1Y13

From XDSwiki

The structure is deposited (http://www.rcsb.org/pdb/explore/explore.do?structureId=1Y13) in the PDB, solved with SAD and refined at a resolution of 2.2 A in spacegroup P4(3)2(1)2 (#96). The data for this project were provided by Jürgen Bosch (SGPP) and are linked to the ACA 2011 workshop website (http://bl831.als.lbl.gov/example_data_sets/ACA2011/DPWTP-website/index.html) . There are two high-resolution (2 Å) datasets E1 (wavelength 0.9794Å) and E2 (@ 0.9174Å) collected (with 0.25° increments) at an ALS beamline on June 27, 2004, and a weaker dataset collected earlier at a SSRL beamline. We will only use the former two datasets here.

Contents

Dataset E1

Use generate_XDS.INP and run xds once. Based on R-factors in the resulting CORRECT.LP, and an inspection of BKGPIX.cbf, I modified XDS.INP to have

```
INCLUDE_RESOLUTION_RANGE=40 2.1                      ! too weak beyond 2.1 Å
VALUE_RANGE_FOR_TRUSTED_DETECTOR_PIXELS=8000. 30000.  ! raised from 7000 30000 to mask beamstop
```

and ran xds again.

What's the problem?

This is the excerpt from CORRECT.LP :

```
SPACE-GROUP         UNIT CELL CONSTANTS            UNIQUE   Rmeas  COMPARED  LATTICE-
  NUMBER      a      b      c   alpha beta gamma                             CHARACTER

      5     145.8  145.7  131.4  90.0  90.0  90.0    9735    24.5    23176    10 mC
     75     103.1  103.1  131.4  90.0  90.0  90.0    5262    23.4    27649    11 tP
     89     103.1  103.1  131.4  90.0  90.0  90.0    2911    22.8    30000    11 tP
     21     145.7  145.8  131.4  90.0  90.0  90.0    5270    23.2    27641    13 oC
      5     145.7  145.8  131.4  90.0  90.0  90.0    9681    24.2    23230    14 mC
      1     102.9  103.2  131.4  90.0  90.0  89.9   18040     6.9    14871    31 aP
 *   16     102.9  103.2  131.4  90.0  90.0  90.0    5568     9.1    27343    32 oP
      3     103.2  102.9  131.4  90.0  90.0  90.0   10536     9.5    22375    35 mP
      3     102.9  103.2  131.4  90.0  90.0  90.0   10496     8.3    22415    33 mP
      3     102.9  131.4  103.2  90.0  90.1  90.0    9770     7.3    23141    34 mP
      1     102.9  103.2  131.4  90.0  90.0  90.1   18040     6.9    14871    44 aP

...

REFINED PARAMETERS:  DISTANCE BEAM ORIENTATION CELL AXIS
USING  219412 INDEXED SPOTS
STANDARD DEVIATION OF SPOT    POSITION (PIXELS)     1.01
STANDARD DEVIATION OF SPINDLE POSITION (DEGREES)    0.11
CRYSTAL MOSAICITY (DEGREES)     0.191
DIRECT BEAM COORDINATES (REC. ANGSTROEM)  -0.004789  0.003758  1.021015
DETECTOR COORDINATES (PIXELS) OF DIRECT BEAM    1027.25   1064.20
DETECTOR ORIGIN (PIXELS) AT                     1036.84   1056.68
CRYSTAL TO DETECTOR DISTANCE (mm)       209.38
LAB COORDINATES OF DETECTOR X-AXIS  1.000000  0.000000  0.000000
LAB COORDINATES OF DETECTOR Y-AXIS  0.000000  1.000000  0.000000
LAB COORDINATES OF ROTATION AXIS  0.999997  0.000527  0.002187
COORDINATES OF UNIT CELL A-AXIS    21.922    52.895    85.337
COORDINATES OF UNIT CELL B-AXIS     3.771    87.158   -54.992
COORDINATES OF UNIT CELL C-AXIS  -128.130    18.914    21.191
REC. CELL PARAMETERS   0.009731  0.009697  0.007620  90.000  90.000  90.000
UNIT CELL PARAMETERS    102.766   103.125   131.241  90.000  90.000  90.000
E.S.D. OF CELL PARAMETERS  1.3E-01 8.6E-02 9.3E-02 0.0E+00 0.0E+00 0.0E+00
SPACE GROUP NUMBER     16
```

So CORRECT chooses an orthorhombic spacegroup.

The file continues:

```
...
    a        b          ISa
6.058E+00  3.027E-04   23.35

...

     NOTE:      Friedel pairs are treated as different reflections.

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION
RESOLUTION     NUMBER OF REFLECTIONS    COMPLETENESS R-FACTOR  R-FACTOR COMPARED I/SIGMA   R-meas  Rmrgd-F  Anomal  S
  LIMIT     OBSERVED  UNIQUE  POSSIBLE     OF DATA   observed  expected                                Corr

   6.23       17389    5807      6045       96.1%       2.4%      2.8%    17277   35.83     3.0%     2.0%    66%   1
   4.43       32116   10536     10787       97.7%       2.7%      3.0%    32057   33.78     3.3%     2.4%    55%   1
   3.62       41900   13700     13961       98.1%       3.4%      3.4%    41793   27.98     4.1%     3.6%    38%   1
   3.14       51146   16371     16513       99.1%       5.4%      5.3%    50967   18.89     6.6%     7.2%    20%   0
   2.81       59159   18627     18675       99.7%      12.7%     13.2%    58877    9.82    15.4%    18.0%     8%   0
   2.56       65525   20596     20651       99.7%      28.5%     30.2%    65130    5.19    34.5%    40.4%     3%   0
   2.37       71579   22491     22533       99.8%      62.6%     67.1%    71068    2.60    75.6%    88.8%     1%   0
   2.22       74065   23837     24094       98.9%      97.9%     97.0%    73444    1.59   118.8%   139.8%    11%   0
   2.09       65776   24379     25674       95.0%     133.3%    140.6%    63647    0.90   166.4%   216.0%     1%   0
  total      478655  156344    158933       98.4%       6.5%      6.8%   474260   10.65     7.9%    22.5%    16%   0


NUMBER OF REFLECTIONS IN SELECTED SUBSET OF IMAGES  492346
NUMBER OF REJECTED MISFITS                           13342
NUMBER OF SYSTEMATIC ABSENT REFLECTIONS                  0
NUMBER OF ACCEPTED OBSERVATIONS                     479004
NUMBER OF UNIQUE ACCEPTED REFLECTIONS               157108
```

Some comments:

- the "STANDARD DEVIATION OF SPOT POSITION (PIXELS)" is significantly higher (1.01) than those reported for the 5°-batches in INTEGRATE.LP (about 0.6) . This suggests that the geometry refinement has to deal with inconsistent data.
- CORRECT obviously indicates an orthorhombic spacegroup.

- the number of MISFITS is higher than 1%. From the first long table (fine-grained in resolution) table in CORRECT.LP we learn that the misfits are due to faint high-resolution ice rings - so this is a problem intrinsic to the data, and not to their mode of processing.

To my surprise, pointless does not agree with CORRECT's standpoint:

```
Scores for each symmetry element

Nelmt  Lklhd  Z-cc    CC        N  Rmeas    Symmetry & operator (in Lattice Cell)

  1   0.959   9.91   0.99   65030  0.034     identity
  2   0.959   9.91   0.99  132222  0.035 *** 2-fold l ( 0 0 1)  {-h,-k,+l}
  3   0.958   9.87   0.99  110073  0.044 *** 2-fold h ( 1 0 0)  {+h,-k,-l}
  4   0.942   9.55   0.96  132646  0.109 *** 2-fold   ( 1 1 0)  {+k,+h,-l}
  5   0.958   9.87   0.99  111819  0.043 *** 2-fold k ( 0 1 0)  {-h,+k,-l}
  6   0.941   9.54   0.95  131842  0.109 *** 2-fold   ( 1-1 0)  {-k,-h,-l}
  7   0.937   9.50   0.95  224393  0.107 *** 4-fold l ( 0 0 1)  {-k,+h,+l} {+k,-h,+l}
```

and

```
    Laue Group        Lklhd   NetZc  Zc+   Zc-    CC    CC-  Rmeas   R-  Delta ReindexOperator

> 1  P 4/m m m  ***  1.000   9.73  9.73  0.00   0.97  0.00   0.07  0.00   0.2 [h,k,l]
- 2    P m m m       0.000   0.35  9.88  9.53   0.99  0.95   0.04  0.11   0.0 [h,k,l]
  3    C m m m       0.000  -0.02  9.72  9.74   0.97  0.97   0.07  0.07   0.2 [h+k,-h+k,l]
  4      P 4/m       0.000   0.07  9.77  9.70   0.98  0.97   0.06  0.08   0.2 [h,k,l]
  5  P 1 2/m 1       0.000   0.25  9.91  9.66   0.99  0.97   0.03  0.08   0.0 [-h,-l,-k]
  6  P 1 2/m 1       0.000   0.22  9.89  9.67   0.99  0.97   0.04  0.08   0.0 [h,k,l]
  7  P 1 2/m 1       0.000   0.21  9.88  9.67   0.99  0.97   0.04  0.08   0.0 [-k,-h,-l]
  8  C 1 2/m 1       0.000  -0.01  9.72  9.73   0.97  0.97   0.07  0.07   0.2 [h-k,h+k,l]
  9  C 1 2/m 1       0.000  -0.02  9.71  9.73   0.97  0.97   0.07  0.07   0.2 [h+k,-h+k,l]
 10       P -1       0.000   0.21  9.91  9.70   0.99  0.97   0.03  0.08   0.0 [h,k,l]
```

and

```
  Spacegroup         TotProb SysAbsProb     Reindex         Conditions

  <P 41 21 2> ( 92)    0.823  0.823                        00l: l=4n, h00: h=2n (zones 1,2)
  <P 43 21 2> ( 96)    0.823  0.823                        00l: l=4n, h00: h=2n (zones 1,2)
   ..........
   <P 4 21 2> ( 90)    0.095  0.095                        h00: h=2n (zone 2)
   ..........
  <P 42 21 2> ( 94)    0.077  0.077                        00l: l=2n, h00: h=2n (zones 1,2)
```

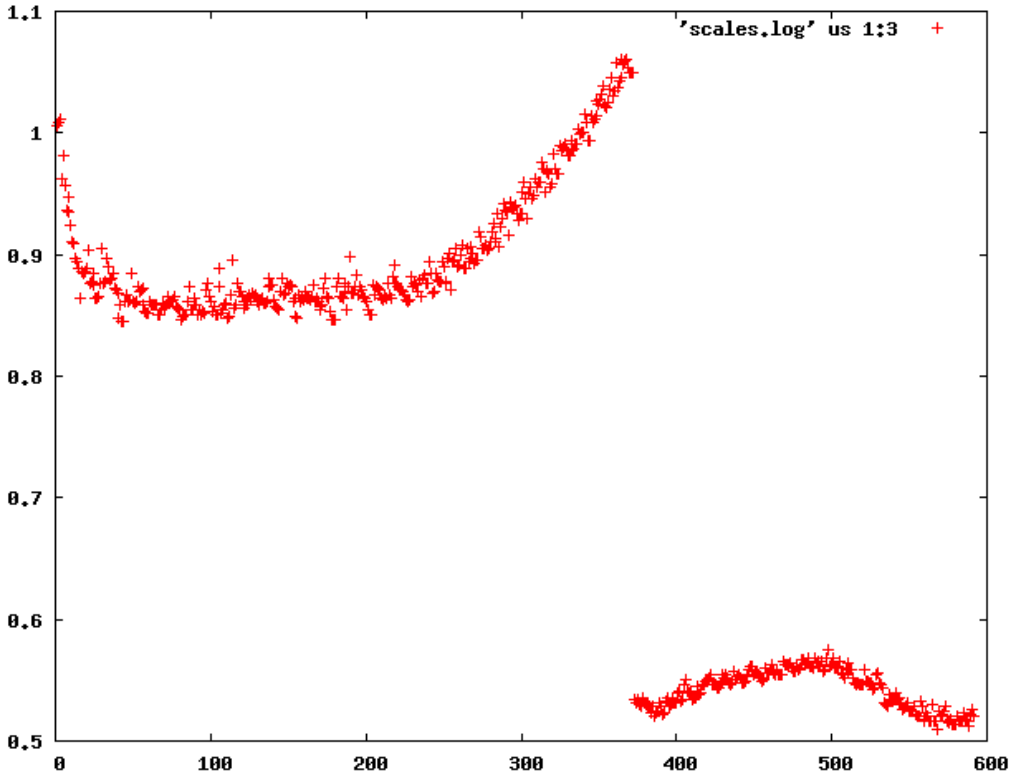
Thus suggesting #92 or #96 - the latter of which agrees with the PDB deposition. However, running CORRECT in #96 and specifying 103 103 130 90 90 90 as cell parameters, we obtain:

```
REFINED PARAMETERS:  DISTANCE BEAM ORIENTATION CELL AXIS
USING  220320 INDEXED SPOTS
STANDARD DEVIATION OF SPOT    POSITION (PIXELS)     1.17
STANDARD DEVIATION OF SPINDLE POSITION (DEGREES)    0.14
CRYSTAL MOSAICITY (DEGREES)     0.191
DIRECT BEAM COORDINATES (REC. ANGSTROEM)  -0.004790  0.004009  1.021014
DETECTOR COORDINATES (PIXELS) OF DIRECT BEAM    1027.19   1064.23
DETECTOR ORIGIN (PIXELS) AT                     1036.79   1056.20
CRYSTAL TO DETECTOR DISTANCE (mm)       209.52
LAB COORDINATES OF DETECTOR X-AXIS  1.000000  0.000000  0.000000
LAB COORDINATES OF DETECTOR Y-AXIS  0.000000  1.000000  0.000000
LAB COORDINATES OF ROTATION AXIS  0.999996  0.000901  0.002534
COORDINATES OF UNIT CELL A-AXIS    21.926    53.087    85.553
COORDINATES OF UNIT CELL B-AXIS     3.794    87.060   -54.995
COORDINATES OF UNIT CELL C-AXIS  -128.212    18.926    21.115
REC. CELL PARAMETERS   0.009704  0.009704  0.007616  90.000  90.000  90.000
UNIT CELL PARAMETERS    103.045   103.045   131.310  90.000  90.000  90.000
E.S.D. OF CELL PARAMETERS  2.1E-01 2.1E-01 2.1E-01 0.0E+00 0.0E+00 0.0E+00
SPACE GROUP NUMBER     96

...

   a        b          ISa
7.890E+00  8.793E-04   12.01

...

    NOTE:      Friedel pairs are treated as different reflections.

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION
RESOLUTION     NUMBER OF REFLECTIONS    COMPLETENESS R-FACTOR  R-FACTOR COMPARED I/SIGMA   R-meas  Rmrgd-F  Anomal  S
  LIMIT     OBSERVED  UNIQUE  POSSIBLE     OF DATA   observed  expected                                 Corr

    6.23       16770    2983      3017       98.9%       5.2%      6.1%    16752   26.20     5.7%     2.6%    55%   1
    4.43       30598    5392      5393      100.0%       5.8%      6.2%    30596   25.25     6.3%     3.0%    50%   1
    3.62       39822    6992      6994      100.0%       6.9%      6.6%    39820   22.27     7.6%     4.0%    32%   0
    3.14       49620    8240      8242      100.0%       9.2%      8.7%    49619   17.14    10.1%     6.2%    19%   0
    2.81       59388    9379      9379      100.0%      17.7%     18.1%    59387   10.44    19.3%    12.3%     0%   0
    2.56       65652   10308     10310      100.0%      34.6%     39.1%    65652    6.08    37.7%    23.6%    -1%   0
    2.37       71744   11258     11259      100.0%      71.3%     83.8%    71744    3.23    77.6%    52.1%    -2%   0
    2.22       74888   12065     12082       99.9%     111.0%    116.9%    74888    1.98   121.2%    86.9%     2%   0
    2.09       65727   12386     12874       96.2%     151.3%    176.1%    65517    1.12   168.0%   148.4%    -3%   0
   total      474209   79003     79550       99.3%      10.3%     11.0%   473975    9.44    11.3%    17.2%    13%   0


NUMBER OF REFLECTIONS IN SELECTED SUBSET OF IMAGES  492346
NUMBER OF REJECTED MISFITS                           17898
NUMBER OF SYSTEMATIC ABSENT REFLECTIONS                141
NUMBER OF ACCEPTED OBSERVATIONS                     474307
NUMBER OF UNIQUE ACCEPTED REFLECTIONS                79022
```

which is much worse than the spacegroup 19 statistics (compare the ISa values - they differ by a factor of 2 !) so there may be something wrong with some assumptions we were making ...

Identifying a possible cause

The easiest thing one can do is to inspect INTEGRATE.LP - this lists scale factor, beam divergence and mosaicity for every reflection. There's a jiffy called "scalefactors" which grep's the relevant lines from INTEGRATE.LP ("scalefactors > scales.log"). This shows the scale factor (column 3):



demonstrating that "something happens" between frame 372 and 373 (of course one has to look at the table to find the exact numbers).

It should be noted that any abrupt change in conditions during the experiment is going to spoil the resulting data in one way or another. This is most true for a SAD experiment which is supposed to give accurate values for the tiny differences in intensities between Friedel-related reflections.

A solution

At this point it is good to look at the data for experiment E2. Here, we find exactly the same problems of bad ISa and high "STANDARD DEVIATION OF SPOT POSITION (PIXELS)" when reducing frames 1-591 in one run of xds.

With this knowledge, we are lead, for E1, to reduce frames 1-372 and 373-592 separately, in spacegroup 96. For E2, we use frames 1-369 and 371-591, respectively. Frame E2-370 has a very high scale factor so we leave it out altogether.

This is also a good time to closely inspect the headers of the frames:

```
% grep --binary-files=text DATE j1603b3PK_1_E1_37?.img
```

gives

```
j1603b3PK_1_E1_370.img:DATE=Sun Jun 27 08:55:51 2004;
j1603b3PK_1_E1_371.img:DATE=Sun Jun 27 08:56:00 2004;
j1603b3PK_1_E1_372.img:DATE=Sun Jun 27 08:56:08 2004;
j1603b3PK_1_E1_373.img:DATE=Sun Jun 27 09:19:45 2004;
j1603b3PK_1_E1_374.img:DATE=Sun Jun 27 09:19:54 2004;
j1603b3PK_1_E1_375.img:DATE=Sun Jun 27 09:20:02 2004;
j1603b3PK_1_E1_376.img:DATE=Sun Jun 27 09:20:10 2004;
j1603b3PK_1_E1_377.img:DATE=Sun Jun 27 09:20:58 2004;
j1603b3PK_1_E1_378.img:DATE=Sun Jun 27 09:21:08 2004;
j1603b3PK_1_E1_379.img:DATE=Sun Jun 27 09:21:17 2004;
```

and

```
% grep --binary-files=text DATE j1603b3PK_1_E2_3[67]?.img
```

gives

```
j1603b3PK_1_E2_366.img:DATE=Sun Jun 27 08:55:15 2004;
j1603b3PK_1_E2_367.img:DATE=Sun Jun 27 08:55:23 2004;
j1603b3PK_1_E2_368.img:DATE=Sun Jun 27 08:55:32 2004;
j1603b3PK_1_E2_369.img:DATE=Sun Jun 27 08:56:19 2004;
j1603b3PK_1_E2_370.img:DATE=Sun Jun 27 08:56:28 2004;
j1603b3PK_1_E2_371.img:DATE=Sun Jun 27 09:19:26 2004;
j1603b3PK_1_E2_372.img:DATE=Sun Jun 27 09:19:34 2004;
j1603b3PK_1_E2_373.img:DATE=Sun Jun 27 09:20:22 2004;
j1603b3PK_1_E2_374.img:DATE=Sun Jun 27 09:20:30 2004;
j1603b3PK_1_E2_375.img:DATE=Sun Jun 27 09:20:38 2004;
j1603b3PK_1_E2_376.img:DATE=Sun Jun 27 09:20:47 2004;
```

thus proving that both datasets were interrupted for 20 minutes around frame 370.

The really weird thing here is that both datasets appear to be collected at the same time, but at different wavelengths (E1 at 0.9794 Å, E2 at 0.9184 Å), and yet the individual parts merge as follows: using the following XSCALE.INP:

```
UNIT_CELL_CONSTANTS=103.316   103.316   131.456  90.000  90.000  90.000
SPACE_GROUP_NUMBER=96
OUTPUT_FILE=temp.ahkl
INPUT_FILE=../e1_1-372/XDS_ASCII.HKL
INPUT_FILE=../e1_373-592/XDS_ASCII.HKL
INPUT_FILE=../e2_1-369/XDS_ASCII.HKL
INPUT_FILE=../e2_371-591/XDS_ASCII.HKL
```

and running xscale, we obtain in XSCALE.LP:

```
   CORRELATIONS BETWEEN INPUT DATA SETS AFTER CORRECTIONS

DATA SETS  NUMBER OF COMMON  CORRELATION   RATIO OF COMMON   B-FACTOR
 #i   #j     REFLECTIONS     BETWEEN i,j  INTENSITIES (i/j)  BETWEEN i,j

   1    2       15943           0.978            1.0002         0.0106
   1    3       22366           1.000            1.0012        -0.0008
   2    3       15801           0.977            0.9983         0.0557
   1    4       15648           0.979            0.9988         0.0541
   2    4       14862           0.999            1.0024        -0.0007
   3    4       15524           0.978            0.9999        -0.0015
```

which means that e1_1-372 correlates well (1.000) with e2_1-369, and e1_373-59 well (0.999) with e2_371-591, but the crosswise correlations are consistently low (0.978, 0.977, 0.979, 0.978). The adjustment to the error model proves this:

```
    a        b          ISa    ISa0   INPUT DATA SET
6.112E+00  1.429E-03   10.70   22.37 ../e1_1-372/XDS_ASCII.HKL
1.074E+01  1.825E-03    7.14   23.79 ../e1_373-592/XDS_ASCII.HKL
5.707E+00  1.621E-03   10.40   22.82 ../e2_1-369/XDS_ASCII.HKL
8.547E+00  1.796E-03    8.07   24.17 ../e2_371-591/XDS_ASCII.HKL
```

telling us that "if we merge these datasets together, their error estimates have to be increased a lot". However, if we switch to

```
UNIT_CELL_CONSTANTS=103.316   103.316   131.456  90.000  90.000  90.000
SPACE_GROUP_NUMBER=96

OUTPUT_FILE=firstparts.ahkl
INPUT_FILE=../e1_1-372/XDS_ASCII.HKL
INPUT_FILE=../e2_1-369/XDS_ASCII.HKL

OUTPUT_FILE=secondparts.ahkl
INPUT_FILE=../e1_373-592/XDS_ASCII.HKL
INPUT_FILE=../e2_371-591/XDS_ASCII.HKL
```

we obtain

```
    a        b          ISa    ISa0   INPUT DATA SET
6.120E+00  3.673E-04   21.09   22.37 ../e1_1-372/XDS_ASCII.HKL
5.713E+00  3.819E-04   21.41   22.82 ../e2_1-369/XDS_ASCII.HKL
5.639E+00  3.151E-04   23.72   23.79 ../e1_373-592/XDS_ASCII.HKL
5.289E+00  3.258E-04   24.09   24.17 ../e2_371-591/XDS_ASCII.HKL
```

proving that the second parts of datasets E1 and E2 should be treated separately from the first parts.

Upon inspection of the cell parameters, we find that the cell axes of the second "halfs" are shorter by a factor of 0.9908 when compared with the first parts. This suggests that they were collected at a longer wavelength! But then the wavelength values in the headers are most likely completely wrong: we can speculate that the two first parts were collected at the SeMet peak wavelength, and the two second parts at the inflection wavelength.

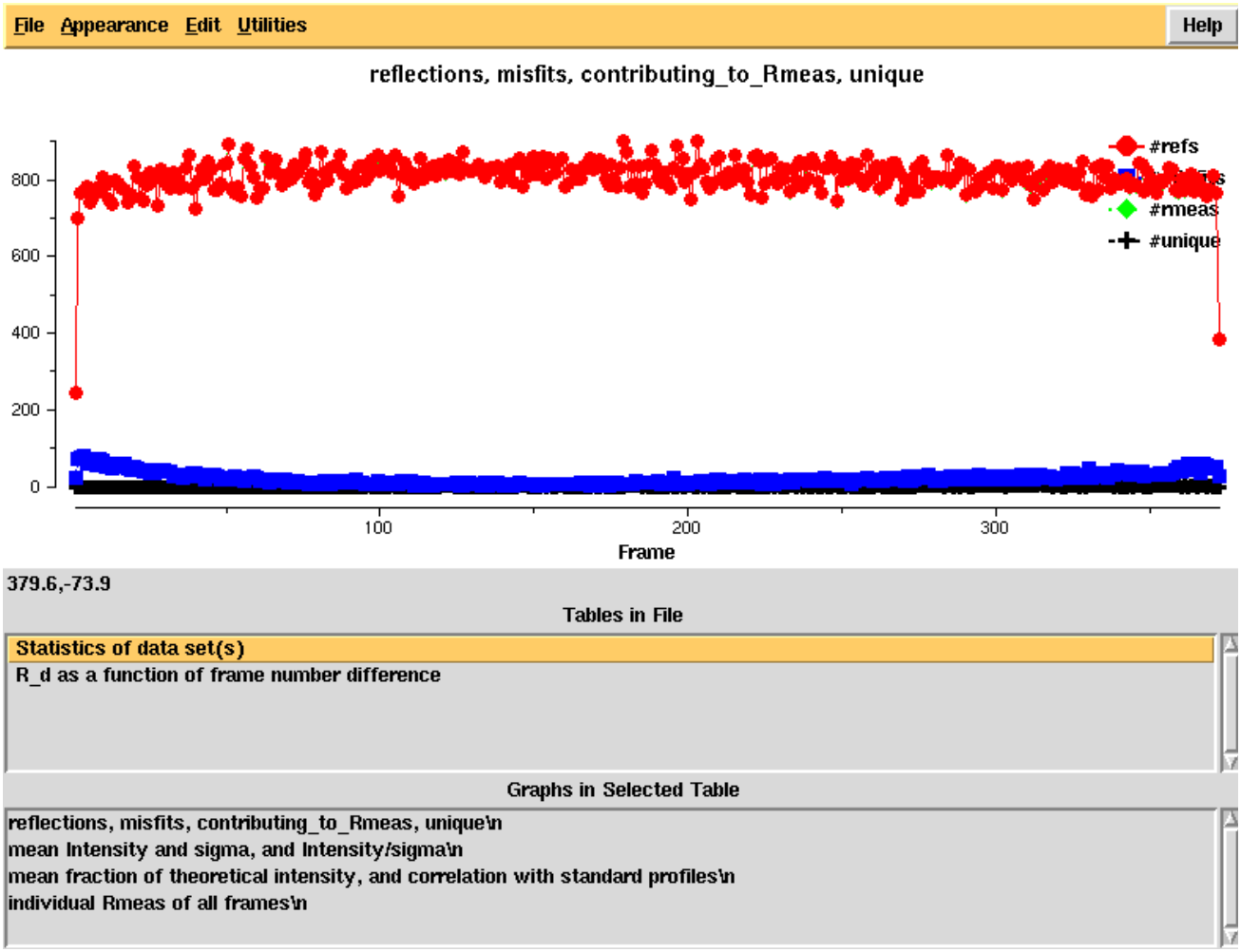
The almost-simultaneous DATEs in the headers may be explained by an inverse-beam measuring strategy which alternatingly collects 4 frames in one orientation as E1, then rotates the spindle by 180° and collects 4 frames into E2. For some reason, the beamline software did not write the correct wavelength into the headers.

So this little detective work appears to tell us what happened in the morning of Sunday June 27, 2004 at ALS beamline 821.

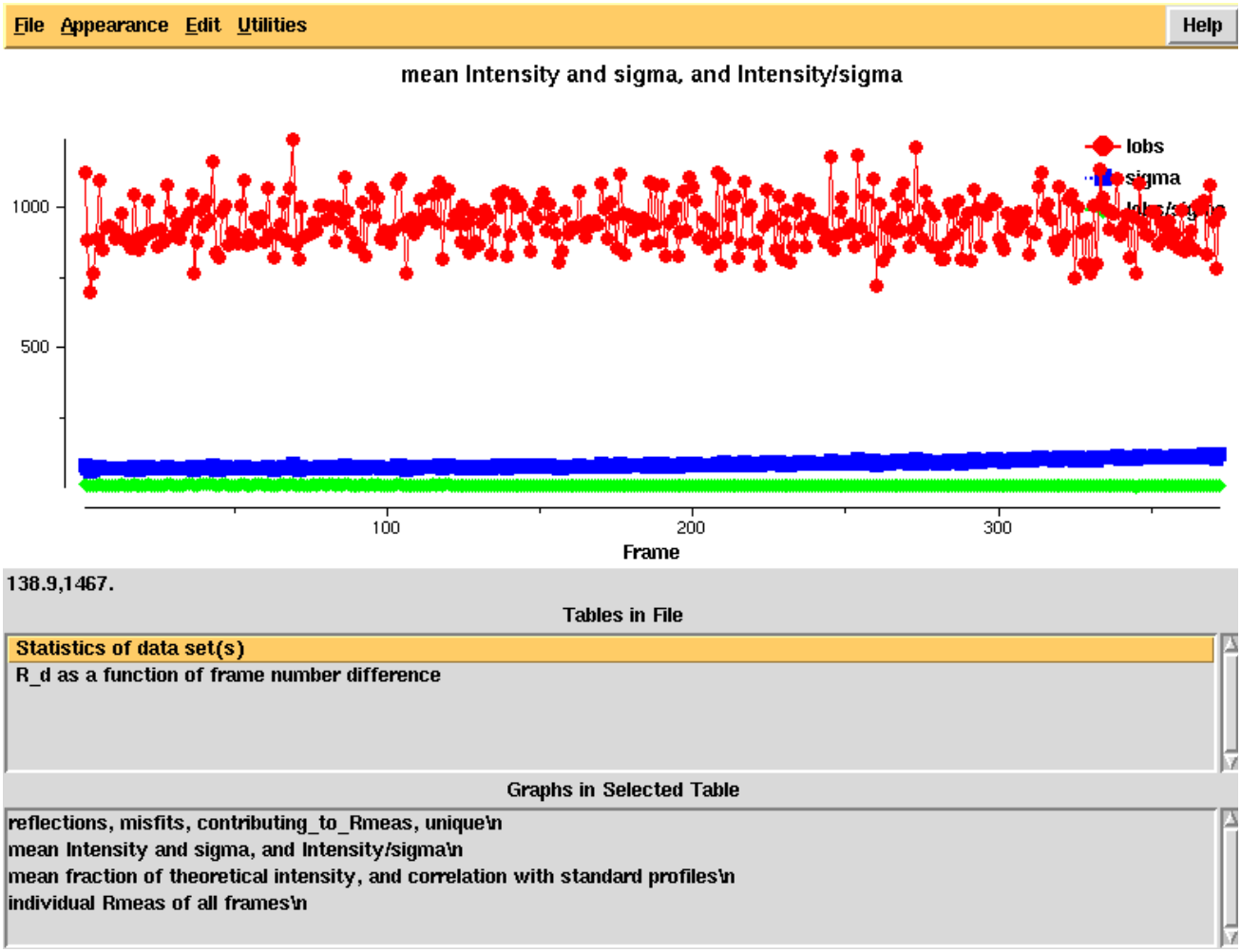
Further analysis of datasets E1 and E2

Here, we try to learn more about the constituents of "firstparts".

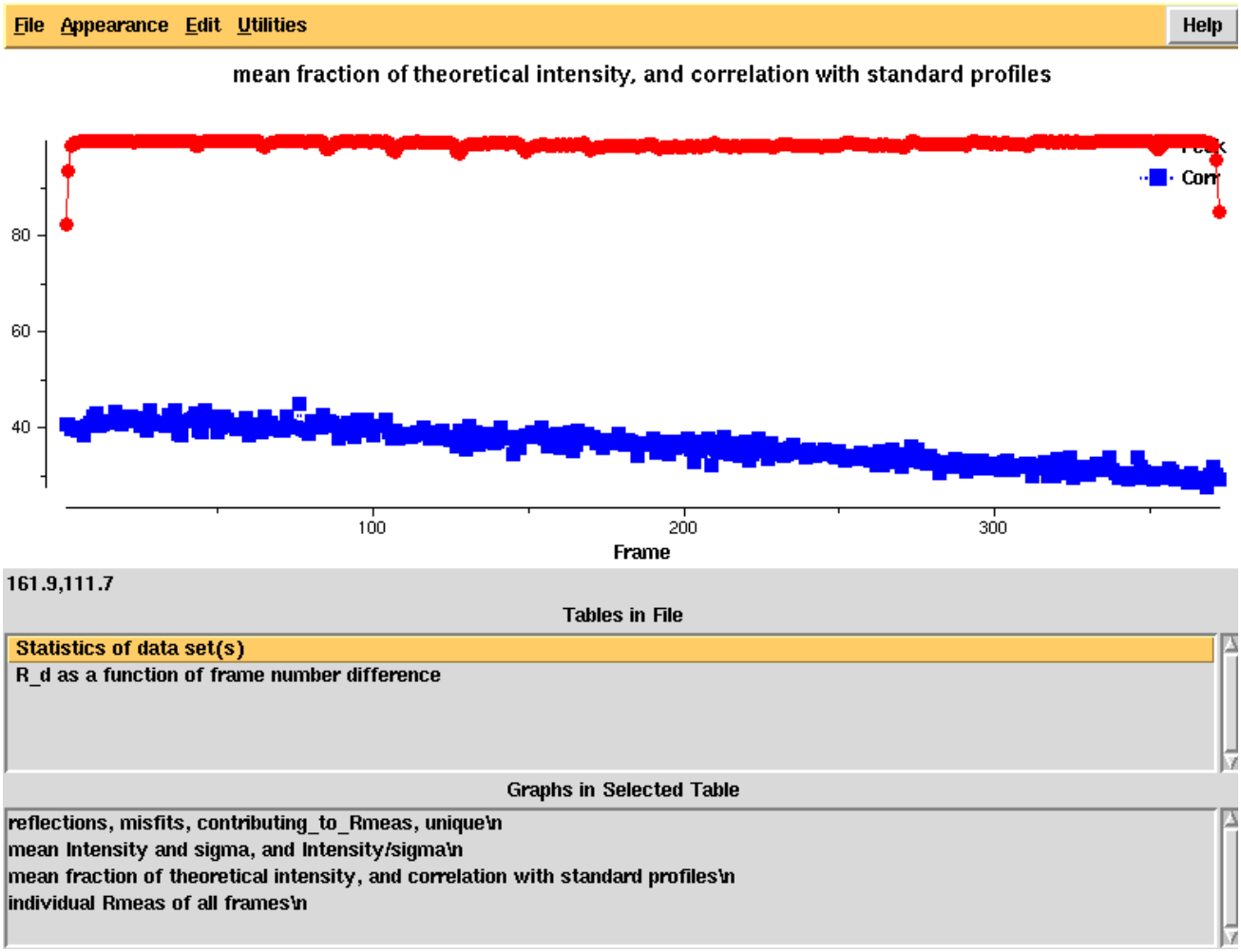
Running "xdsstat > XDSSTAT.LP" in the e1_1-372 and e2_1-369 directories, we obtain statistics output not available from CORRECT. We open XDSSTAT.LP with the CCP4 program "loggraph", and take a look at misfits.pck, rf.pck, and the other files produced by xdsstat, using VIEW or XDS-Viewer:



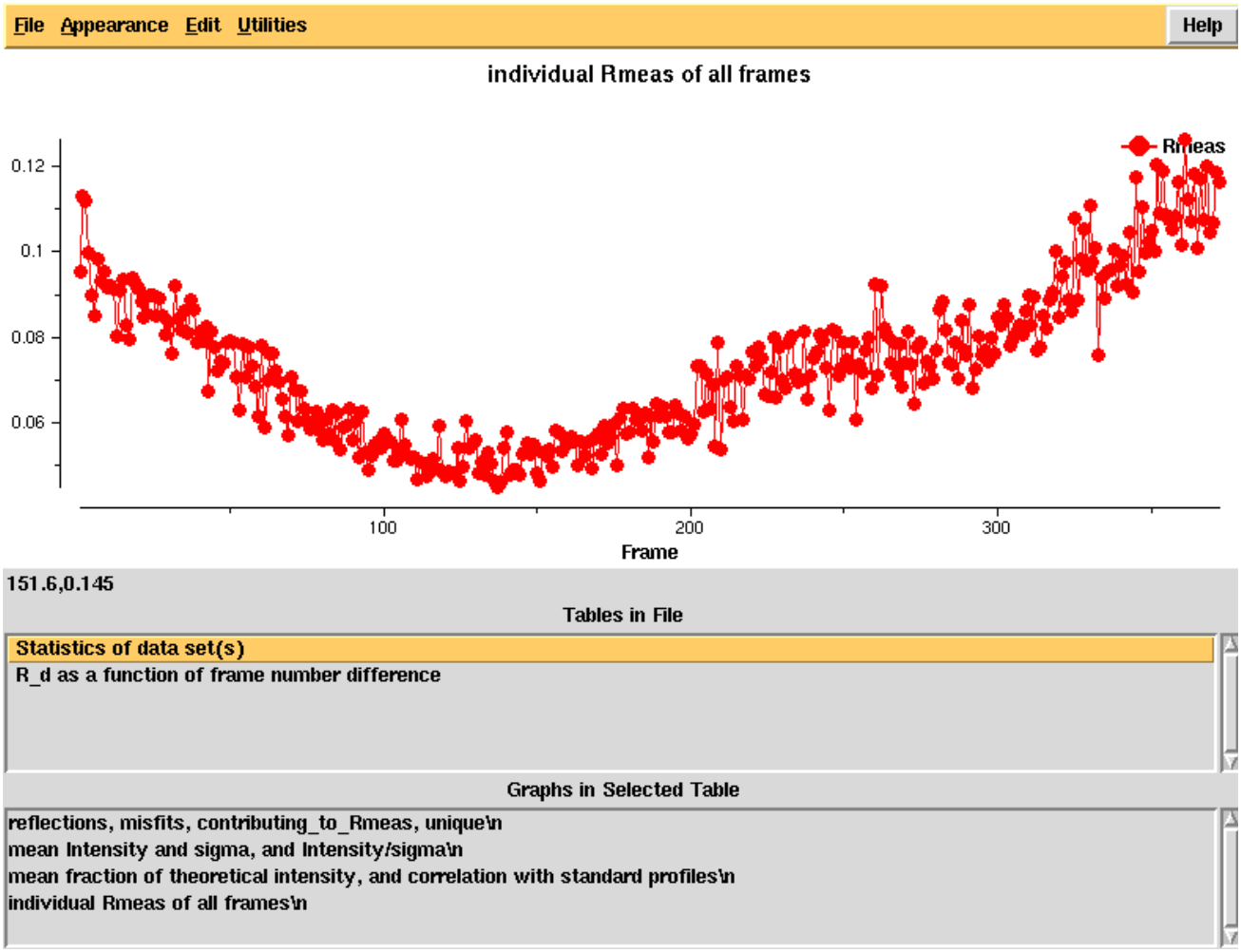
Reflections and misfits, by frame - looks normal



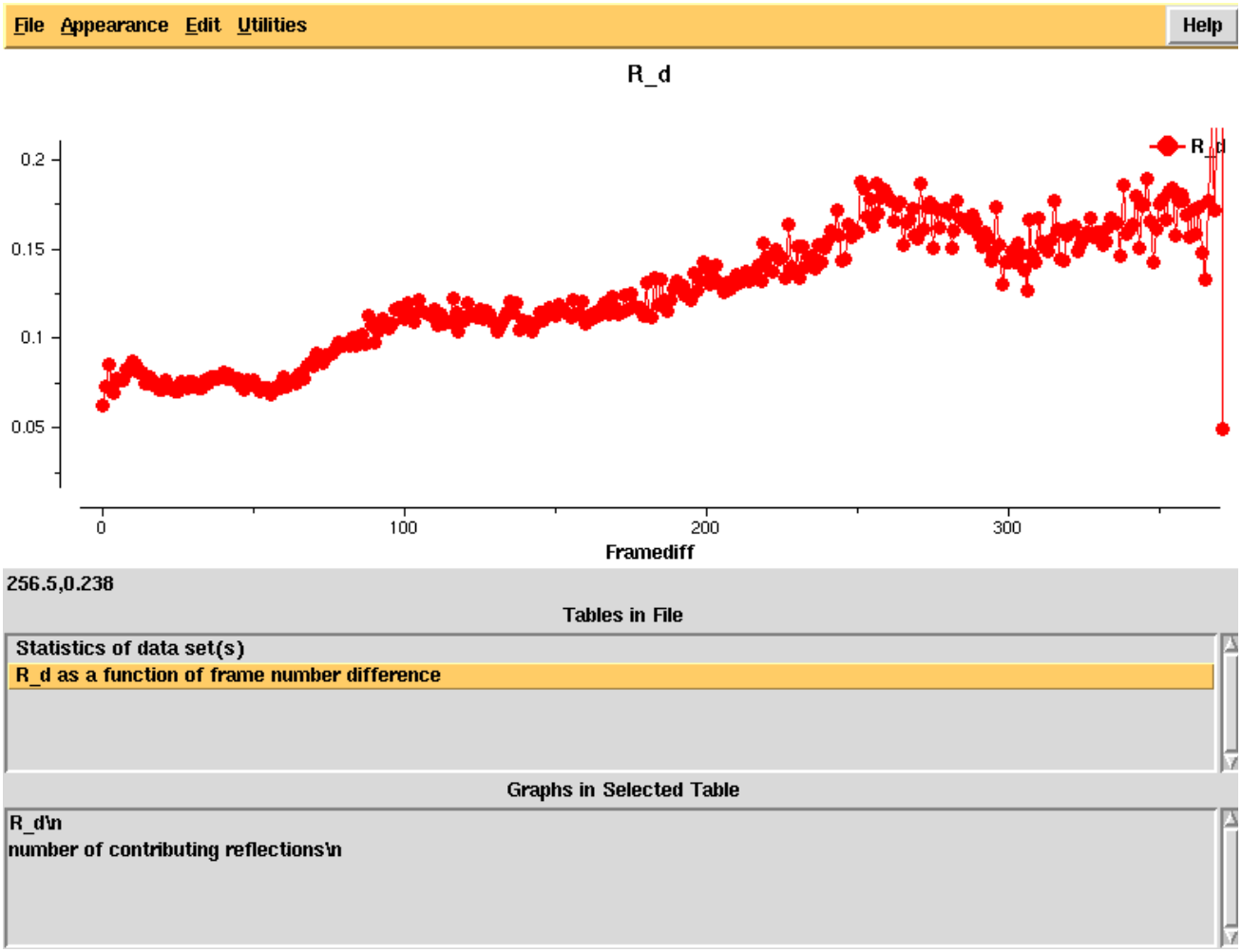
Intensity and sigma by frame - looks normal



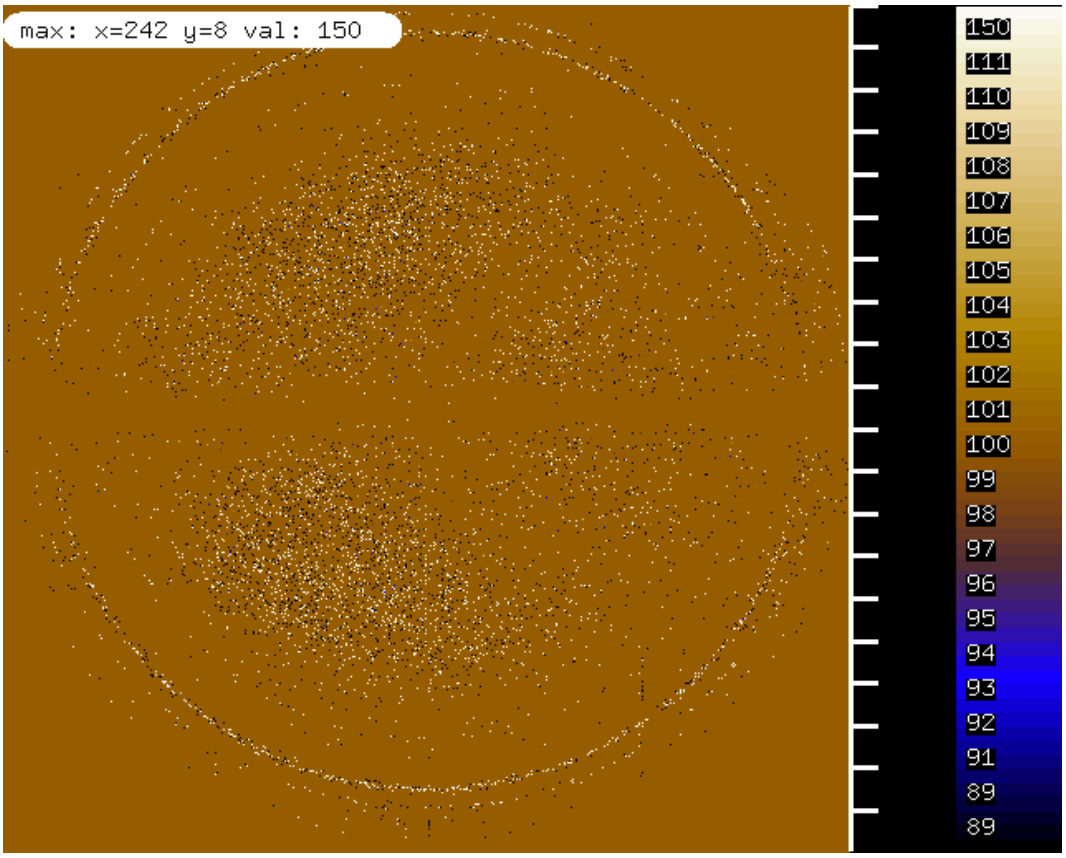
"partiality" and profile agreement, by frame - looks good but it's clear that the profiles at high frame number agree worse with the average profiles, possibly due to radiation damage



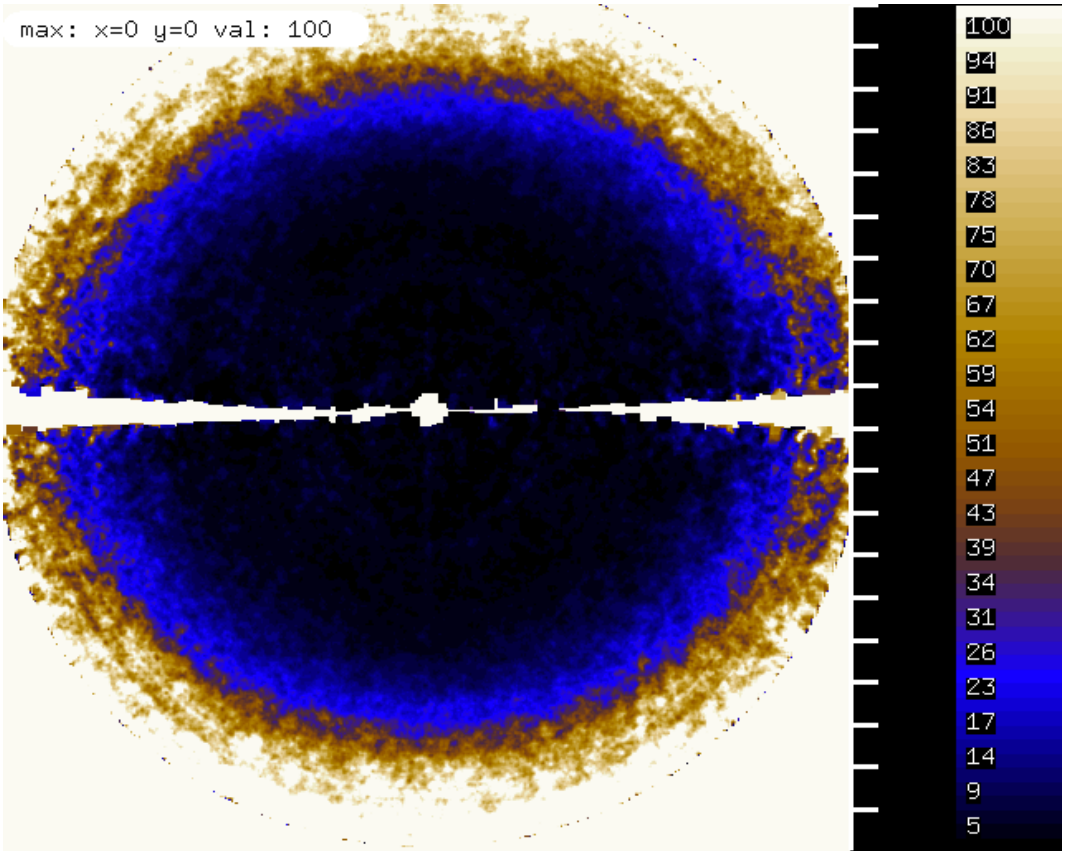
R_meas, by frame, clearly showing good R_meas in the middle of the dataset



R_d - an R-factor which directly depends on radiation damage. This is calculated as a function of frame number difference and the linear rise indicates significant radiation damage that should be correctable in XSCALE, using the CRYSTAL_NAME keyword.



misfits mapped on the detector, showing ice rings.



R_meas mapped on the detector, showing elevated R_meas at the location of the ice rings.

Solving the structure

Although we could now think of using these two files ("firstparts" and "secondparts" merged) and assume that they are peak and inflection wavelengths, it appears more reasonable to try and solve the structure with SAD - which means using "firstparts" only.

First try

Let's look at the XSCALE statistics for "firstparts":

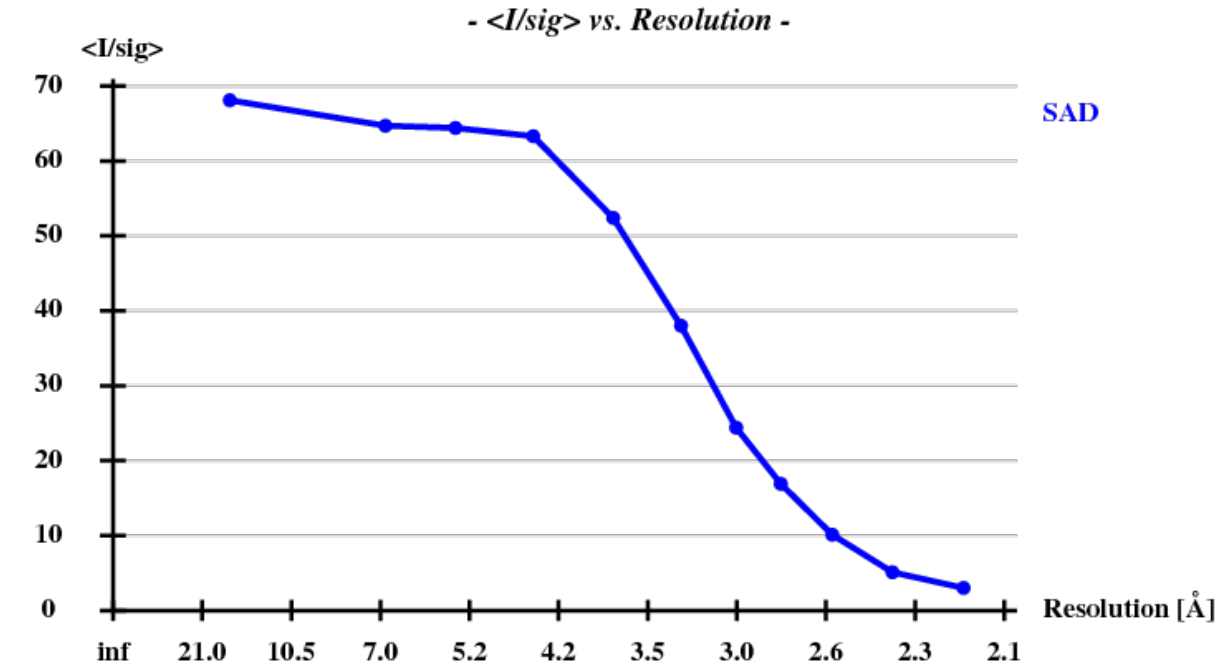
```
     NOTE:      Friedel pairs are treated as different reflections.

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION
RESOLUTION     NUMBER OF REFLECTIONS    COMPLETENESS R-FACTOR  R-FACTOR COMPARED I/SIGMA   R-meas  Rmrgd-F  Anomal  S
  LIMIT     OBSERVED  UNIQUE  POSSIBLE     OF DATA   observed  expected                                 Corr

   9.40        6122     844       883       95.6%       2.9%      3.5%     6111   54.76     3.2%     1.4%    79%   2
   6.64       12037    1611      1621       99.4%       2.9%      3.6%    12035   51.54     3.1%     1.5%    80%   2
   5.43       15348    2065      2086       99.0%       3.5%      3.7%    15347   47.79     3.7%     1.7%    78%   2
   4.70       18714    2487      2498       99.6%       3.0%      3.7%    18711   49.55     3.2%     1.5%    72%   1
   4.20       21104    2797      2821       99.1%       3.1%      3.7%    21102   47.24     3.3%     1.7%    72%   1
   3.84       23316    3095      3117       99.3%       3.8%      4.0%    23313   42.74     4.1%     2.1%    65%   1
   3.55       25693    3345      3366       99.4%       4.4%      4.5%    25693   37.93     4.7%     2.6%    50%   1
   3.32       28017    3633      3653       99.5%       5.2%      5.2%    28015   32.89     5.6%     3.6%    40%   1
   3.13       30266    3842      3848       99.8%       7.2%      7.2%    30264   25.87     7.7%     4.8%    36%   1
   2.97       32595    4114      4118       99.9%      10.4%     10.4%    32594   19.26    11.1%     7.7%    30%   1
   2.83       34384    4315      4320       99.9%      14.3%     14.8%    34382   14.88    15.3%    10.3%    20%   0
   2.71       35654    4475      4478       99.9%      18.3%     19.1%    35652   12.13    19.5%    13.1%    15%   0
   2.61       37307    4705      4710       99.9%      27.5%     28.8%    37304    8.44    29.4%    19.8%    11%   0
   2.51       38997    4893      4896       99.9%      35.5%     38.0%    38997    6.78    38.0%    26.0%    10%   0
   2.43       40036    5026      5027      100.0%      51.3%     55.1%    40032    4.92    54.8%    38.0%     2%   0
   2.35       39975    5180      5222       99.2%      71.3%     68.9%    39967    3.78    76.4%    52.7%    21%   0
   2.28       42041    5385      5423       99.3%      93.7%     93.1%    42037    2.90   100.3%    66.7%    11%   0
   2.21       43012    5538      5541       99.9%      85.7%     88.3%    43011    2.87    91.8%    58.8%    10%   0
   2.16       42610    5701      5703      100.0%     113.6%    120.7%    42607    2.13   122.0%    85.4%     4%   0
   2.10       38996    5634      5912       95.3%     146.1%    153.9%    38944    1.50   157.8%   122.7%     3%   0
  total      606224   78685     79243       99.3%       6.7%      7.2%   606118   16.88     7.2%    12.0%    29%   1
```

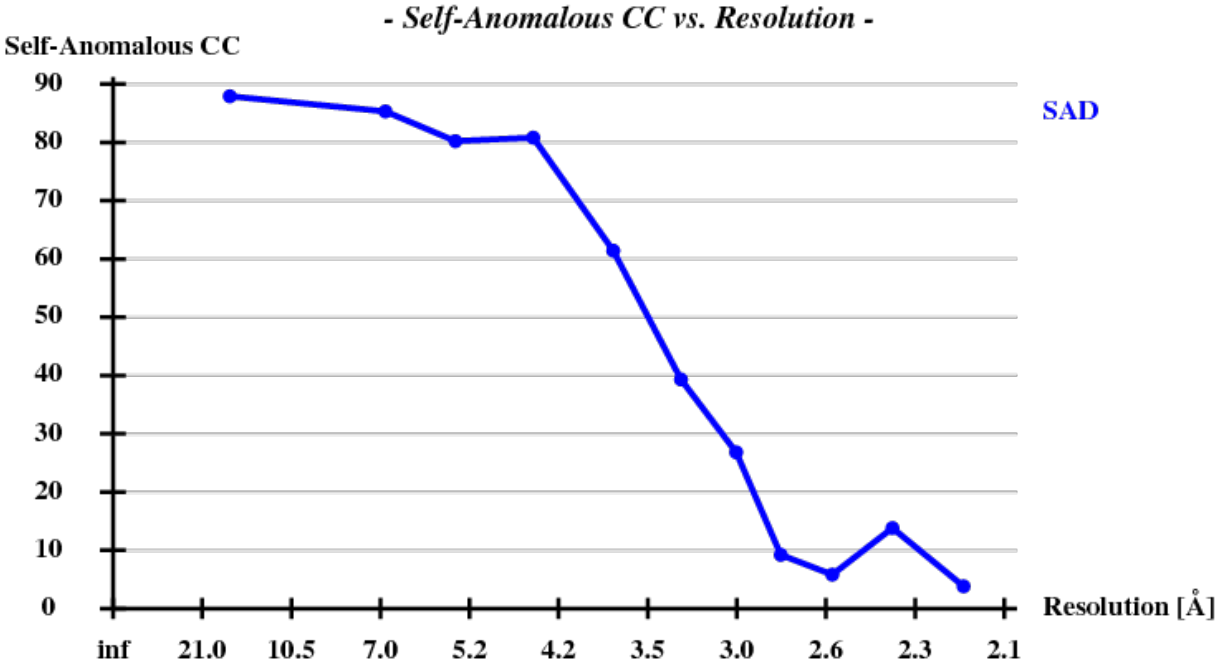
The anomalous correlation is good at low resolution, though not outstanding. At high resolution it rises again but this is presumably due to the ice rings.

I like to use hkl2map which runs SHELXC, SHELXD and SHELXE from its GUI. Before doing so, we have to run XDSCONV with the following XDSCONV.INP:

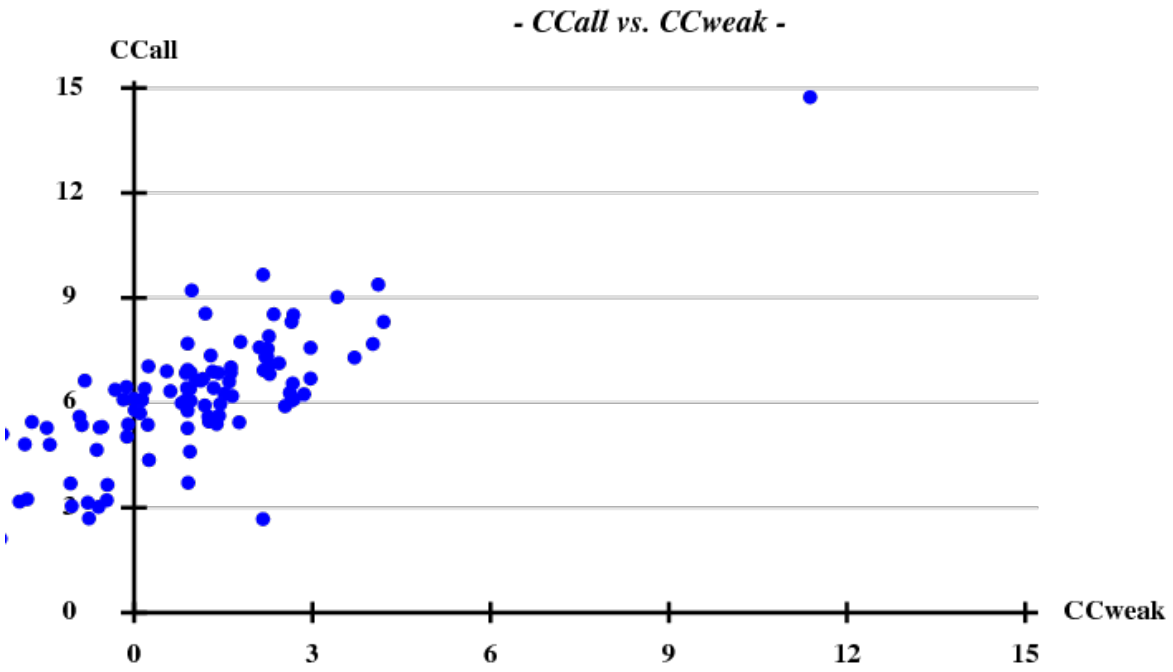
```
INPUT_FILE=firstparts.hkl
OUTPUT_FILE=temp.hkl SHELX
```

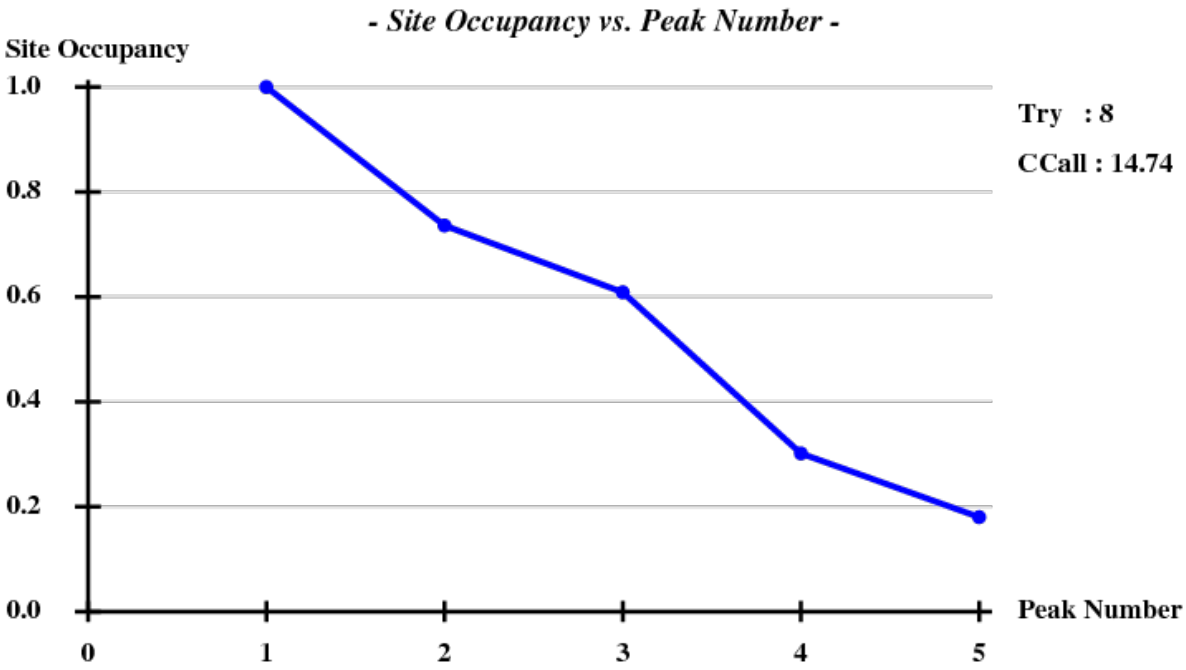
First, the shelxc output which shows that these data are quite good:





And then we show the result of 100 trials at substructure solution of shelxd, trying to find 3 Se atoms at 30 - 3.3Å resolution (I also tried 3.0 3.1 3.2 3.4 3.5 Å but 3.3 Å was best).





This looks reasonable although the absolute value of CCall is so low that there is little hope that the structure can be solved with this amount of information. And indeed, SHELXE did not show a difference between the two hands (in fact we even know that the "original hand" is the correct one since the inverted had would correspond to spacegroup #92 !).

Second try: correcting radiation damage by 0-dose extrapolation

Since we noted significant radiation damage we could try to correct that. All we have to do is ask XSCALE to do zero-dose extrapolation:

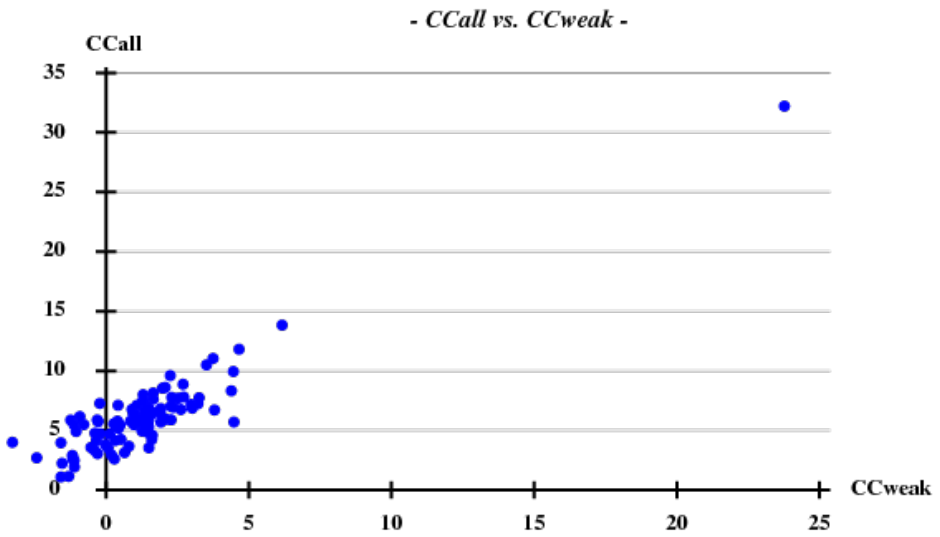
```
UNIT_CELL_CONSTANTS=103.316   103.316   131.456  90.000  90.000  90.000
SPACE_GROUP_NUMBER=96

OUTPUT_FILE=temp.ahkl
INPUT_FILE=../e1_1-372/XDS_ASCII.HKL
CRYSTAL_NAME=a
INPUT_FILE=../e2_1-369/XDS_ASCII.HKL
CRYSTAL_NAME=a
```

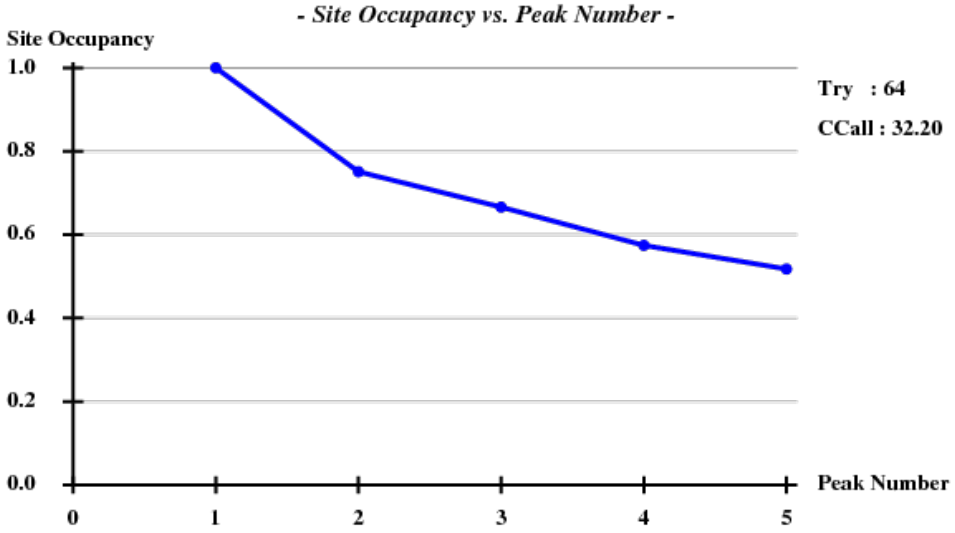
As a result we obtain:

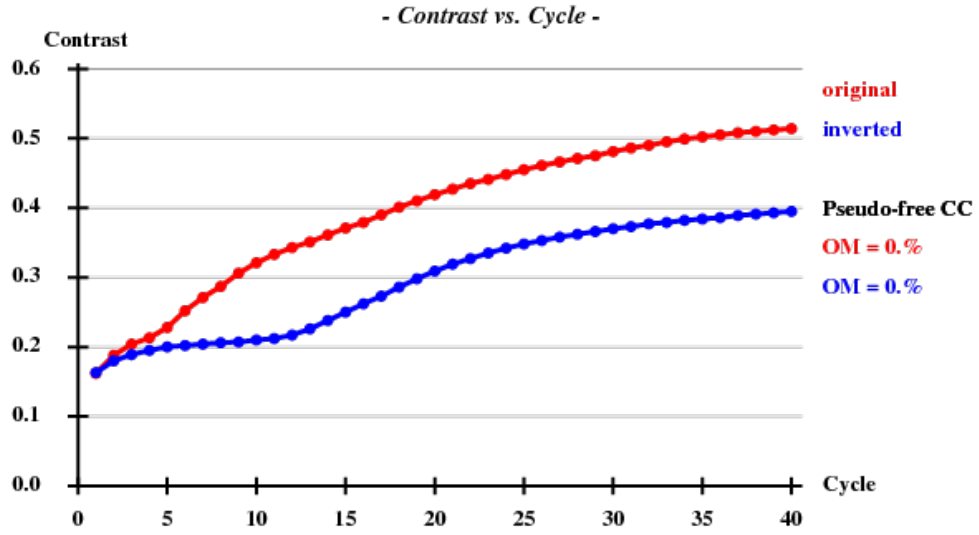
```
*****************************************************************************
         RESULTS FROM ZERO-DOSE EXTRAPOLATION OF REFLECTION INTENSITIES

                      for reference on this subject see:
K. Diederichs, S. McSweeney & R.B.G. Ravelli, Acta Cryst. D59, 903-909(2003).
"Zero-dose extrapolation as part of macromolecular synchrotron data reduction"
*****************************************************************************

Radiation damage can lead to localized modifications of the structure.
To correct for this effect, XSCALE modifies the intensity measurements
I(h,i) by individual correction factors,

                     exp{-b(h)*dose(h,i)}

where h,i denotes the i-th observation with unique reflection indices
h, and dose(h,i) the X-ray dose accumulated by the crystal when the
reflection was recorded. Assuming a constant dose for each image
(dose_rate), the accumulated dose when recording image_number(i), on
which I(h,i) was observed, is then

dose(h,i) = starting_dose + dose_rate * (image_number(i)-first_image)

The decay factor b(h) is determined from the assumption that symmetry
related reflections in a data set taken from the same crystal should
have the same intensity after correction. Moreover, b(h) is assumed to
be the same for Friedel-pairs and independent of the X-ray wavelength.

To avoid overfitting the data, XSCALE starts with the hypothesis that
b(h)=0 and rejects this assumption if its probability is below 10.0%.



CORRELATION OF COMMON DECAY-FACTORS BETWEEN INPUT DATA SETS
------------------------------------------------------------


First  INPUT_FILE= ../e2_1-369/XDS_ASCII.HKL
     CRYSTAL_NAME= a
Second INPUT_FILE= ../e1_1-372/XDS_ASCII.HKL
     CRYSTAL_NAME= a

RESOLUTION    NUMBER    CORRELATION
  LIMIT      OF PAIRS      FACTOR

    9.40         210        0.955
    6.64         441        0.955
    5.43         587        0.940
    4.70         692        0.969
    4.20         750        0.949
    3.84         836        0.920
    3.55         809        0.942
    3.32         775        0.925
    3.13         663        0.888
    2.97         557        0.837
    2.83         375        0.681
    2.71         302        0.812
    2.61         212        0.625
    2.51         163        0.508
    2.43          95        0.291
    2.35         139        0.722
    2.28         110        0.688
    2.21          91        0.734
    2.16          88        0.561
    2.10          54        0.126
   total        7949        0.788


          X-RAY DOSE PARAMETERS USED FOR EACH INPUT DATA SET
          --------------------------------------------------


CRYSTAL_NAME= a
       STARTING_DOSE             DOSE_RATE       NAME OF INPUT FILE
    initial    refined      initial    refined

  0.000E+00   8.557E+00   1.000E+00   1.000E+00  ../e1_1-372/XDS_ASCII.HKL
  0.000E+00   0.000E+00   1.000E+00   1.024E+00  ../e2_1-369/XDS_ASCII.HKL


          STATISTICS OF 0-DOSE CORRECTED DATA FROM EACH CRYSTAL
          -----------------------------------------------------

NUNIQUE = Number of unique reflections with enough symmetry-
          related observations to determine a decay factor b(h)
```

We not that the "CORRELATION OF COMMON DECAY-FACTORS BETWEEN INPUT DATA SETS" are really high which confirms the hypothesis that this is a valid procedure to perform.

Comparison of the last table with that of the previous paragraph, i.e. without zero-dose extrapolation, shows that the I/sigma, the anomalous correlation coefficients and the SigAno are significantly higher. Does this translate into better structure solution? It does:







Automatically building the main chain of 452 out of 519 residues

Based on the sites obtained by SHELXD, we run

```
shelxe.beta -a -q -h -b -s0.585 -m40 raddam raddam_fa
```

This already builds a significant number of residues, but also gives an improved list of heavy atom sites - there are actually 6 sites instead of the 5 that SHELXD wrote out (yes, we had asked SHELXD for 3 sites since there are 3 Met residues, but SHELXD as always was smarter than we are). We "mv raddam.hat raddam_fa.res" for another run of SHELXE:

```
shelxe.beta -a -q -h6 -b -s0.585 -m40 -n3 raddam raddam_fa
```

and get

```
  452 residues left after pruning, divided into chains as follows:
A:  15   B:   5   C:  22   D:  22   E:  27   F:  62   G: 263   H:  36

CC for partial structure against native data =  39.83 %

------------------------------------------------------------------------------

Global autotracing cycle   4

<wt> = 0.300, Contrast = 0.447, Connect. = 0.705 for dens.mod. cycle 1
<wt> = 0.300, Contrast = 0.660, Connect. = 0.781 for dens.mod. cycle 2
<wt> = 0.300, Contrast = 0.723, Connect. = 0.801 for dens.mod. cycle 3
<wt> = 0.300, Contrast = 0.762, Connect. = 0.807 for dens.mod. cycle 4
Pseudo-free CC = 64.88 %
<wt> = 0.300, Contrast = 0.785, Connect. = 0.810 for dens.mod. cycle 5
<wt> = 0.300, Contrast = 0.806, Connect. = 0.813 for dens.mod. cycle 6
<wt> = 0.300, Contrast = 0.820, Connect. = 0.815 for dens.mod. cycle 7
<wt> = 0.300, Contrast = 0.831, Connect. = 0.817 for dens.mod. cycle 8
<wt> = 0.300, Contrast = 0.839, Connect. = 0.819 for dens.mod. cycle 9
Pseudo-free CC = 69.74 %
<wt> = 0.300, Contrast = 0.845, Connect. = 0.820 for dens.mod. cycle 10
<wt> = 0.300, Contrast = 0.849, Connect. = 0.821 for dens.mod. cycle 11
<wt> = 0.300, Contrast = 0.851, Connect. = 0.822 for dens.mod. cycle 12
<wt> = 0.300, Contrast = 0.853, Connect. = 0.823 for dens.mod. cycle 13
<wt> = 0.300, Contrast = 0.854, Connect. = 0.823 for dens.mod. cycle 14
Pseudo-free CC = 70.80 %
<wt> = 0.300, Contrast = 0.854, Connect. = 0.824 for dens.mod. cycle 15
<wt> = 0.300, Contrast = 0.855, Connect. = 0.824 for dens.mod. cycle 16
<wt> = 0.300, Contrast = 0.855, Connect. = 0.824 for dens.mod. cycle 17
<wt> = 0.300, Contrast = 0.854, Connect. = 0.824 for dens.mod. cycle 18
<wt> = 0.300, Contrast = 0.854, Connect. = 0.824 for dens.mod. cycle 19
Pseudo-free CC = 71.03 %
<wt> = 0.300, Contrast = 0.854, Connect. = 0.824 for dens.mod. cycle 20

Estimated mean FOM and mapCC as a function of resolution
d    inf - 4.62 - 3.64 - 3.17 - 2.88 - 2.67 - 2.51 - 2.38 - 2.27 - 2.18 - 2.11
<FOM>   0.736  0.786  0.768  0.721  0.701  0.681  0.618  0.595  0.587  0.540
<mapCC> 0.862  0.932  0.946  0.934  0.924  0.924  0.922  0.913  0.882  0.858
N        4206   4227   4214   4135   4185   4207   4292   4406   4320   3702

Estimated mean FOM = 0.674   Pseudo-free CC = 71.18 %

Density (in map sigma units) at input heavy atom sites

 Site     x        y        z     occ*Z    density
   1   0.2276   0.7578   0.1189  34.0000    29.98
   2   0.1568   0.6345   0.3049  32.2898    30.44
   3   0.1767   0.5344   0.2160  32.2388    29.67
   4   0.3059   0.4535   0.1297  26.0746    23.51
   5   0.0280   0.8243   0.1410  22.7324    21.02
   6   0.0383   0.9748   0.0492  21.5050    21.18

Site    x       y       z  h(sig) near old  near new
  1  0.1569  0.6345  0.3048  30.4  2/0.02  9/13.36 3/15.73 2/19.52 7/22.13
  2  0.2278  0.7578  0.1188  30.0  1/0.02  1/19.52 6/21.97 7/22.48 9/25.02
  3  0.1767  0.5345  0.2158  29.7  3/0.03  9/2.90 1/15.73 4/19.45 2/26.88
  4  0.3060  0.4536  0.1292  23.5  4/0.07  3/19.45 9/21.16 8/26.49 5/26.83
  5  0.0382  0.9748  0.0490  21.2  6/0.02  8/2.63 8/15.66 5/15.88 6/19.80
  6  0.0278  0.8240  0.1416  21.1  5/0.08  5/19.80 8/21.59 7/21.87 2/21.97
  7  0.1854  0.9571  0.1787  -5.0  5/21.86  6/21.87 1/22.13 2/22.48 8/22.57
  8  0.0427  0.9993  0.0530  -5.0  6/2.62  5/2.63 8/15.31 5/15.66 6/21.59
  9  0.1787  0.5611  0.2228  -4.7  3/2.91  3/2.90 1/13.36 4/21.16 2/25.02
```

At this point the structure is obviously solved, and we could use buccanneer or Arp/wArp to add side chains and the rest of the model. 3-fold NCS surely helps!

Could we do better?

Yes, of course (as always). I can think of four things to try:

- an optimization round of running xds for the two datasets
- using a negative offset for STARTING_DOSE in XSCALE.INP, as documented in the XSCALE wiki article.
- use MERGE=TRUE in XDSCONV.INP. I tried it and this gives 20 solutions with CCall+CCweak > 25 out of 1000 trials, whereas MERGE=FALSE (the default) gives only 4 solutions!

- adding the "secondparts" data assuming this is a longer wavelength

But this time we learn that one has to take special care of the data in particular when they were measured by someone else who does not tell us everything we need to know. Second, zero-dose extrapolation made the day.

Availability of data

The XDS/XSCALE - produced data are available at 1y13-raddam-F.mtz (ftp://turn5.biologie.uni-konstanz.de/pub/xds-datared/1y13xds-1y13-raddam-F.mtz) (amplitudes) and 1y13-raddam-I.mtz (ftp://turn5.biologie.uni-konstanz.de/pub/xds-datared/1y13xds-1y13-raddam-I.mtz) (intensities). In addition I provide [1] (ftp://turn5.biologie.uni-konstanz.de/pub/xds-datared/e1_1-372_XDS_ASCII.HKL.bz2) and [2] (ftp://turn5.biologie.uni-konstanz.de/pub/xds-datared/e2_1-369_XDS_ASCII.HKL.bz2) to enable investigating based on the original XDS data.