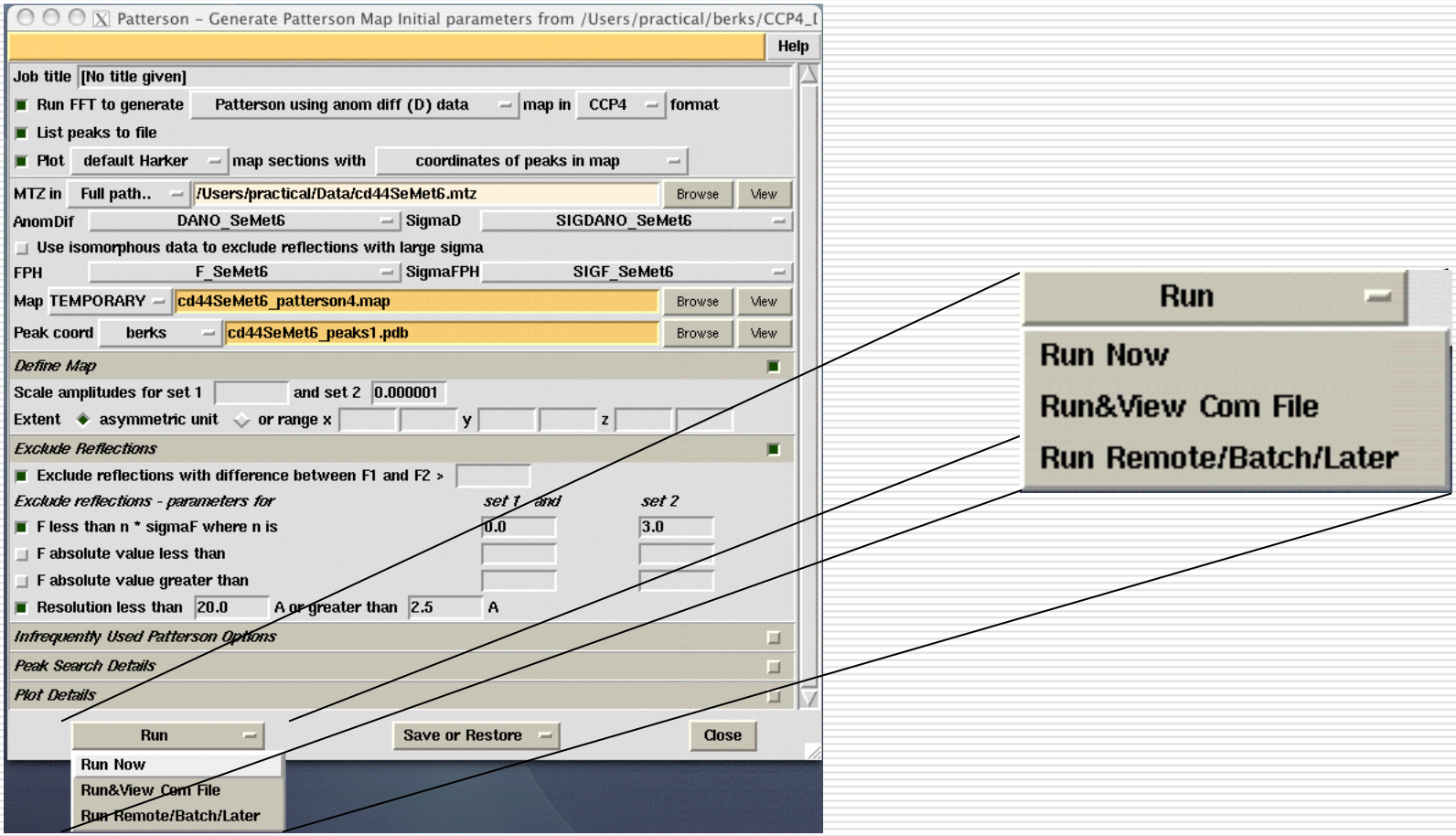
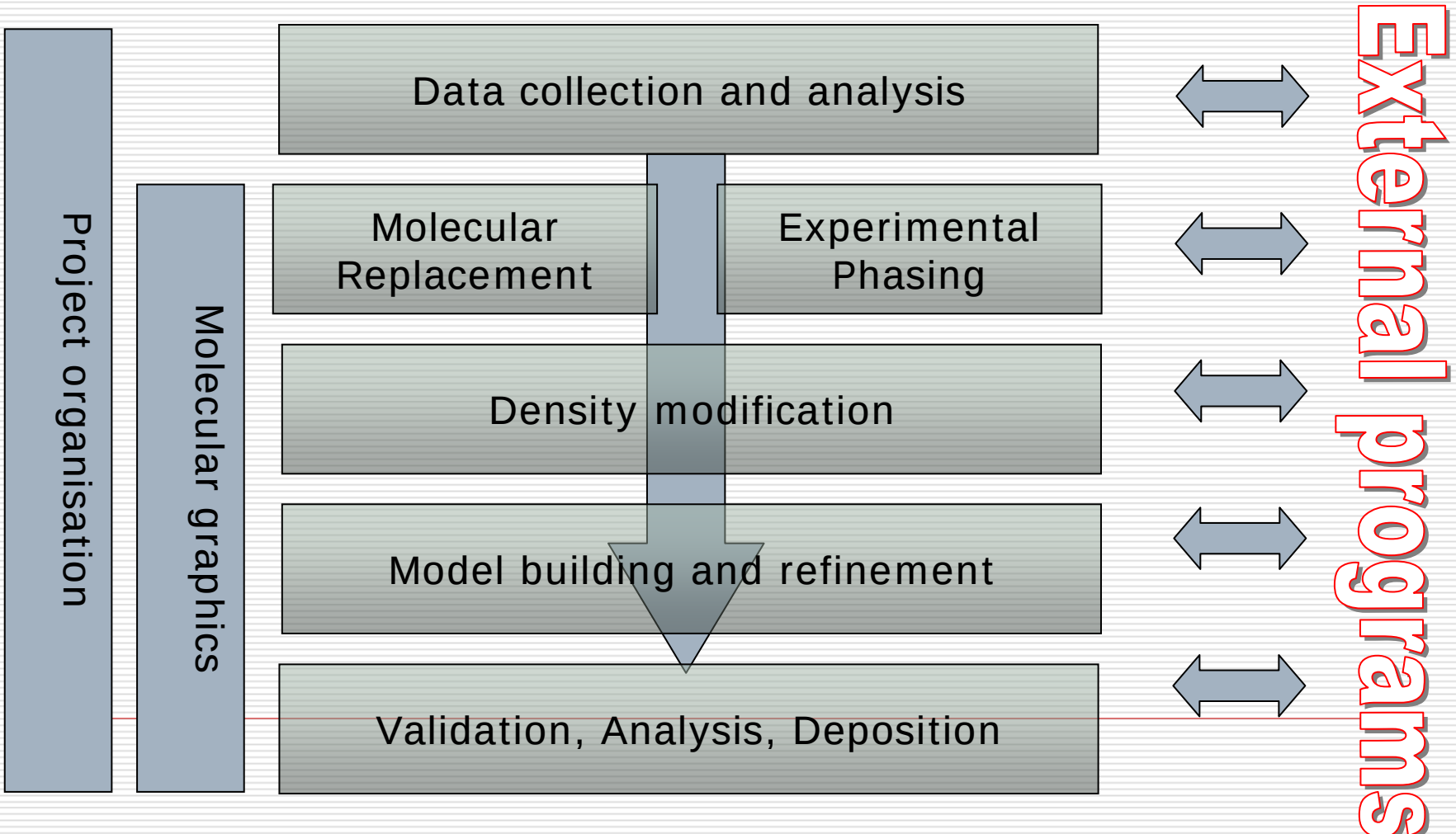
Using CCP4 for PX

Martin Noble, Oxford University
and CCP4

Running programs locally or remotely

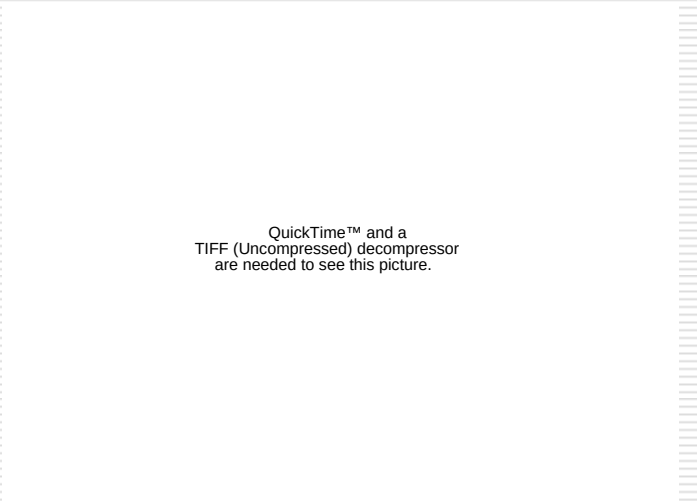


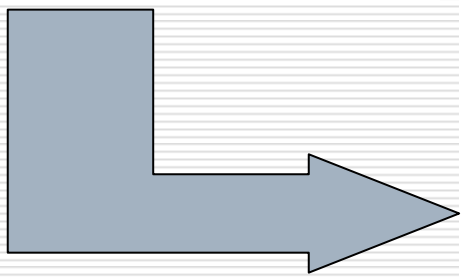
Overview


Project organisation
Molecular graphics
Data collection and analysis
Molecular Replacement
Experimental Phasing
Density modification
Model building and refinement
Validation, Analysis, Deposition
External programs

Project Organisation

- 2) Workflows



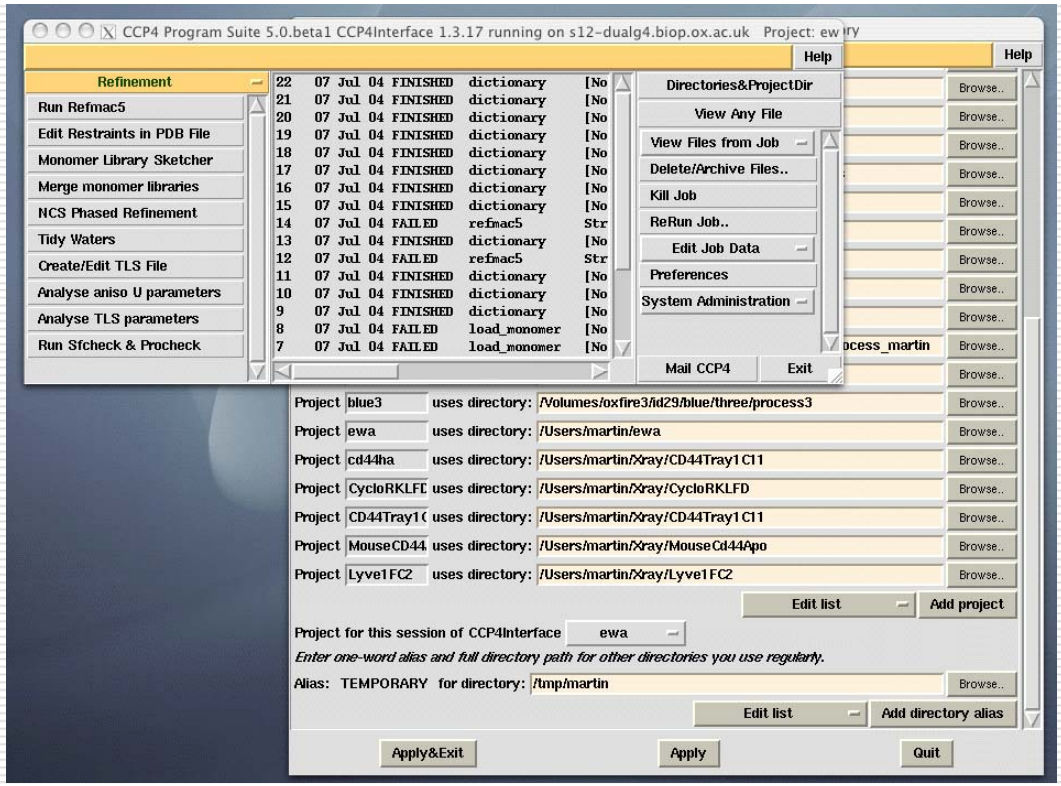


Experimental Phasing

- Data Reduction
- Experimental Phasing
- Molecular Replacement
- Density Improvement
- Model Building
- Refinement
- Structure Analysis
- Validation & Deposition
- Map & Mask Utilities
- Reflection Data Utilities
- Coordinate Utilities
- Graphics and Viewing Utilities

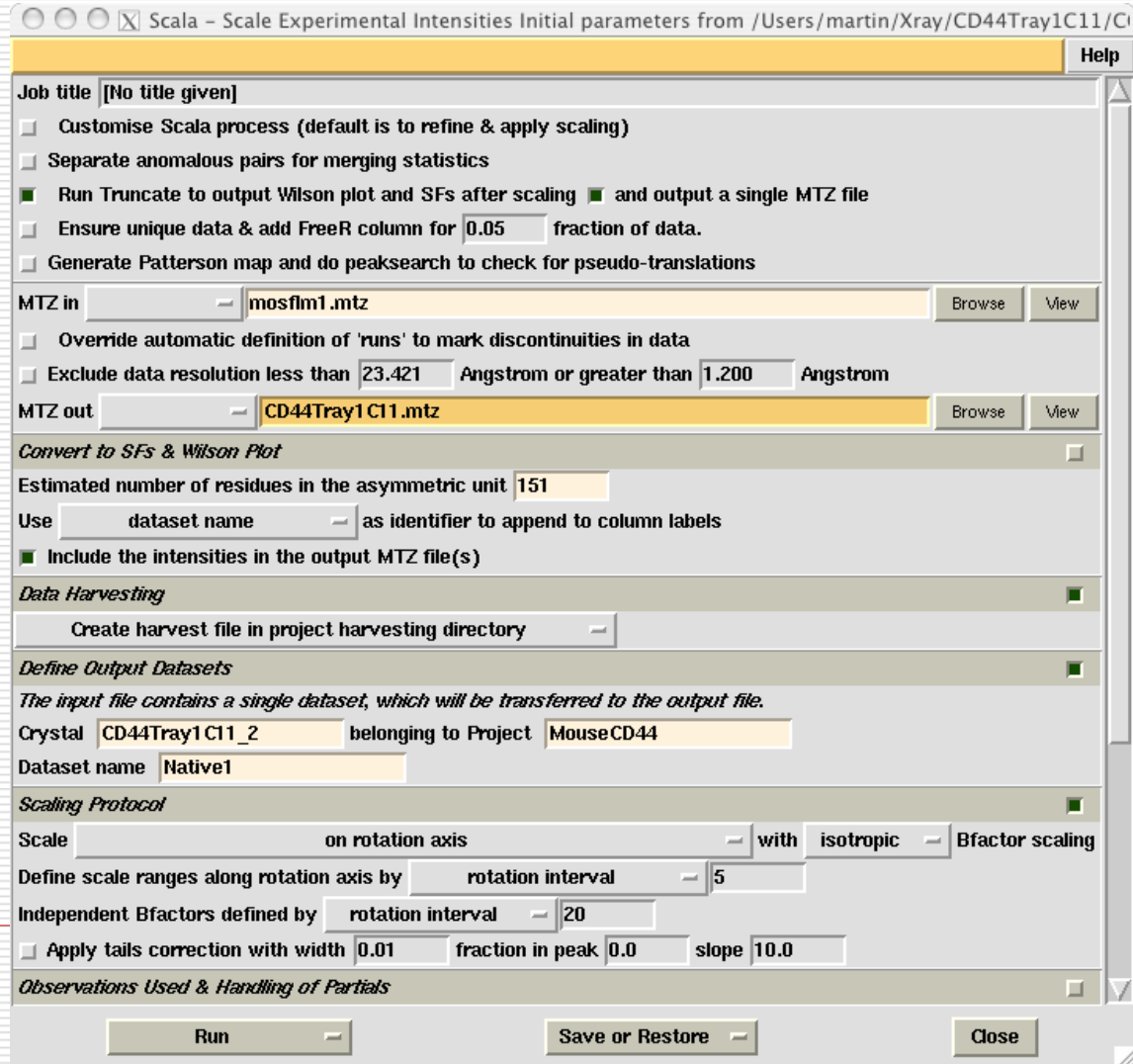
Project Organisation

1) Graphical

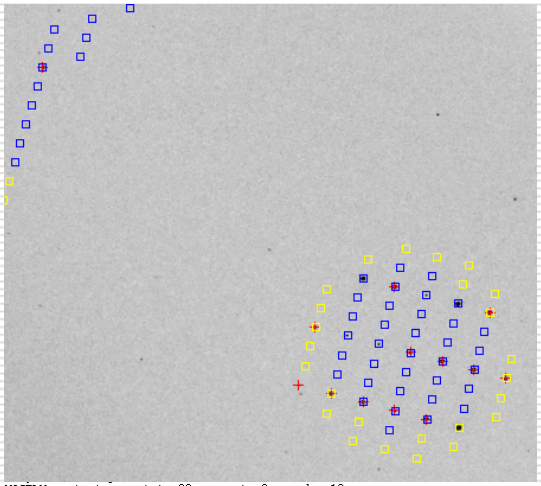


Project Organisation

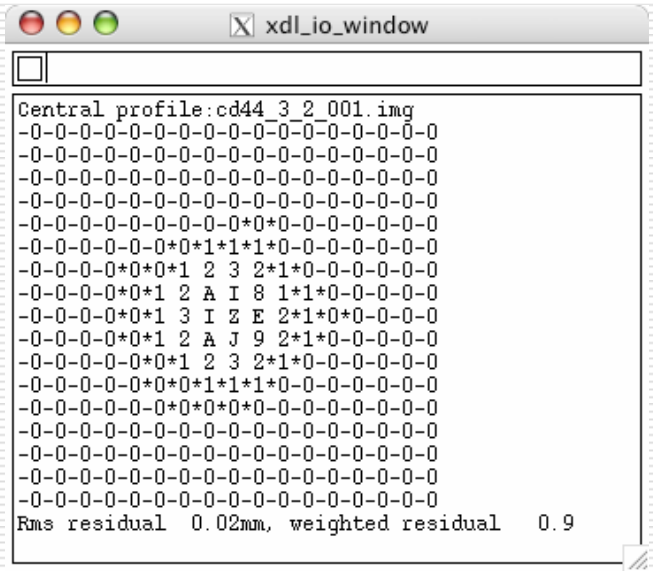
- 3) Data
  - Project
  - Crystal
  - Dataset

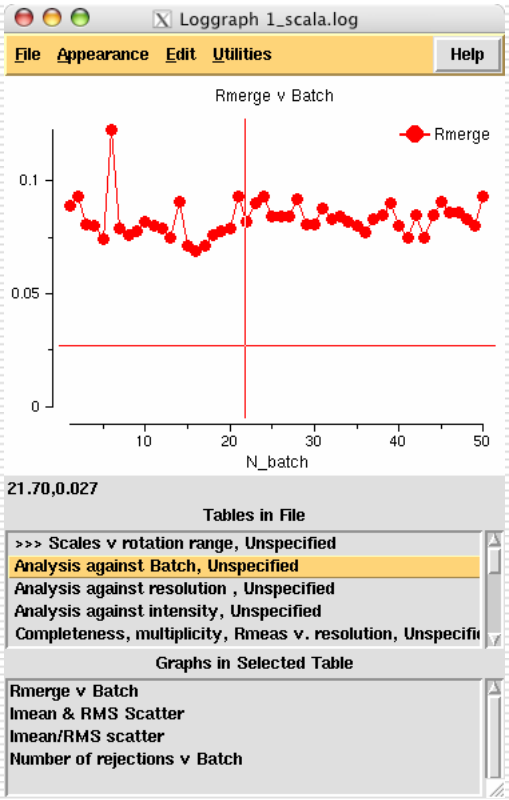


Data collection and analysis



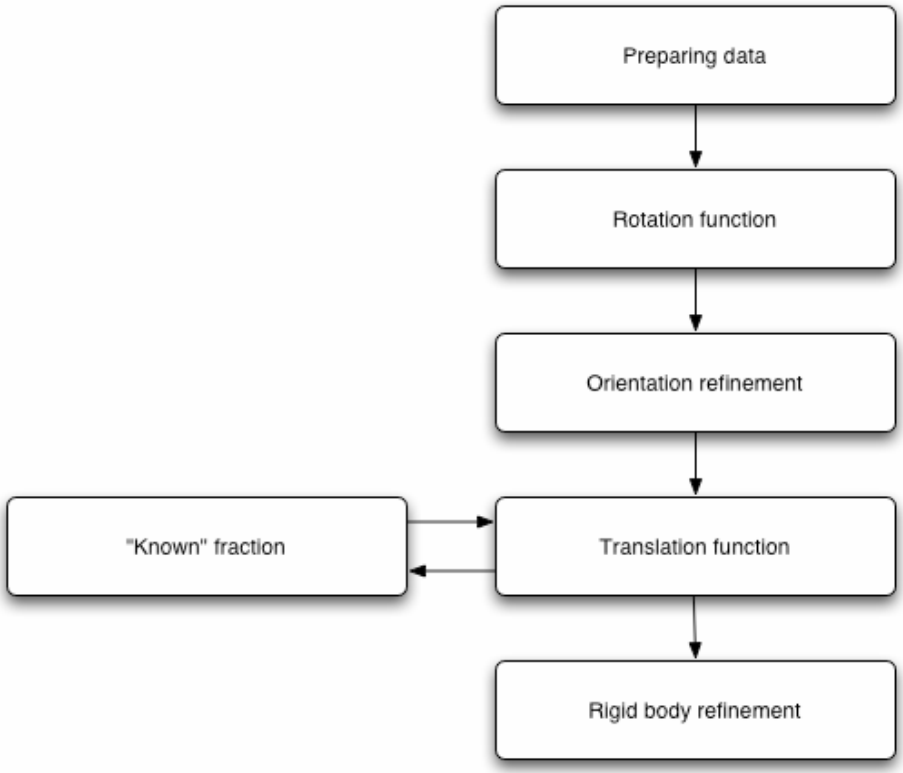
```
MOSFLM =>strategy rotate 90 segments 2 speedup 10
MOSFLM =>go

Angles between a axis and X,Y,Z axes   36.630 125.294   81.437
Angles between b axis and X,Y,Z axes   57.913  36.340   74.775
Angles between c axis and X,Y,Z axes  105.763  97.565   17.570
The c axis is closest to the rotation axis (angle 17.57)
Rotation angle to get the a axis in XZ plane    35.75
Rotation angle to get the b axis in XZ plane   -56.60
Generating reflection list

Generated reflections for phi range   27.0 to  135.0
Sorting the generated list.
Generating the list of unique reflections.
Merging  the generated and unique reflection list.

Optimum rotation gives 100.0% of unique data.
This corresponds to the following rotation range(s):
From   32.0 to  77.0 degrees
From   77.0 to 122.0 degrees
Type "STATS" at prompt for full statistics.

Use the TESTGEN option to determine appropriate oscillation angles to avoid
spatial overlaps.
STRATEGY =>
```





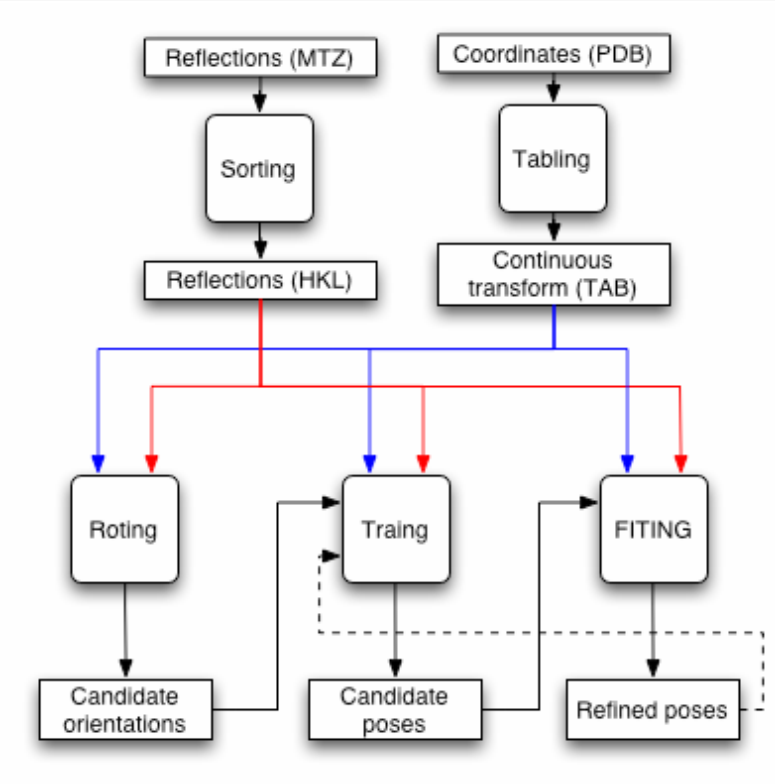
MOSFLM → SCALA

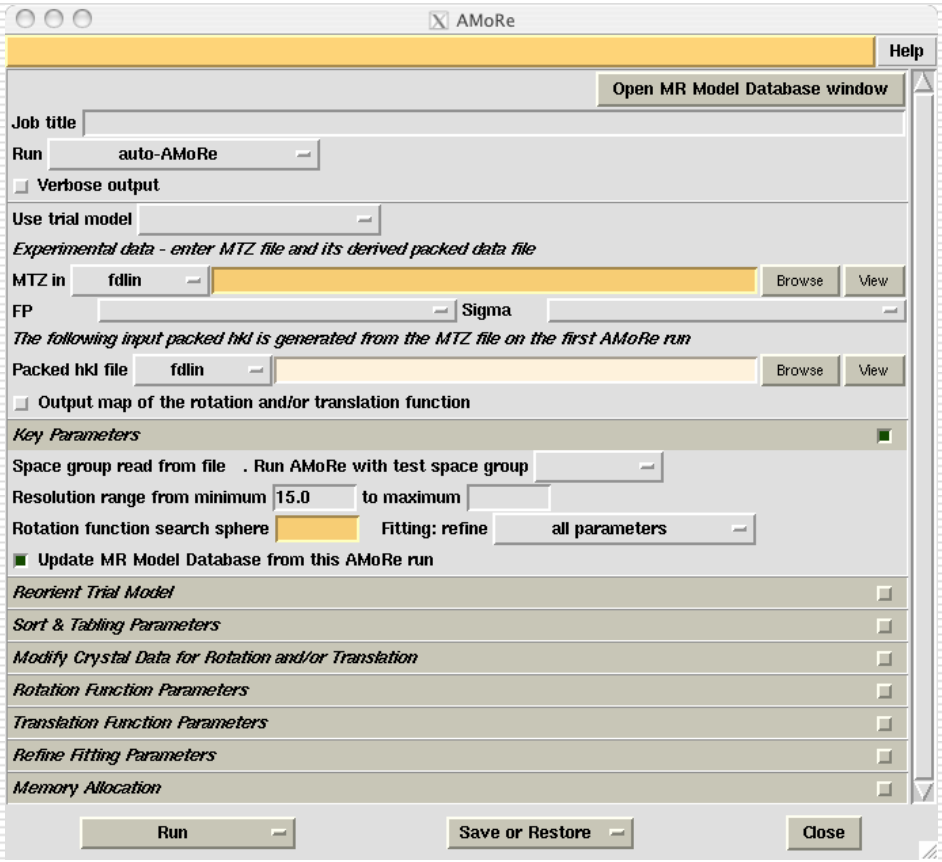
Molecular Replacement



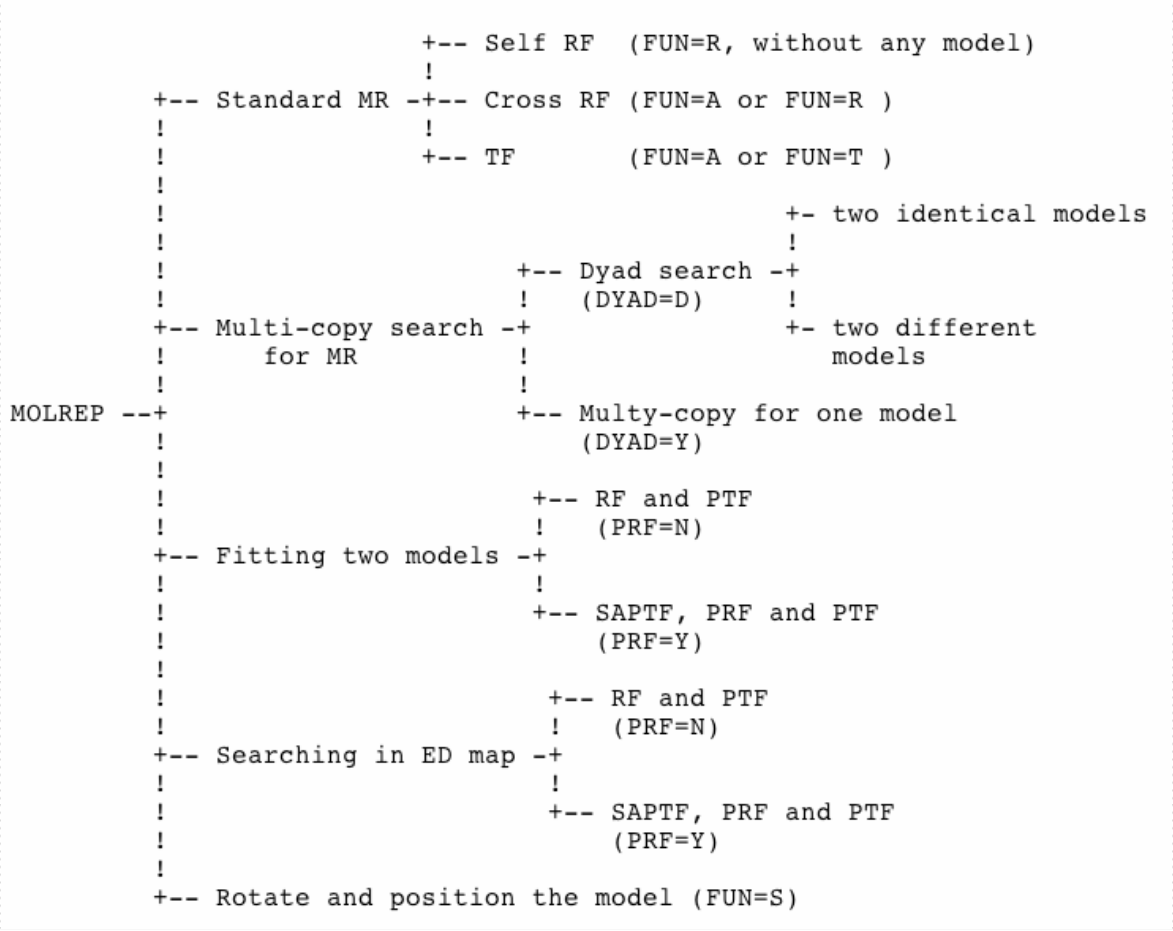
[Phaser, BP3]

Amore


Reflections (MTZ)
Coordinates (PDB)
Sorting
Tabling
Reflections (HKL)
Continuous transform (TAB)
Roting
Traing
FITING
Candidate orientations
Candidate poses
Refined poses


AMoRe
Help
Open MR Model Database window
Job title
Run
auto-AMoRe
Verbose output
Use trial model
Experimental data - enter MTZ file and its derived packed data file
MTZ in
fdlin
Browse
View
FP
Sigma
The following input packed hkl is generated from the MTZ file on the first AMoRe run
Packed hkl file
fdlin
Browse
View
Output map of the rotation and/or translation function
Key Parameters
Space group read from file . Run AMoRe with test space group
Resolution range from minimum
15.0
to maximum
Rotation function search sphere
Fitting: refine
all parameters
Update MR Model Database from this AMoRe run
Reorient Trial Model
Sort & Tabling Parameters
Modify Crystal Data for Rotation and/or Translation
Rotation Function Parameters
Translation Function Parameters
Refine Fitting Parameters
Memory Allocation
Run
Save or Restore
Close

Molrep capabilities



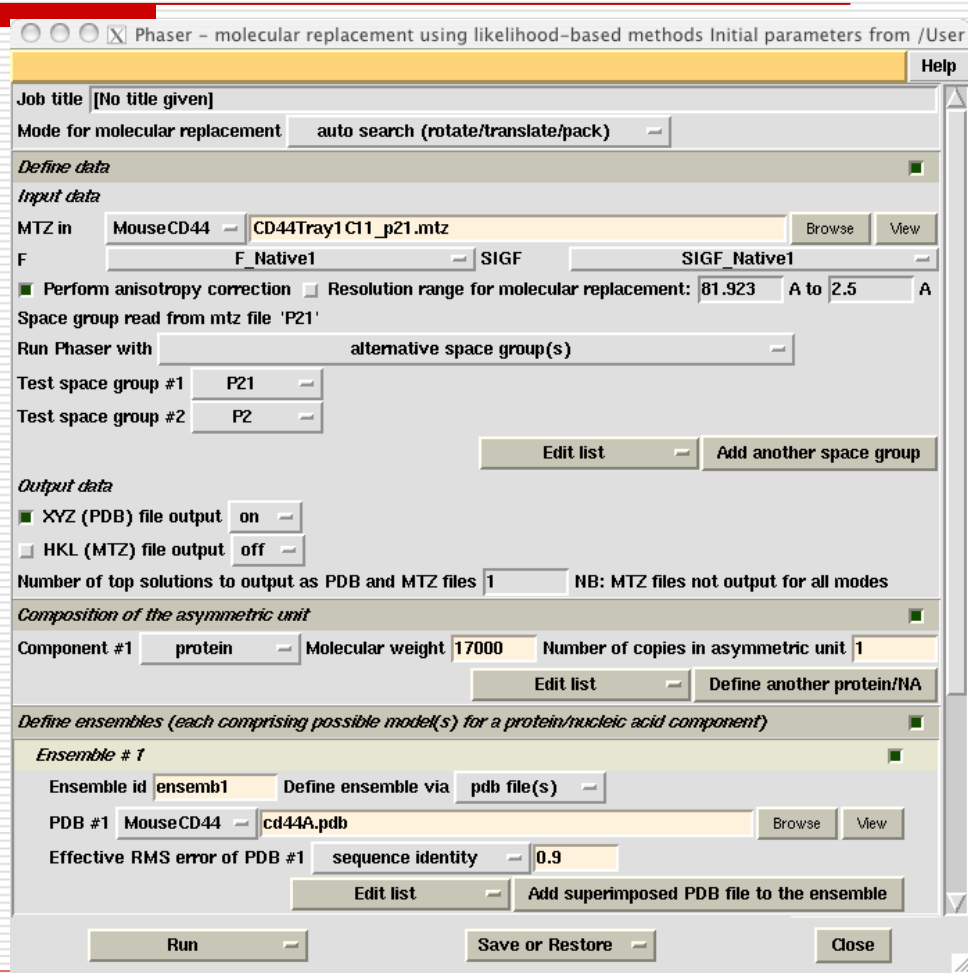
Molrep

- • **scaling by Patterson** origin peak,soft low resolution cut-off, anisotropic correction of data
- • **standard molecular replacement method** by rotation function (RF), full-symmetry translation function (**TF**) and packing function (PF)
- • allows input of **a priori knowledge** of similarity and completeness of the model (By pseudo B-factor).
- • performs rigid body refinement
- • can check and manage **pseudo-translation**
- • can compute **Self Rotation Function** with PostScript plots
- • Spherically averaged phased translation function (**SAPTF**)
- • Phased Rotation(**PRF**) and Phased Translation functions (PTF)
- • (**superimposing two models)**
- • can evaluate R-factor, CC for a proposed rotation and translation
- • **multi-copy searh**
- • can **improve** the model before use, model correction by sequence alignment
- • can choose from symmetry-related models closest to which was found before
- • can use **modified stucture factors** instead of Fobs for RF
- • can use **NMR** models

- *J. Appl. Cryst.* (1997). **30**, 1022-1025 ***MOLREP*: an Automated Program for Molecular Replacement**A. Vagin **and A. Teplyakov**

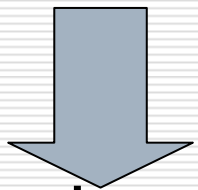
Phaser

- Likliehood targets
  - Multiple search models
  - Test multiple space groups
  - Rapid likliehood target (FRF) prescreen, followed by higher accuracy rescoring
  - Packing function screening of translation function solutions



Acorn

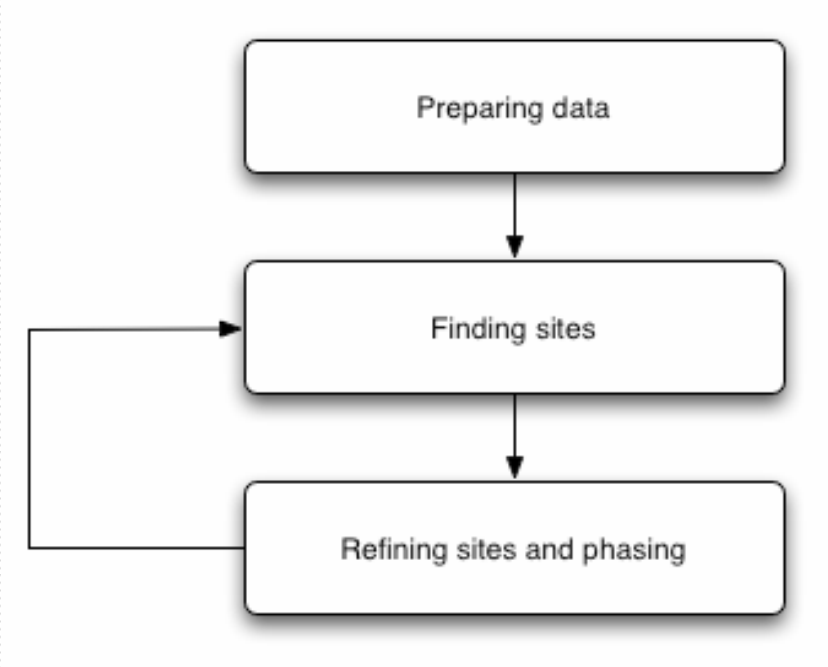
- Patterson-based heavy atom sub structure determination
- Molecular replacement of secondary structural elements



Complete determination of structures at atomic resolution

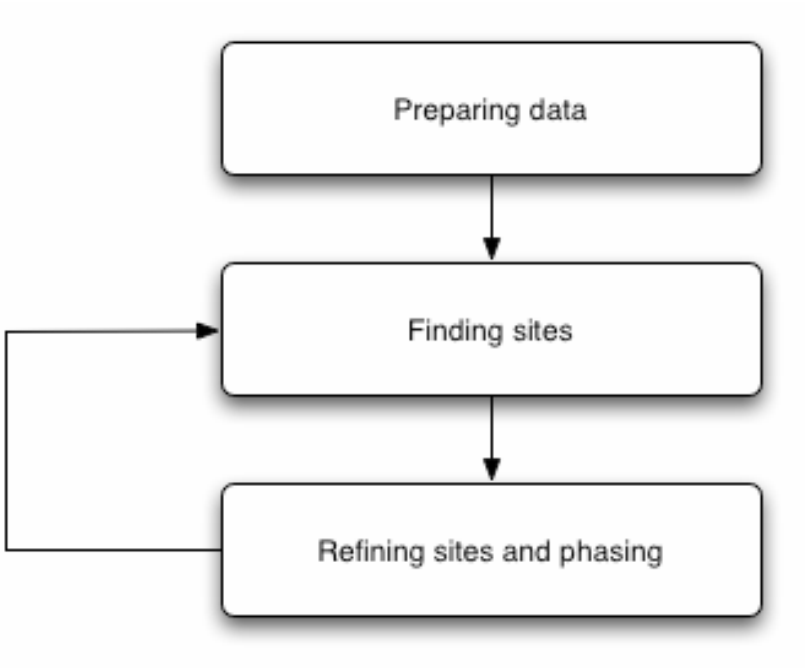
Foadi,J., Woolfson,M.M., Dodson,E.J., Wilson,K.S., Yao Jia-xing and Zheng Chao-de (2000) *Acta. Cryst.* **D56**, 1137-1147.

Experimental Phasing



Experimental Phasing
Merge Datasets (CAD)
Scale and Analyse Datasets
Prepare Data for HA Search
Acorn - ab initio Phasing
Shelx - Heavy Atom Search
Rantan - Direct Methods
Professs - NCS from HA
Run Mlphare
Oasis - SAD/SIR phasing
Generate Patterson Map
Real Space Patterson Search

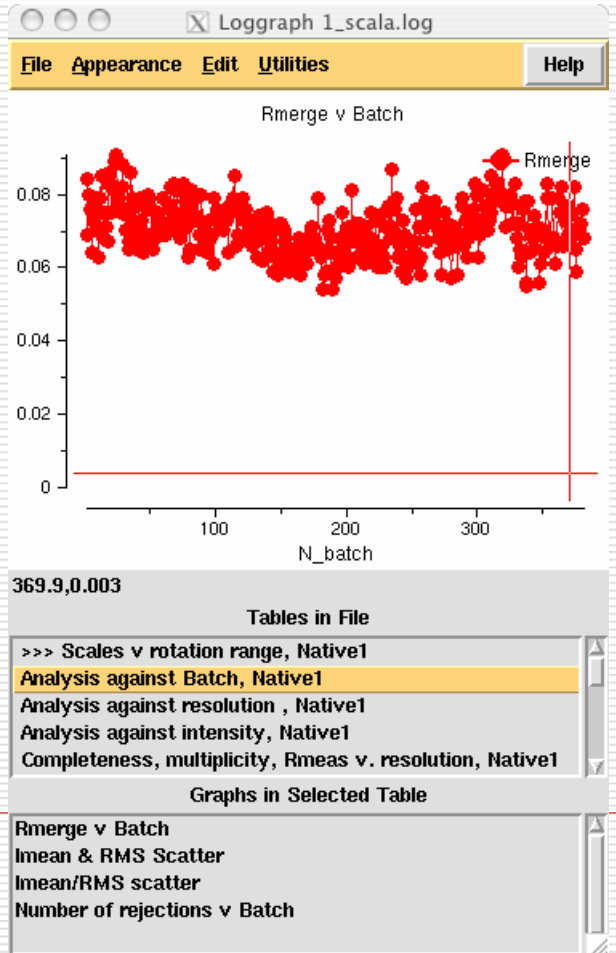
Experimental phasing

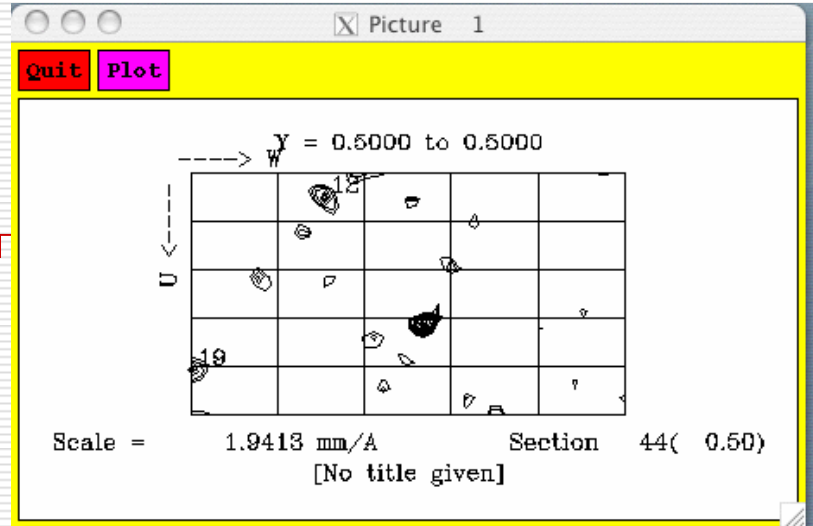


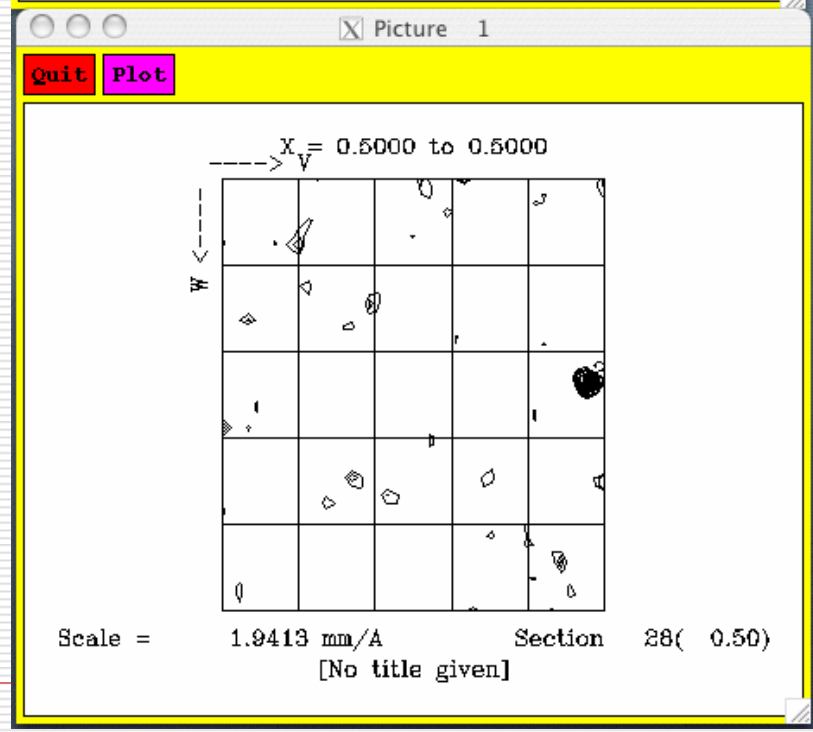
CCP4	Others
ECALC REVISE	XPREP
RSPS ACORN RANTAN	SHELX AUTOSHARP SOLVE
MLPHARE	SOLVE SHARP AUTOSHARP

Using CCP4

- Graphical Feedback

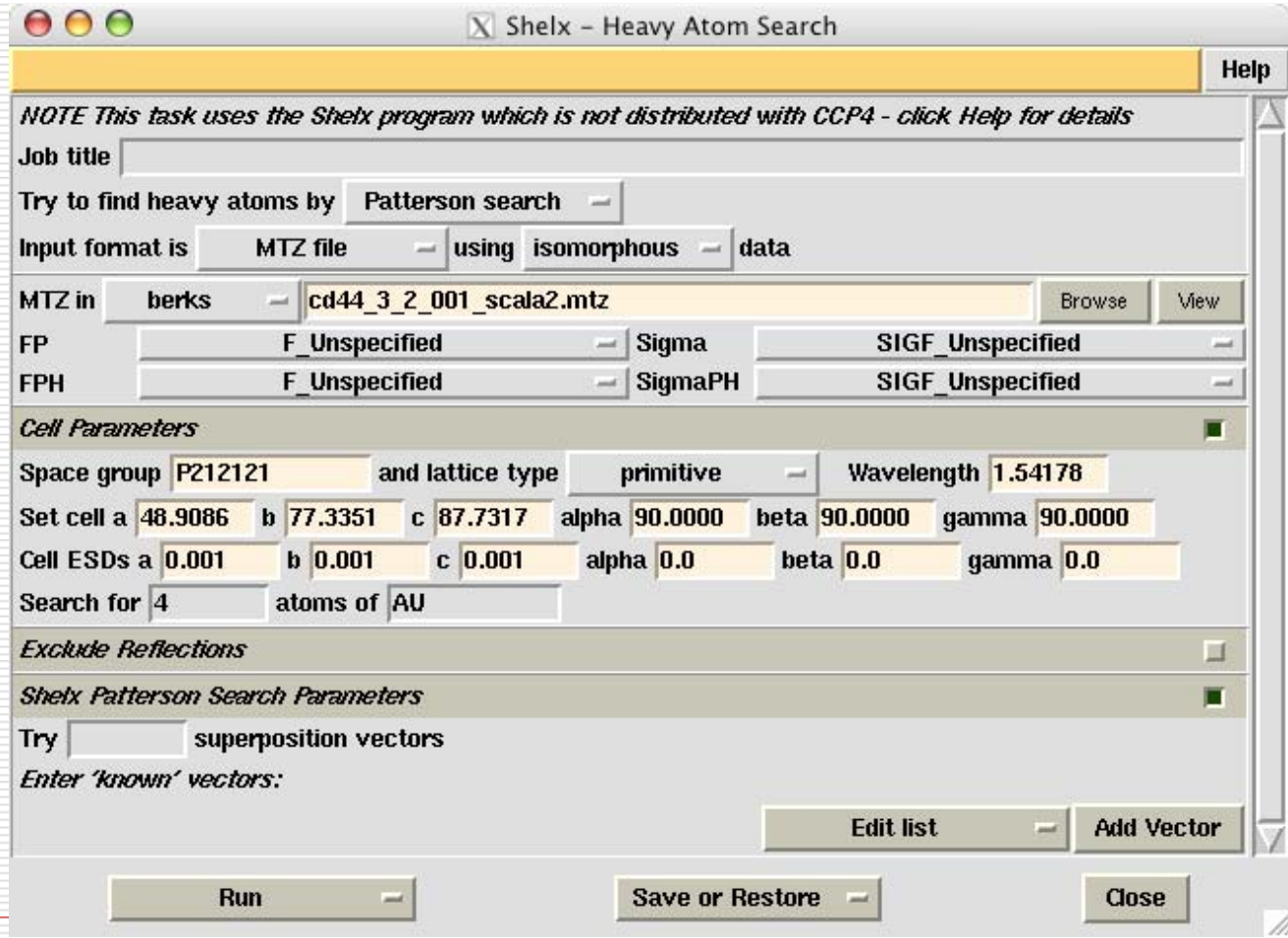






Finding Sites (External)

- SHELX



Finding sites

- RSPS
  - Patterson search
    - Harker section searches combined by sum, mean or harmonic mean
    - Cross peaks used to relate multiple sites to the same origin
  - Can exploit NCS
    - Full transformation to generate “pseudo Harker”
    - Can search for pairs of atoms with a known separation

Knight, S.D. (2000): *RSPS version 4.0: a semi-interactive vector-search program for solving heavy-atom derivatives*, Acta Cryst. D **52**, 42-47

Finding sites

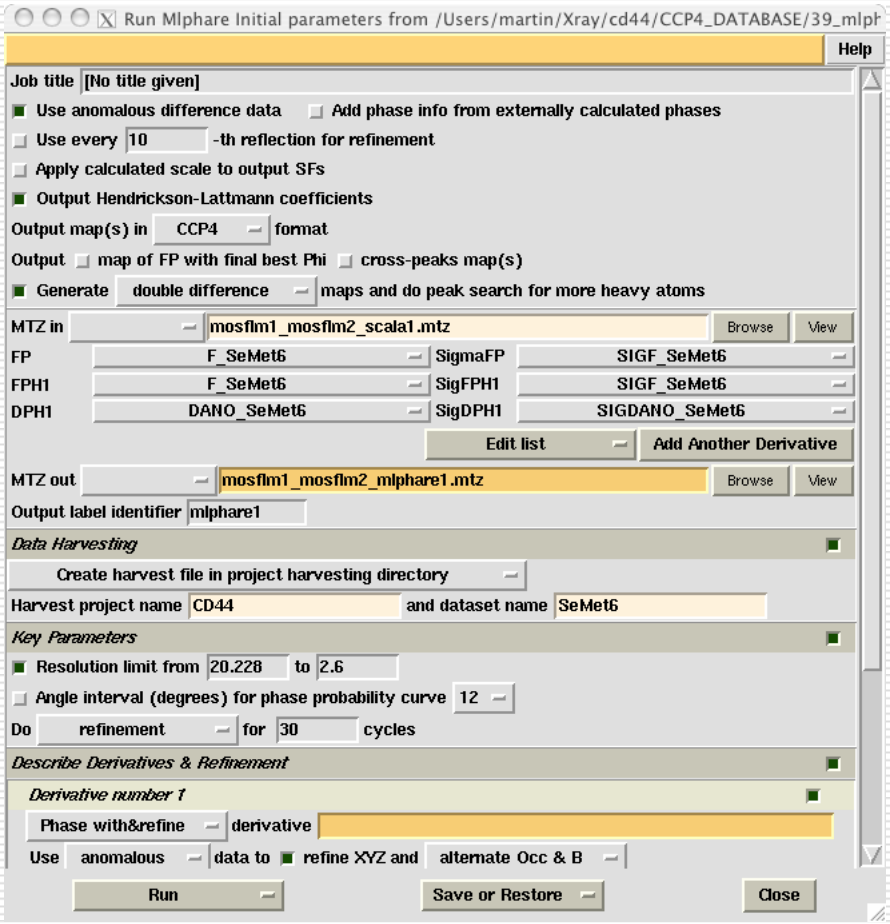
- Rantan
  - Data prepared by (Revise and) ECALC
  - Tangent formula refinement of random phase sets for a large number of triplets

1. Yao Jia-xing, (1981). *Acta. Cryst.* **A**37, 642-644.

MLPHARE

Phased refinement suitable for SAD, MAD, SIR, SIRAS, MIRAS

- Some maximum likliehood concepts included to minimise phase bias
- Generates coefficients for use in double difference Fouriers for model completion



Z.Otwinowski: Daresbury Study Weekend proceedings, 1991

OASIS

- To break phase ambiguity of SIR (or SAD) experiments by recourse to direct methods and density modification.

Hao, Q.*, Gu, Y.X., Zheng, C.D. & Fan, H.F. (2000) *J. Appl. Cryst.* **33**, 980-981. "OASIS: A Program for Breaking Phase Ambiguity in OAS or SIR".

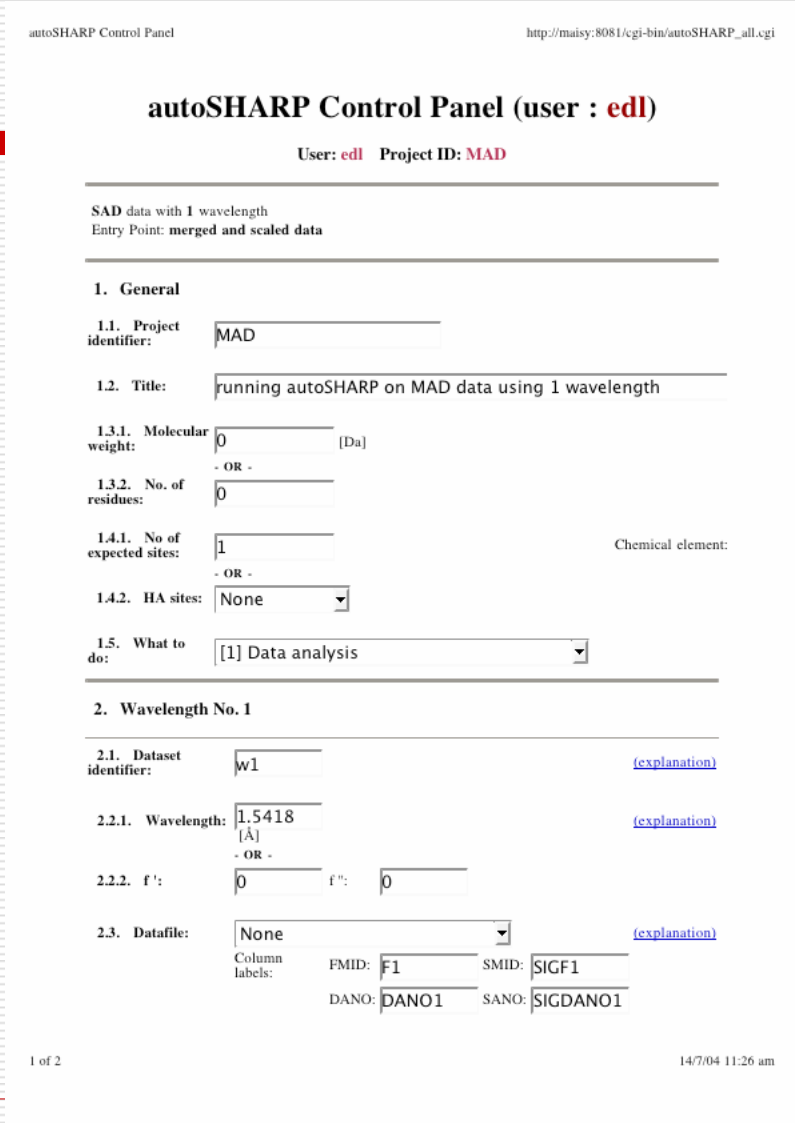
Refining sites

- 2) External interfaces 1
  - 2.1 SOLVE (Terwilliger, solve.lanl.gov)

```
solve <<EOD
!  ccp4 mtz file input
!  solve a 2-deriv MIR dataset
logfile mir.logfile
! write out most information to this file.
! summary info will be written to solve.prt
resolution 20 3
! you need resolution.  space group and cell
! dimensions read from mtz file.
!  get the  mtz file information and read it in:
labin FP=FP SIGFP=SIGFP FPH1=FPH1 SIGFPH1=SIGFPH1 DPH1=DPH1 SIGDPH1=SIGDPH1
labin FPH2=FPH2 SIGFPH2=SIGFPH2 DPH2=DPH2 SIGDPH2=SIGDPH2
hklin mir_fbar-cad.mtz
!  now we're ready with scaled data
```

Refining sites

- 2) External interfaces
  - 2.1 SHARP
  - (Bricogne,
  - babinet.globalphasing.com)



autoSHARP Control Panel http://maisy:8081/cgi-bin/autoSHARP_all.cgi

autoSHARP Control Panel (user : edl)

User: edl **Project ID:** MAD

SAD data with **1** wavelength
Entry Point: **merged and scaled data**

1. General

1.1. Project identifier: MAD

1.2. Title: running autoSHARP on MAD data using 1 wavelength

1.3.1. Molecular weight: 0 [Da]

- OR -

1.3.2. No. of residues: 0

1.4.1. No of expected sites: 1 Chemical element:

- OR -

1.4.2. HA sites: None

1.5. What to do: [1] Data analysis

2. Wavelength No. 1

2.1. Dataset identifier: w1 (explanation)

2.2.1. Wavelength: 1.5418 [Å] (explanation)

- OR -

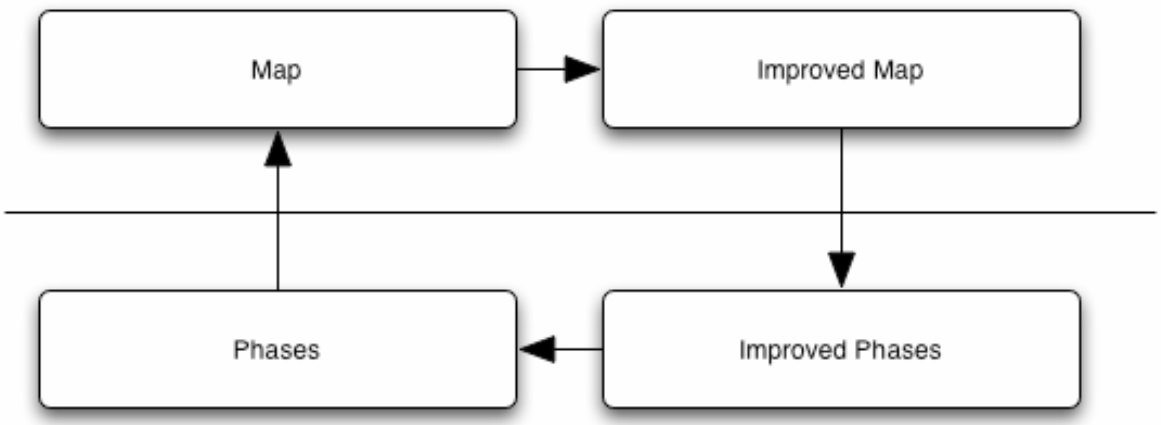
2.2.2. f ': 0 f ": 0

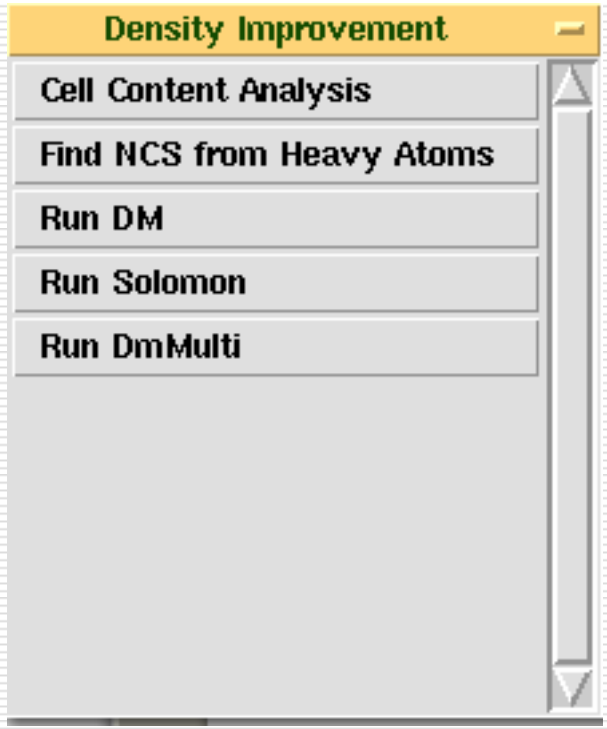
2.3. Datafile: None (explanation)

Column labels: FMID: F1 SMID: SIGF1
DANO: DANO1 SANO: SIGDANO1

1 of 2 14/7/04 11:26 am

Density modification





DM

- Solvent flattening
- NCS averaging (multi-domain)
- Multicrystal averaging
- Histogram matching
- Skeletonisation
- Sayre phase improvement
- Automatic or Manual mask definition
- Inbuilt operator refinement
- *Multiresolution modification and Perturbation gamma correction

• K. Cowtan (1994), Joint CCP4 and ESF-EACBM Newsletter on Protein Crystallography, 31, p34-38.

Solomon

- Solvent flipping
- Protein truncation
- Averaging

Abrahams J. P. and Leslie A. G. W., *Acta Cryst.* **D52**, 30-42 (1996)

Model Building

1) Automatic

Decreasing Resolution

- ACORN
- ARP/wARP
- FFFear/ FFJoin

Model building: Arp/wArp

- 1. Automatic tracing of the density map and model building. (MR-solution refiement and the improvement of MAD and M(S)IR(AS) phases via map interpretation
- 2. Free atoms density modification
- 3. Building of the solvent structure

International Tables for Crystallography, Volume F: Crystallogaphy of biological macromolecules, Eds. Rossmann & Arnold, 2001, 720-722 by Lamzin, Perrakis & Wilson

Model Building

2) Graphical

a) Inhouse

CCP4 Molecular Graphics

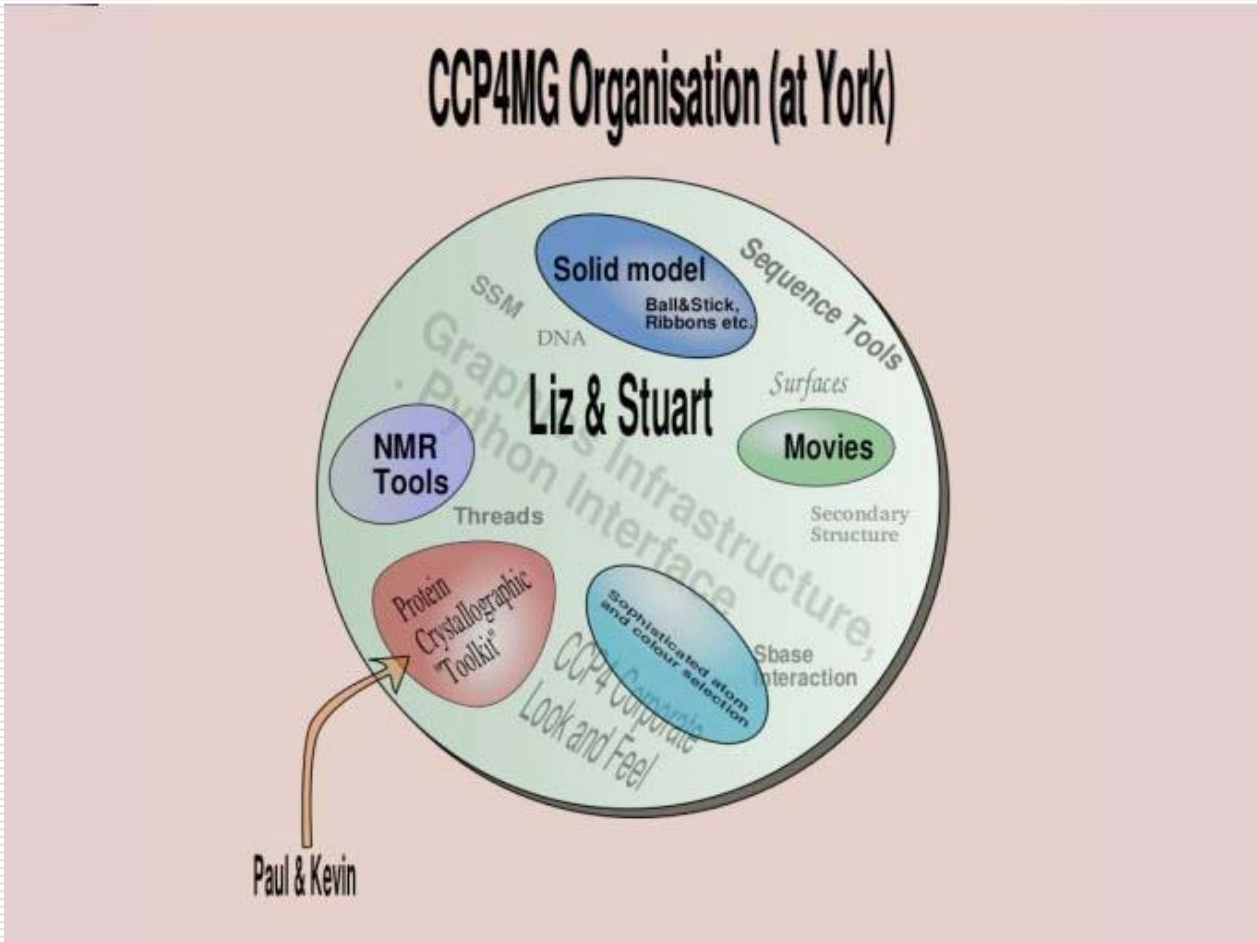
b) Closely linked

COOT

c) Well interfaced

O, mifit

CCP4 Molecular Graphics

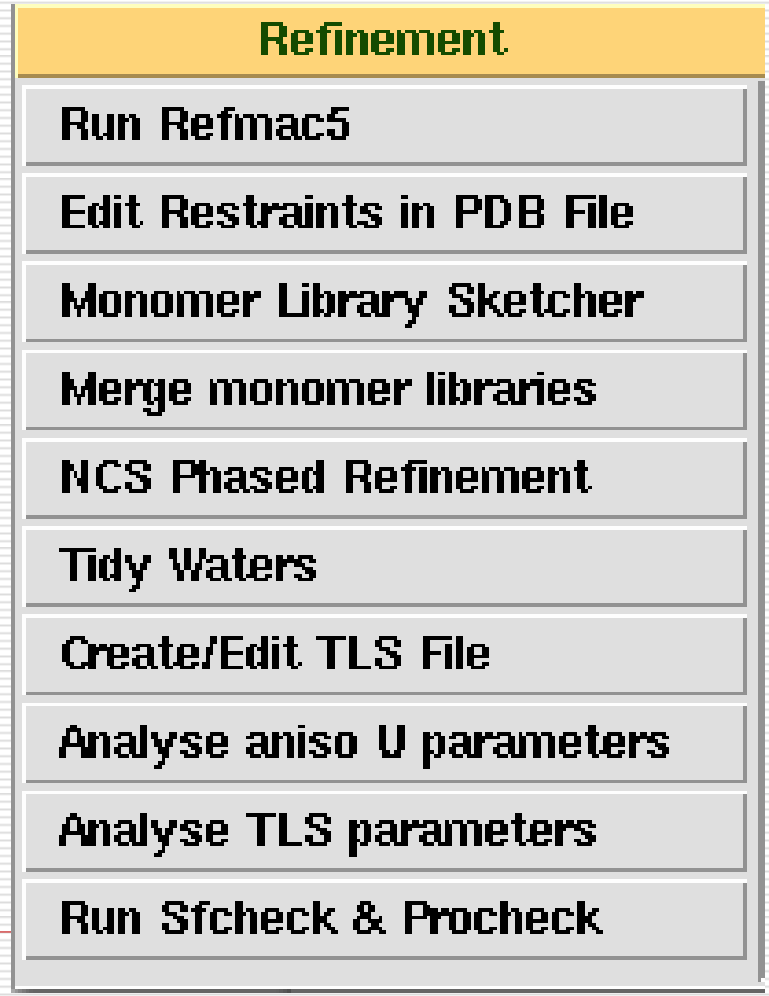


Coot wonders

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

http://www.ysbl.york.ac.uk/~emsley/coot/

Refinement tools



Refinement

- REFMAC strengths
  - Algorithms
  - Monomer libraries (DNA, RNA, Small molecules)

Refinement

- Monomer sketcher