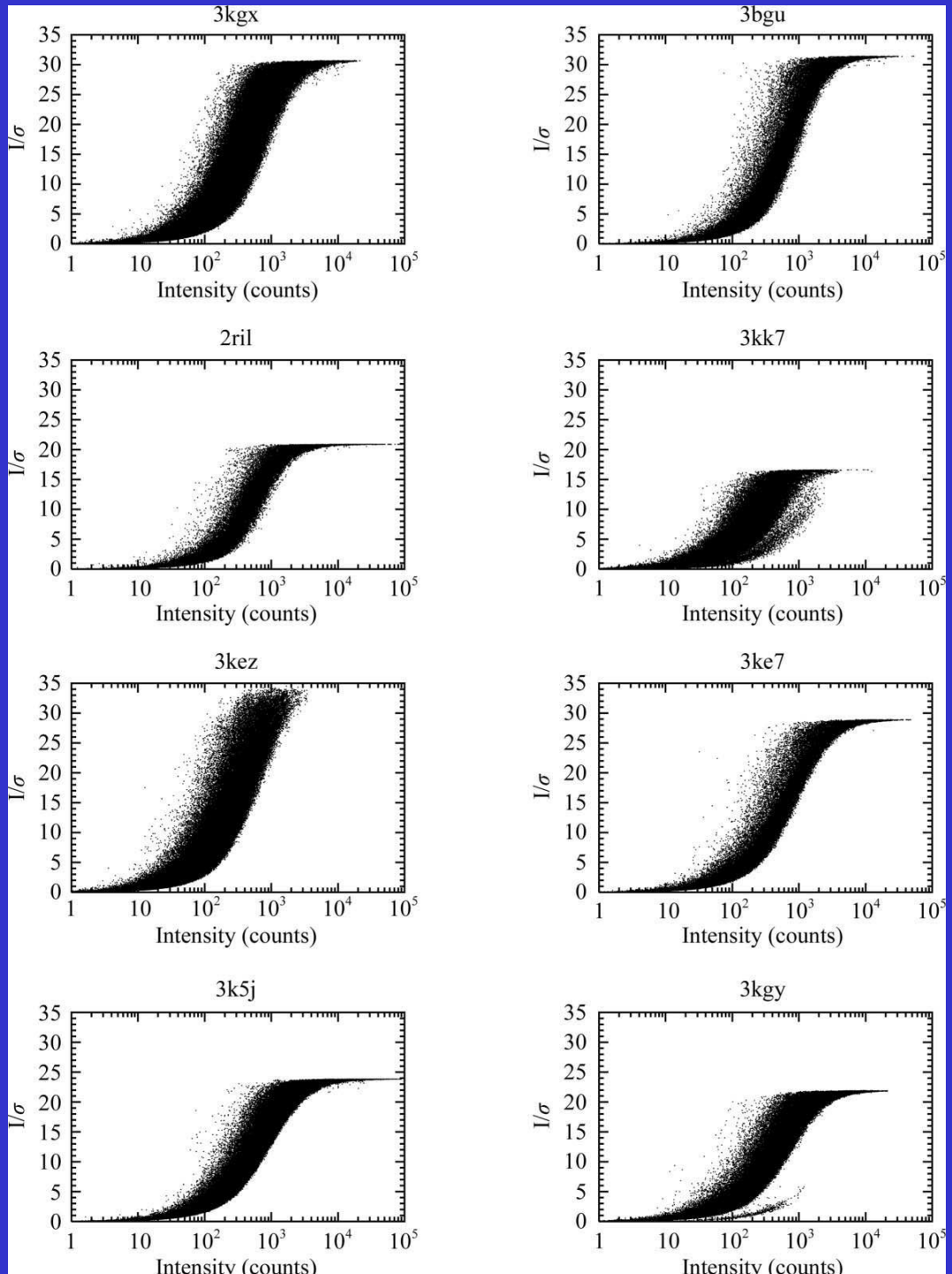
IDXREF.LP - how many lattices? r.m.s.d. between observed and calculated positions?

INTEGRATE.LP - by frame: scales, mosaicity, cell …
FRAME.cbf

CORRECT.LP – shutter stats; spacegroup-related stats; R-factors and I/sigma; twinning; ISa = $(I/\sigma)_{asymptotic}$
MODPIX.cbf, DECAY.cbf, ABSORP.cbf

XDSSTAT.LP (use *loggraph*) – by frame: R-factors, I/sigma, # rejections, ...
scales.pck, rf.pck, R_d

ISa = (I/sigma)$_{asymptotic}$



K. Diederichs

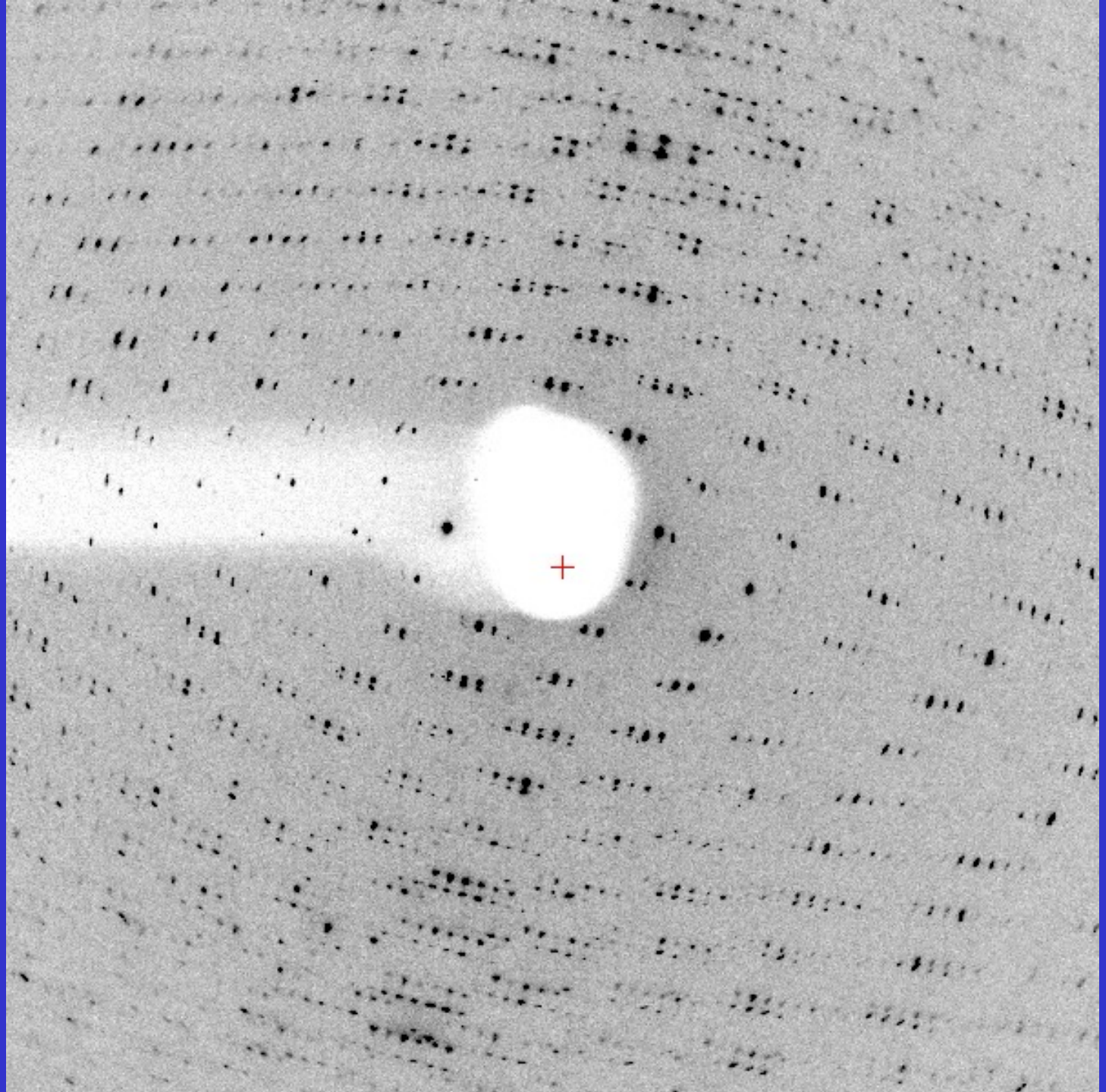
"Quantifying instrument errors in macromolecular X-ray data sets"

Acta Cryst. (2010). D66, 733–740

3CSL: membrane protein complex between heme receptor HasR and its hemophore HasA, plus heme (my lab); SeMet MAD @ 3.5 Å; 2*(865+206) residues / ASU

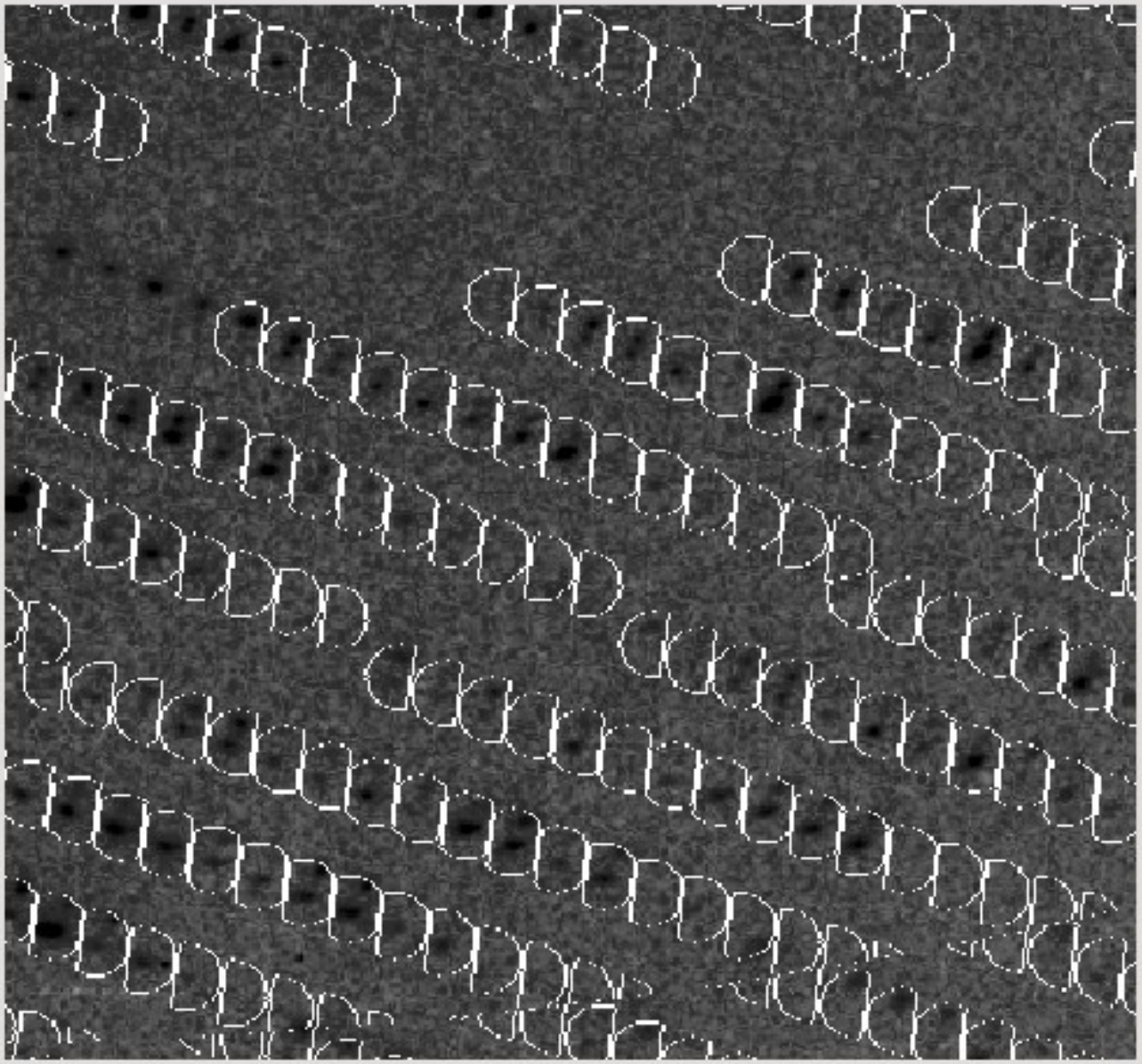
- cracked main xtal, plus smaller xtal
- frames 1-180: IDREF assigns 2898 of the 3000 strongest reflections (59% of all refs) to the major lattice (19% to minor). INTEGRATE is robust enough to give useful data, based on the major lattice.
- 3x3 CCD detector: weaker intensities (80%) at corners of segments; needs special ("MODULATION") correction in scaling, otherwise poor anom signal.

3CSL
frame
closeup



File Help

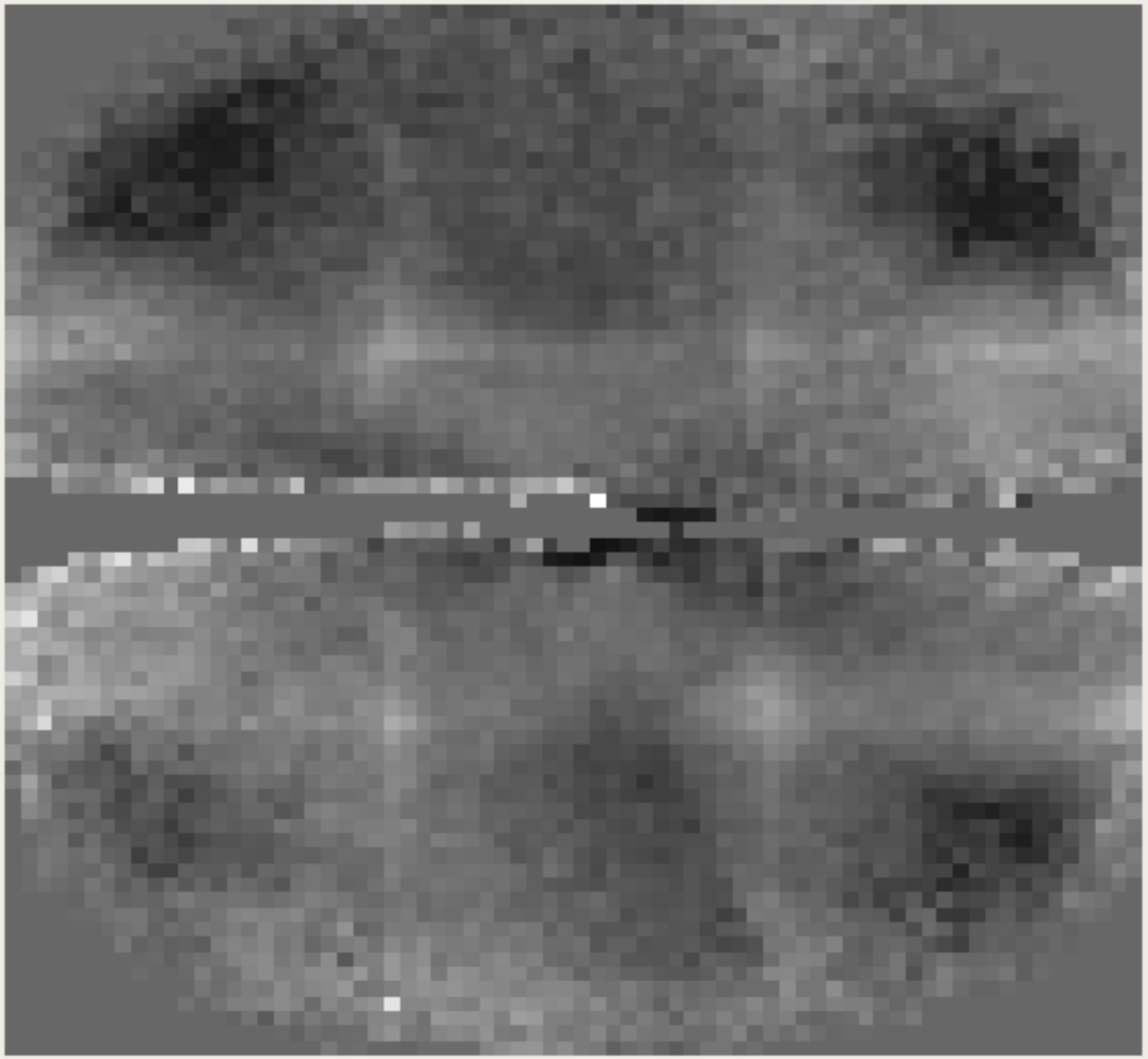
3CSL: FRAME.cbf



3CSL: MODPIX.cbf

File Help

View



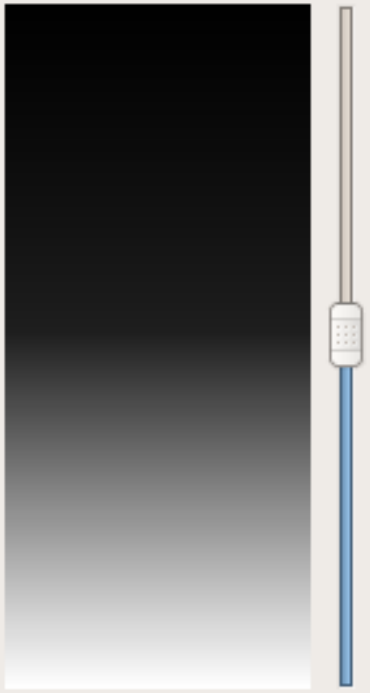
Pixel Value:

x=24, y=23
value=823

Slope:

100 OK

Colors:



1G1C: 2*99 residues / ASU; simulated data (James Holton); 100 datasets of 15 frames each @ 1.8 Å

- batch processing; can easily be scripted
- cell has a=b: don't let the cell fool you w.r.t. the spacegroup decision, it's the symmetry that counts!
- best data with the 100 first frames only
- anom signal incomplete: tweak data processing to include additional weak data & take care of radiation damage

1Y13: 3*181 residues / ASU, SGPP (Jürgen Bosch), SeMet-SAD @ 2.2Å

- problems: information “SeMet data collected at two different wavelengths (and two different beamlines)” inconsistent with results of data processing; radiation damage; ice rings
- diagnostics:
  a) frame-by-frame scalefactors (INTEGRATE): be alerted by jumps
  b) correlation coefficients (XSCALE): be critical towards given information, find better model
  c) R_d plot (XDSSTAT): radiation damage; this can be nicely corrected by XSCALE (0-dose extrapolation)

2QVO: 95 residues/ASU; SECSG (James T. Swindell), S-SAD @ 2.1 Å

- structure was already solved by me 2 years ago; it was indeed more difficult at that time and required more effort
- with current XDS version and default parameters, a straightforward solution is obtained from each dataset; even half of a dataset is enough
- *a posteriori* analyis (documented in XDSwiki): decreasing the number of misfits from the 2.5% of observations obtained with default WFAC1 to the intended 1 % of observations, the best CC All/Weak becomes 44.9 / 22.8 (from 37.3 / 21.4), and the number of successful trials goes from 60% to 91% . **One should note that all internal quality indicators get worse when increasing WFAC1 - but the external ones got significant better!**

2VB1: lysozyme in P1, 0.65Å native data

- straightforward in principle
- don't forget to use corners of detector for low-resol runs (TRUSTED_REGION)
- shadows on the detector need to be removed; there are several keywords for this: UNTRUSTED_RECTANGLE, UNTRUSTED_ELLIPSE, MINIMUM_VALID_PIXEL_VALUE. This requires inspection of frames.

Thank you!

These slides are at
http://strucbio.biologie.uni-konstanz.de/~dikay/XDS_Diederichs_ACA2011.pdf