MX Group @ Swiss Light Source

PRIGO BOOKMARKING & ANOMALOUS COLLECTIONS INSTRUCTIONS

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Changes

Version	Date	Changes	Author
0.1	15.03.2021	Initial	KMS, VO
0.2	18.03.2021 - 25.03.2021	Incorporation of feedback	KMS, VO
1.0	12.04.2021	Published version	KMS, VO

Prior to the crystallographic experiments

- Suggested readings are found here.
- What are the expected:
    - space group
    - resolution
    - number of molecules per asymmetric unit
    - number of sulfurs/phosphorus per molecule
- Are any unexpected ions present, such as Mn, Fe, Co, Ni, Cu, Zn?
    - Do a fluorescence spectrum
- Boundary conditions for Native SAD:
    - 6D beamline is designed for conventional crystals (>50 μm)
    - Resolution of better than ~2.8 Å is usually required
    - Multiple crystal collections may be required if the sample:
        - has low symmetry (i.e. monoclinic or triclinic)
        - insufficient resolution (~3Å)
        - is small (~50 μm in longest direction)
        - especially prone to radiation damage

High Multiplicity Low Dose Data Collection Strategy, the Native-SAD case

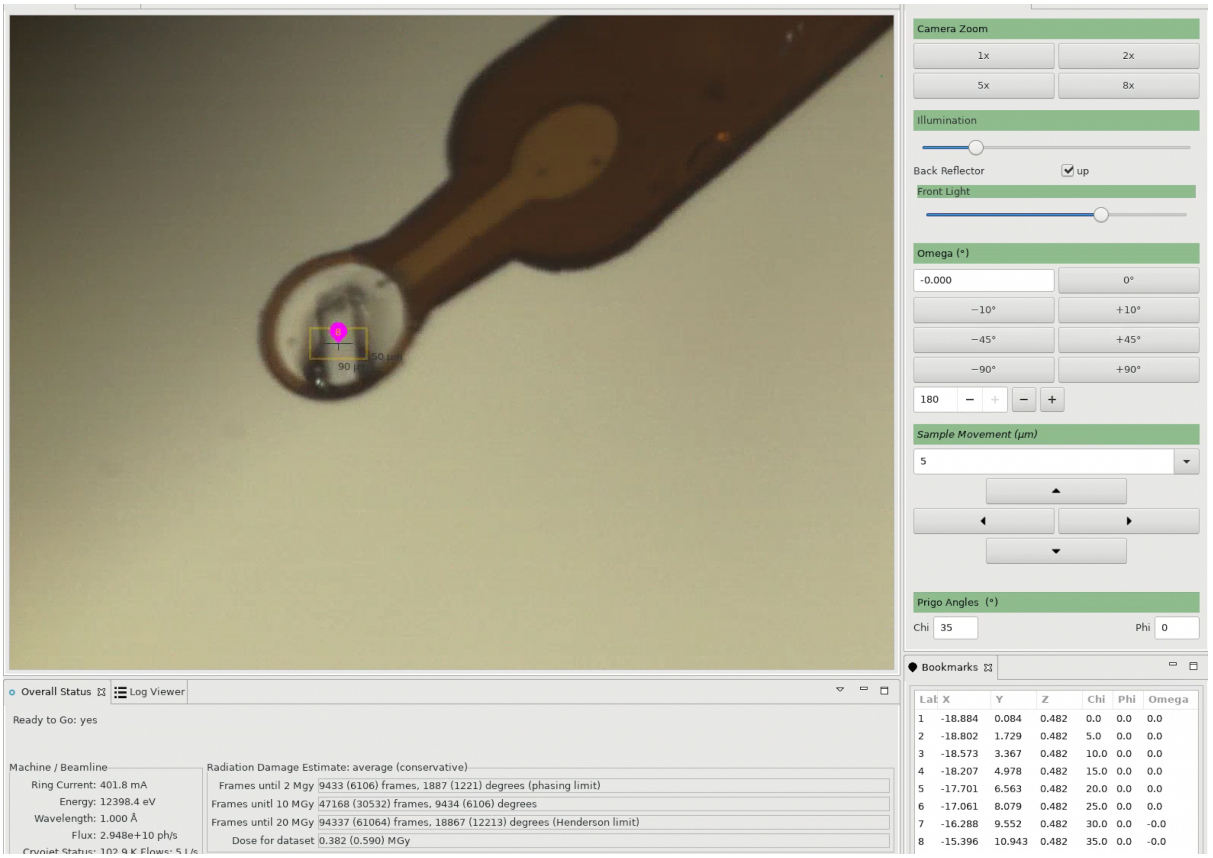
- Data collection at 5975 eV, i.e. 2.075-Å wavelength
- 0.2º/0.1 sec/full beam
- One crystal position
- n × 360º at multiple chi (χ) and phi (φ) PRIGo angles
    - Typically 8 turns at chi(χ)=0º, 5º,..., 35º at phi(φ)=0º
    - You may automate this using bookmarks, see next section.
- On next crystal position chi(χ)=35º, 30º,..., 5º at phi(φ)=45º
- If not solved yet, repeat on the next crystals.

The same strategy applies if a heavy atom is present. Adapt the energy and the dose. In such cases, only a couple of 360º turns should be necessary.

Multiple-orientation data collection automation: Bookmarking with PRIGo

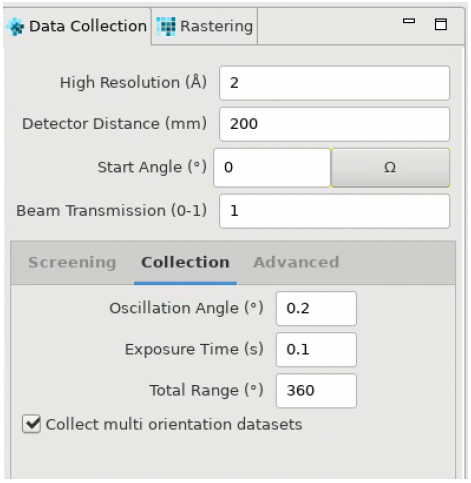
To set bookmarks in DA+ GUI,

1. Center your crystal to the beam position.
2. Right click and in the dropdown select ‘set bookmark’, or on windows press ctrl+b or mac command+b.
3. In the Bookmarks table at the bottom right of DA+ GUI the coordinates of the added bookmark appears.
4. Change chi to next desired collection angle and repeat steps 1 + 2 to set an additional bookmark.
5. After you have added all of the bookmarks for your desired chi angles, please check the bookmarks table at the bottom right to confirm they are all present before moving to step 6.



Warning: DO NOT set too many bookmarks in one go. Bookmarking is fine most of the time but your sample could be moved out of cryo if PRIGo reaches limits. Collecting 1 data set at a time is the safest option.

6. On the left hand panel of DA+ GUI tick “Collect multi-orientation datasets” box in the Data Collection tab. This will collect the Total Range (°) for each of the bookmarks in subfolders named setXX_chiXXphiXX.



Data Processing and Merging

Process as data arrives and play early with hkl2map. You will have to merge data manually, and in case of SG ambiguity, you will have to reprocess with a reference data set. There is a script called go2gether.com which is useful - a merging script that you can manually run.

Usage: `go2gether.com path1/XDS_ASCII.HKL ... [pathn/XDS_ASCII.HKL]`

We recommend creating a 'merging' folder using the sample prefix where you copy the individual XDS_ASCII.HKL files from adp processing and rename them with the respective chi and phi values to parse through the go2gether.com script.

```
e18482@x06da-cn-1 > ls -lrt lyso_7
total 385
-rw-r--r--. 1 e18482 p18482  3896 Mar 15 11:08 lyso_7_1_scanrequest.json
drwxr-sr-x. 3 e18482 p18482 65536 Mar 15 11:12 set01_chi00phi000
drwxr-sr-x. 3 e18482 p18482 65536 Mar 15 11:15 set02_chi05phi000
drwxr-sr-x. 3 e18482 p18482 65536 Mar 15 11:18 set03_chi10phi000
-rw-r--r--. 1 e18482 p18482  2777 Mar 15 11:18 lyso_7.log
```

```
e18482@x06da-cn-1 > ls -lrt lyso_7_merging/
total 145921
-rw-r--r--. 1 e18482 p18482 40887560 Mar 15 11:17 chi00phi00.hkl
-rw-r--r--. 1 e18482 p18482 40957572 Mar 15 11:18 chi05phi00.hkl
```

Change into the merging directory and run go2gether.com

```
e18482@x06da-cn-1 > cd lyso_7_merging/
e18482@x06da-cn-1 > go2gether.com chi00phi00.hkl chi05phi00.hkl
```

The results will be written and saved in the go_SCALE.LP for review.

```
e18482@x06da-cn-1 > ls -lrt
total 145921
-rw-r--r--. 1 e18482 p18482 40887560 Mar 15 11:17 chi00phi00.hkl
-rw-r--r--. 1 e18482 p18482 40957572 Mar 15 11:18 chi05phi00.hkl
-rw-r--r--. 1 e18482 p18482      141 Mar 18 14:09 go_XSCALE.INP
-rw-r--r--. 1 e18482 p18482     4096 Mar 18 14:10 DECAY_001.cbf
-rw-r--r--. 1 e18482 p18482     4096 Mar 18 14:10 DECAY_002.cbf
-rw-r--r--. 1 e18482 p18482    12288 Mar 18 14:10 MODPIX_001.cbf
-rw-r--r--. 1 e18482 p18482    12288 Mar 18 14:10 MODPIX_002.cbf
-rw-r--r--. 1 e18482 p18482     4096 Mar 18 14:10 ABSORP_001.cbf
-rw-r--r--. 1 e18482 p18482     4096 Mar 18 14:10 ABSORP_002.cbf
-rw-r--r--. 1 e18482 p18482 65500522 Mar 18 14:10 go_XSCALE.ahkl
-rw-r--r--. 1 e18482 p18482    24591 Mar 18 14:10 go_XSCALE.LP
```

It is advised to do this in an incremental fashion, merge 2 files, then 3, then 4 etc and to keep an eye on the anomalous signal, high resolution completeness and low resolution R Merge values.

Readings

Basu, S., Finke, A., Vera, L., Wang, M., & Olieric, V. (2019). Making routine native SAD a reality: lessons from beamline X06DA at the Swiss Light Source. Acta Crystallographica Section D: Structural Biology, 75(3), 262-271. https://doi.org/10.1107/S2059798319003103

Finke, A. D., Panepucci, E., Vonrhein, C., Wang, M., Bricogne, G., & Oliéric, V. (2016). Advanced crystallographic data collection protocols for experimental phasing. In E. Ennifar (Ed.), Methods in molecular biology: Vol. 1320. Nucleic acid crystallography. Methods and protocols (pp. 175-191). https://doi.org/10.1007%2F978-1-4939-2763-0_11

Olieric, V., Weinert, T., Finke, A. D., Anders, C., Li, D., Olieric, N., … Wang, M. (2016). Data-collection strategy for challenging native SAD phasing. Acta Crystallographica Section D: Structural Biology, 72(3), 421-429. https://doi.org/10.1107/S2059798315024110

Weinert, T., Olieric, V., Waltersperger, S., Panepucci, E., Chen, L., Zhang, H., … Wang, M. (2015). Fast native-SAD phasing for routine macromolecular structure determination. Nature Methods, 12(2), 131-133. https://doi.org/10.1038/nmeth.3211