Brookhaven National Laboratory, 22 May 2008
Workshop on Raw Image Formats in Structural Biology

The Importance of Standard Image Formats for Scientific Progress

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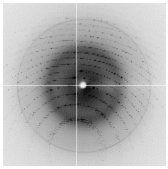
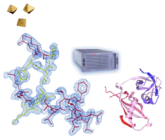
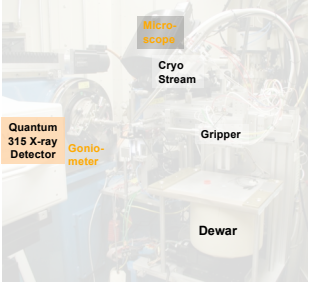




Sector 5 ALS Automounter



High Throughput Screening:
- Screen for best crystal growth conditions
- Select the highest-quality samples from a batch
- Discovery of drug leads and protein-ligand complexes
- Enable multi-crystal dataset acquisition
- Perform initial characterization with minimal radiation dose

Real-time Analysis:
- Autoindex
- Measure the model fit (rmsd)
- Limiting resolution
- Mosaicity
- Ice rings & other artifacts

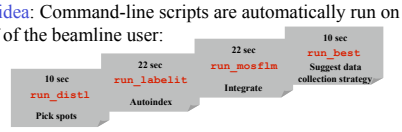
- **ALS-style puck: 112 Crystal Samples**
- **Beamline Operating System (BOS) control**
- **Liquid Nitrogen Autofill**

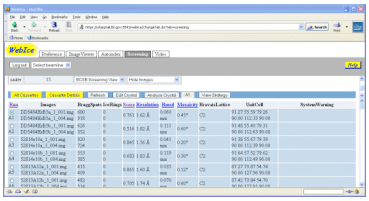
The challenge is to perform this analysis reliably in an automated setting!

Web-Ice: Images and screening results can be viewed both locally & over the Web

González (2008) **J Appl Cryst** 41:176

Basic idea: Command-line scripts are automatically run on behalf of the beamline user:





- *Crystal screening table is visible on a Web page and downloadable as an Excel spreadsheet*
- *Web-based image viewer*
- *The same tabulated results are visible within the beamline GUI: Blu-Ice at SSRL or BOS at ALS*
- *System is under evaluation at APS (GMCA-CAT, IMCA-CAT, NE-CAT)*

Review of Last Year's Presentation: Increasing the Reliability of Automated Image Processing

DISTL Zhang *et al.*(2006) **J Appl Cryst** 39:112

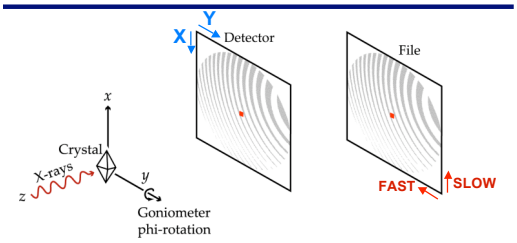
LABELIT Sauter *et al.*(2004) **J Appl Cryst** 37:399

- Macromolecular diffraction patterns are very diverse. Basic well-known algorithms (*e.g.*, cell reduction & autoindexing) had to be rewritten to cover outlying cases. Legacy software (pre-2003) relied heavily on human input to recognize the challenging cases.
- Writing a new set of programs also involved extending support to all of the detector formats that the software users requested.

Detector Vendors
- Successful support
  - ADSC Quantum 4, 210, 315
  - Mar CCD
  - Mar Image Plate
  - Rigaku Raxis IV and HTC
  - Rigaku Raxis II (transformat
  - Rigaku Saturn 92 CCD
  - MacScience DIP 2030b
  - Pilatus-6M
- Very limited success
  - Bruker Proteus CCD (1K x 1K) / proprietary spatial calibration
  - APS SBC 19BM / 19ID requires calibration file

Gerd Rosenbaum: "Does the CBF standard support a data entry that records the incident intensity at various time points during the angular rotation (e.g. at 0.2 second intervals)? This would allow us to apply different scaling factors for reflections on the same image."

Inter-Related Coordinate Systems



- Without a standard file format, the coordinate relationships must be worked out individually for each detector type.

Other Difficulties with Diverse File Formats

- Local keyword dialects. The openness of the ADSC file format has allowed different facilities to utilize conflicting keywords.
  - Berkeley Center uses conflicting "DENZO_BEAM_CENTER" and "BEAM_CENTER" tags
- Coordinate system relationships are unspecified
  - There are 8 possible relationships between Detector and File coordinate systems. For ADSC detectors, two of them are in common use at different synchrotrons. LABELIT needs to maintain a list, keyed by DETECTOR serial number.
- Unit of measure is unspecified
  - ESRF writes MAR CCD beam center in mm instead of pixel units
- Redundant information
  - Start phi, end phi, and delta phi all defined.

New Results: Support for CBF in LABELIT

- LABELIT is built on top of a core library of C++ crystallography algorithms, the "Computational Crystallography Toolbox" or **`cctbx`**. Open source: http://cctbx.sf.net
- An adaptor module, **`cbflib_adaptbx`** (CBF library adaptor toolbox) has been added to **`cctbx`**. CBFlib can now be compiled in as an optional dependency.**
- The complexities of CBF function are encapsulated within wrapper C++ classes. We expose <u>only</u> the limited set of features that will actually be used for file reading and data processing, although this could be extended at any time. Currently 800 lines.
- Memory management is handled by constructors and destructors.
- The C++ wrapper classes are exposed at the Python scripting level with Boost.Python bindings.
- Error-handling macros are redefined so that C++ exceptions will be thrown and handled by the user code; this is propagated up to Python.
- Use Python scripts to rapidly prototype new approaches for data processing.

A Python Example

```
> from iotbx.detectors import ImageFactory
> C = ImageFactory("./MB_LP_1_001.CBF")
> C.show_header()
File: ./MB_LP_1_001.CBF
Number of pixels: slow=3072 fast=3072
Pixel size: 0.102588 mm
Saturation: 65000
Detector distance: 200.00 mm
Detector 2theta swing: 0.00 deg.
Rotation start: 85.00 deg.
Rotation width: 1.00 deg.
Beam center x=157.52 mm  y=157.52 mm
Wavelength: 0.979381 Ang.
```

• *LABELIT* can index the example data provided by Chris Nielson & *MOSFLM* integration works.
• But: I have yet to see a single CBF dataset that is either a complete dataset (for structure solution), or that is collected by a scientific user.

miniCBF support: The Pilatus-6M

- What file formats does the Pilatus detector produce? And why?
- miniCBF format is not CBF.
- CBF examples include convert_miniCBF.c → does not quite work for the example cubic insulin dataset provided on the Pilatus web site.
- Header information is efficiently parsed by a 100-line python script.

```
###CBF: VERSION 1.5, CBFlib v0.7.8 - SLS/DECTRIS PILATUS detectors
data_sun2_1_00001.cif
_array_data.header_convention "SLS_1.0"
_array_data.header_contents

# Detector: PILATUS 6M, 60-0001, X06SA@SLS
# 2007/Oct/22 15:31:17.480
# Pixel_size 172e-6 m x 172e-6 m
# Silicon sensor, thickness 0.000320 m
# Exposure_time 0.395000 s
# Exposure_period 0.400000 s
# Tau = 124.7e-09 s
# Count_cutoff 1563782 counts
# Threshold_setting 6000 eV
# N_excluded_pixels = 949
# Excluded_pixels: BadPixelMap.tif
# Flat_field: (nil)
# Trim_directory: (nil)
# Wavelength 1.0332 A
# Energy_range (0, 0) eV
# Detector_distance 0.30000 m
# Detector_Voffset 0.00000 m
# Beam_xy (1230.10, 1310.55) pixels
# Flux 39.516 phs
# Filter_transmission 0.3560
# Start_angle 45.0000 deg.
# Angle_increment 0.2000 deg.
# Detector_2theta 0.0000 deg.
# Polarization 0.990
# Alpha 0.0000 deg.
# Kappa 0.0000 deg.
# Phi 0.0000 deg.
# Chi 0.0000 deg.
# Oscillation_axis X, CW
# N_oscillations 1
;
_array_data.data
;
--CIF-BINARY-FORMAT-SECTION--
Content-Type: application/octet-stream;
    conversions="x-CBF_BYTE_OFFSET"
Content-Transfer-Encoding: BINARY
X-Binary-Size: 6225323
X-Binary-ID: 1
X-Binary-Element-Type: "signed 32-bit integer"
X-Binary-Element-Byte-Order: LITTLE_ENDIAN
Content-MD5: ygx+qLaZTENsKK05endpXw==
X-Binary-Number-of-Elements: 6224000
X-Binary-Size-Fastest-Dimension: 2463
X-Binary-Size-Second-Dimension: 2527
```

miniCBF support: The Pilatus-6M

- The binary data is read using the crystallographic binary API

```
private_file = std::fopen(filename.c_str(),"rb");
cbf_read_widefile (cbf_h, private_file, MSG_DIGEST);

/* Find the binary data */
cbf_find_tag (cbf_h, "_array_data.data");
cbf_rewind_row (cbf_h);

/* Read data array parameters */
cbf_get_integerarrayparameters_wdims (
     cbf_h, &compression, &binary_id, &elsize, &elsigned, &elunsigned,
     &elements, &minelement, &maxelement,(const char **) &byteorder,
     &dim1, &dim2, &dim3, &padding);

ASSERT(elements == slow*fast); ASSERT(elsize == sizeof(int)); ASSERT(elsigned==1);

/* Read the binary data */
cbf_get_integerarray (cbf_h, //cbf handle
&id,                         //ptr to binary section identifier
begin,                       //array ptr
sizeof (int),                //element size
1,                           //flag of signed data type
sz,                          //elements requested
&nelem_read                  //elements read
                 );
```

Other issues with the Pilatus & its file format

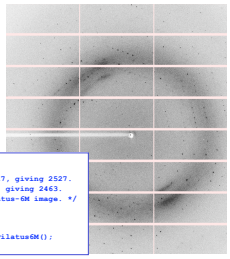
- The data are not spatially corrected. Dectris says the spot centroid **σ** is 0.3mm

Cubic Insulin Results:

	Solution	Metric fit	Indexing rmsd	Integration rmsd	crystal_system	unit_cell		
:)	22	0.4819 dg	0. 672	0.153	cubic cI	76.9	76.9	76.9
:)	1	0.0000 dg	0.755	0.158	triclinic aP	66.3	66.5	66.6

- The image has inactive pixels.
  -1 = dead area
  -2 = invalid pixel

- Pilatus-6M has 60 rectangular pixel arrays. Derive a class for identifying inactive area, which is then accessed in run time by a virtual function call:



```
class ActiveAreaPilatus6M: public ActiveAreaDefault {
  virtual bool is_active_area(int x,int y){
    /* x vertical: 12 blocks of 195, separated by 11 stripes of 17, giving 2527.
       y horizont:  5 blocks of 487, separated by 4 stripes of 7, giving 2463.
       Takes 0.02 seconds extra to apply this test to entire Pilatus-6M image. */
    return ( (x%212<195) && (y%494<487) ); }
};
...
if (vendortype=="Pilatus-6M") detector_location = new ActiveAreaPilatus6M();
if (!detector_location->is_active_area(x,y)) { /* make pink */ }
```

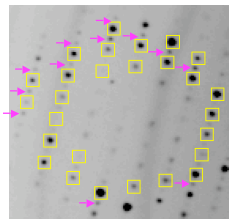
Advantages of Image Standardization

- The idea of permanently archiving raw data has recently regained currency [Baker, Dauter, Guss & Einspahr (2008). *Acta Cryst.* D**64**, 337].
- Ashley Deacon of the JCSG [Joint Center for Structural Genomics] has made over 100 datasets available (out of a total of ~600 PDB structures).
- The desire to make these datasets available for outside analysis also provides a rationale for requiring spatial correction information and beamline conventions to be recorded with the original data file.
- Image formatting questions naturally enter into this discussion.
- The JCSG experience provides a window into the types of discoveries possible when revisiting archived datasets.

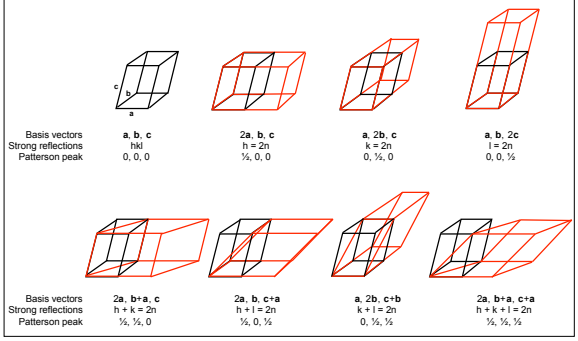
Findings:
- Sublattices
- Non-merohdral twinning
- Spot shape indicating phase transition

Pseudocentering: systematically weak Bragg spots

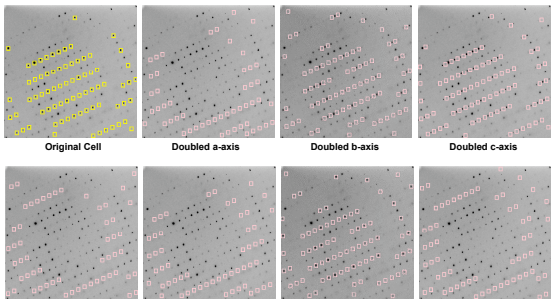
- The true symmetry is $P2_1$ with two protein molecules per asymmetric unit, related by a non-crystallographic translation.
- The NCS translation is ½ the cell length, approximating an additional symmetry operator, giving rise to alternating weak spots (Hauptman & Karle, 1953).
- If weak spots are ignored, the symmetry is *C*-centered orthorhombic with one protein molecule per asymmetric unit.
- Automatic indexing relies on picking the brightest spots, so it is easy to pick the oC cell by chance. [*Disclaimer: I don't know any example of this type of misindexing in published JCSG work.*]
- Lowering the spot-picking threshhold to find the weak spots is counterproductive – existing cases indexed by hand?



Construction of the Sublattice: Cell Doubling



Evidence for Cell Doubling in the Raw Data

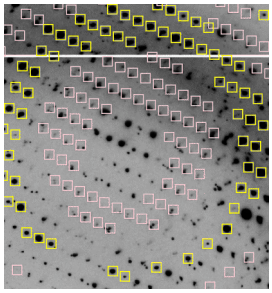


*

Filtering out the decoy signals

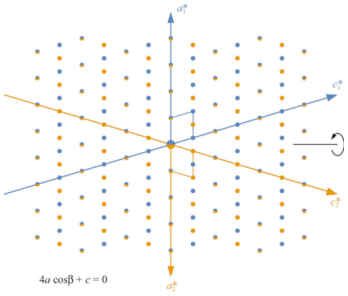
Weak spots do not match the profile of the main lattice

- Mismatched positions
- Split spots



Non-merohedral twinning

- Non-merohedral twin laws involve a symmetry operation belonging to a higher symmetry supercell, not to the point group of the diffraction pattern.



- In this example from Dauter (2003), twin domains (blue and orange) are related by the black two-fold rotation.
- A coincidental relationship in the unit cell measurements causes half of the blue spots to overlap with half of the orange spots.
- The apparent symmetry of the ensemble is higher than the intrinsic symmetry of each twin domain; but there is an unusual patten of absences—missing every other reflection on alternate layers.
- This is difficult to index; but having done so, easy to detwin.
- It is possible for the two domains to have unequal twin fractions, leading to a pattern of strong and weak reflections.

Non-merohedral twinning

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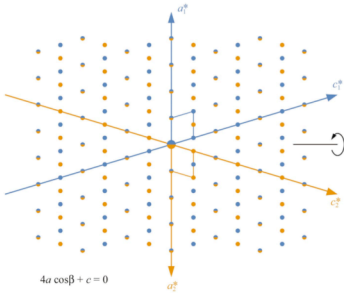
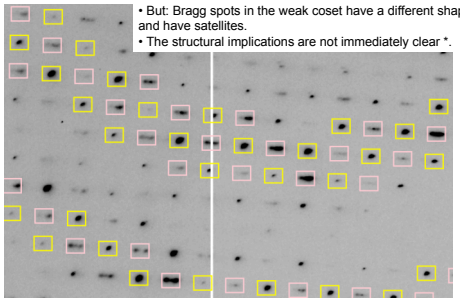


- If the "blue" twin domain is predominant, the pattern can be correctly indexed and the structure correctly solved. However, the R factor will be degraded.
- The key to fixing it is to observe the weak "orange" reflections in the original data.
- An initial survey finds at least two such cases (of 100) in the JCSG database, so this phenomenon is fairly common.

Other unusual phenomena

• JCSG structure published as $P2_12_12_1$, with 2 trimers/asymmetric unit. The weak sublattice (shown in pink) was taken into account, the yellow-only lattice would lead to $C222_1$ with 1 trimer/asymmetric unit.



• But: Bragg spots in the weak coset have a different shape and have satellites.
• The structural implications are not immediately clear *.

In Summary

- There is still work to be done to demonstrate that real CBF-formatted data can be processed with *LABELIT* as part of an automated pipeline.
- There is information in the raw dataset that is not captured in the processed structure factor file deposited with the Protein Data Bank. Follow up analysis could potentially lead to re-refinement and an improved understanding of particular structures.
- The inclusion of spatial correction data with the CBF-formatted file is potentially critical for follow up analysis.

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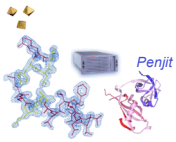

CBF Compile: Summary of Multi-Platform Warnings

```
Intel cc compiler 9.1
cbflib_0.7.9/ src/cbf.c(7351): warning #186: pointless comparison of unsigned integer with zero
      if (sec[axsize-1]<0) extrabits = -0;

Irix MIPS Pro 7.4.1
cbflib_0.7.9/src/cbf.c, Line = 6530 The variable "ltest" is set but never used.
cbflib_0.7.9/src/cbf.c, Line = 6532 The variable "dtest" is set but never used.
cbflib_0.7.9/src/cbf.c, Line = 5972 The variable "catname" is set but never used.
cbflib_0.7.9/src/cbf.c, Line = 7405 The variable "sign" is set but never used.
cbflib_0.7.9/src/cbf_byte_offset.c, Line = 284 The variable "borrow" is set but never used.
cbflib_0.7.9/src/cbf_byte_offset.c, Line = 992 The variable "errorcode" is set but never used.
cbflib_0.7.9/src/cbf_byte_offset.c, Line = 992 The variable "carry" is set but never used.
cbflib_0.7.9/src/cbf_canonical.c, Line = 1440 The variable "endcode" is set but never used.
cbflib_0.7.9/src/cbf_canonical.c, Line = 1446 The variable "code" is set but never used.
cbflib_0.7.9/src/cbf_codes.c, Line = 600 The variable "tmp" is set but never used.
cbflib_0.7.9/src/cbf_codes.c, Line = 1312 The variable "mab" is set but never used.
cbflib_0.7.9/src/cbf_simple.c, Line = 4912 The variable "ncenter1" is set but never used.
cbflib_0.7.9/src/cbf_simple.c, Line = 4912 The variable "ncenter2" is set but never used.
cbflib_0.7.9/src/cbf_simple.c, Line = 5083 The variable "ncenter1" is set but never used.
cbflib_0.7.9/src/cbf_simple.c, Line = 5083 The variable "ncenter2" is set but never used.

Tru64 CC
Info: cbflib_0.7.9/examples/img.c, line 1334: In this statement, an array subscript expressio n is either less than zero or greater than
the largest value that can be repre sented by the size_t type. (badsubscript)
      for (C = C64+strlen(C64)-1; (C != C64-1 && *C == ' '); C-- ) *C = '\0';
-----------------------------------^

Warning: cbflib_0.7.9/src/cbf_uncompressed.c, line 262: The redefinition of the macro "_XOPEN_SOURCE" conflicts with a current definition
because the replacement lists differ. The redefinition is now in effect. (macroredef)
#define _XOPEN_SOURCE
---------------------^
Warning: /usr/include/unistd.h, line 59: Syntax error in constant expression . (badconstexpr)
#if defined (_XOPEN_SOURCE) && (_XOPEN_SOURCE>=500)
---------------------------------------------------^

Windows VC 8.0
C:\AUTOBU~1\sources\labelit_modules\cbflib_0.7.9\src\cbf_uncompressed.c(263) : fatal error C1083:
Cannot open include file: 'unistd.h': No such file or directory
```