Rigaku FR-X Usage

Training on 2015.03.30

FR-X generator:

- 45 kV,
- 66 mA,
- 70 µ cross-section,
- 180 µ beam,
- 1.7 miliradians divergence

XG Control tells you the settings values (45 kV and 66 mA vs 20 kV and 10 mA when not used) . You can screen as soon as the parameters have reached the target values, but wait one more hour to collect, so that the beam is stable.

Pilatus 300K detector:

- 3 chips,
- shorter exposure time,
- no readout (no shutter closing),
- almost never overloaded (>1.5 millions),
- water circulation at room temperature,
- dry air

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>thread
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command on camserver gives the temperature and humidity level Green light at the back of the detector to check before use.

Cryostream should be at 100 K

Close 3 glass doors, push the green light on the left [1)]

Run HKL_3000_P300KChi [2)]

Collect/Connect to initialize (also open an instrument server window in the background)

0.25° oscillation by default As a “rule of thumb” set the detector distance (in mm) to half the longest unit cell parameter (in Å) [3)]

Align/Zero goniostat resets all positions (parameters in /hkl_dc.ini)

2θ always swings away (clockwise)

Set the directory /data/user/project1_crystal1

dtdisplay

- middle click > zoom area

- middle click > reset zoom
- control + left click > move around
- measure /Measure Left 1x, 2x, 3x or 4x (to average)

Never collect at 2θ = 0, use at least 2θ = 2 to offset the center horizontally and avoid thus avoid missing data due to the gaps between detectors

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Index

- Refine in triclinic (P1)
- Fit Basic
- Fit All
- Mosaicity (by default it cannot estimate well the mosaicity[4)], so you need to select)
- With a 3D Window >1, the mosaicity option with already be checked
- To process up to the corner, you need to index up to the corner!

Strategy

- HKL_3000 strategy only works for single axis
- Rigaku_Strategy is specially made for FR-X on the 4 axis
- Choose I want to make changes
- Completeness 99% (to reduce collect time)
- Redundancy 3.0 (4.0 by default)
- Distance (the distance from the first image is set)
- Suggested distance is not useful
- Edit preferences is possible

You can swing the slider on Time window to adjust/reduce the number of collected frames Don't forget to adjust the Mosaicity value! If anomalous data is required, adjust the redundancy accordingly

For some pins, Chi 60° may be too high and should be restricted to 50°

Close the window and choose *Setup DC* to transfer the information to HKL data collection window
Exposure time is not imported from the strategy and should adjusted manually

multi mode is useful to find the best detector distance and exposure time

Check diffraction images go to the data directory

> dtdisplayhkl *.img &

30 to 50 strong reflections are needed for indexing. If it is not the case, increase the exposure time. It's possible to index non consecutive images if they have the same scan id

At 2θ ω goes from -90° to +90°, so don't put 90° step with 0° as ω start

Open display, do the peak search there, frame ↑ (middle click), the peak search has automatically changed, OK

Chi geometry goniometer, omega angle 180° follows the 2θ angle

If you press ABORT, the connexion will be lost and you will need to re-initialize Check the log on the MSCservdect window. To resume, check the parameters in the display. The scan number will be implemented.

With a 3D window >1, the mosaicity box will automatically be checked

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Zoom window

Box size: (16 by default) usually not necessary to change, even if there are neighbor spots in the box Int_box: red ellipsoid to integrate spot. The reject area is between the red and the white. Is it necessary to increase the size for the red ellipsoid (0.35 by default)?

Changing box size also changes the spot size. Use the box size that gives the highest resolution for the red area (it depends if the number is odd or even).

Profile fitting radius is the yellow circle. When ON, it display only the strongest reflexions. Increase (from 8 to 10) when there are less spots to have enough strong reflexions within the circle, otherwise HKL might reject too many weak reflexions. Don't use a too big radius because spot profile is different at the center and in the edges.

3D window: number of consecutive images put together to estimate the mosaicity. Usually it covers the spread of the mosaicity ONCE, but set it to cover it TWICE.

Set shadow region: the Mask for the beamstop is not critical.

Integrate

All scans are processed χ^2 should be close to 1

Scale

Check the boxes:

- Scale restrains
- Absorption correction
- Anomalous
- B restrains (HKL recommended)

Have low reflexions marked for rejection. Scale several times until the number don't change anymore. If χ^2 is too low, decrease the error scale factor. Exclude frames if necessary.

For SAD phasing: if χ^2 is high: increase the scale factor (up to 1.6) and the error model (up to 0.6-0.7), but it might kill the anomalous signal. Check the orange(merged)/blue(unmerged) plot on χ^2 : higher signal means higher gap

Use auto correction: will try to reach a χ^2 of 1, but we don't know how, so be careful.

Report

The log file contains merged I+/I- an the end of the file and tables with unmerged I+/I- in the middle of the file.

SAD-Phasing

The sequence is necessary. Give the source or the signal or define if you have a heavy atom. Software used: Arp/Warp, CCP4-6.3.0, CCP4-6.4.0(Coot), ShelX

Data analysis: Grey area 1.3 for anomalous signal (can be used up to 1.0)

Find sites: Choose the resolution limit (>1.8 Å to distinguish disulfide bonds, test 0.1 Å increments) Check the absolute value and the spread of solutions (a few in the up-right corner)

Site view: Sphere size correlates with the occupancy

Phase: High resolution limit; try different resolution cutoffs

Check hand (*ShelXE*): You should see the difference, dark red/dark blue, difference of one graduation in scale. If necessary, increase the cycle number so that a plateau is reached.

Phase: *Mlphase*, *DM-phase extension*

NCS: *DM* or *Parrot* (a bit better) At least try it if you have more than 1 molecule in the Asymmetric Unit

Build: *ARP/WARP* for high resolution *Buccaner* for lower resolution (~ 3 Å) Let ARP/WARP build/refine several times if has difficulties building. Start several times witha few cycles rather that once with many cycles.

Molecular Replacement

Analyze data

Prepare Model: Edit the PDB file (delete ligands, waters, side-chains if you want)

Run MR (*MolRep*): 3 Å by default; rigid body at 3.0 Å, then refine at max resolution

1)

HKL does get info on the shutter status

2)

the other HKL_3000 version is for data integration only

3)

2 slits can be tweaked to reduce the divergence with the middle wheel, but at some point the beam decreased and the exposure time will need to be increased to compensate

4)

default value 0.3