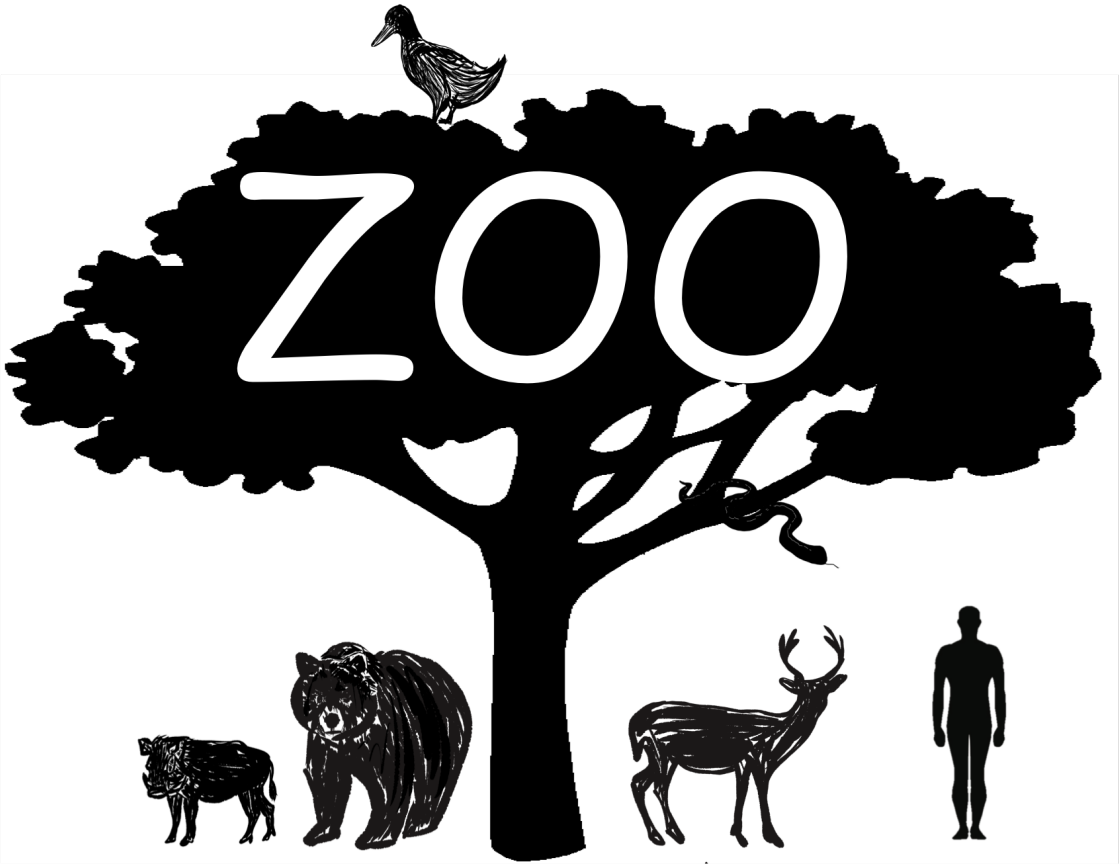
Current status of automatic data collection by ZOO at SPring-8

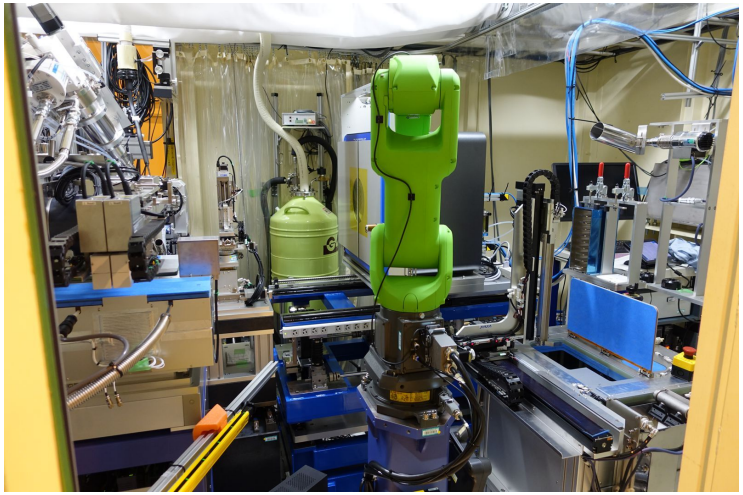


Kunio Hirata
(RIKEN/SPring-8 Center)

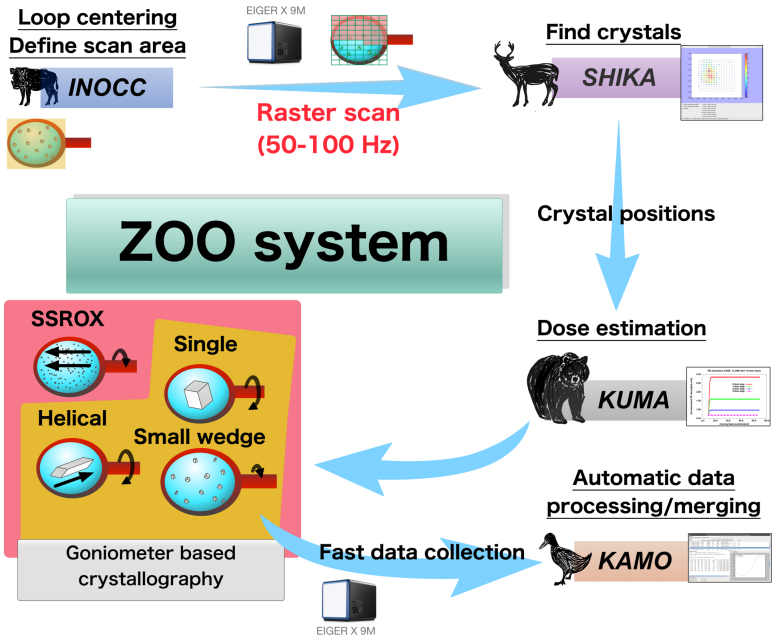
Beamline BL32XU at SPring-8

SPring-8

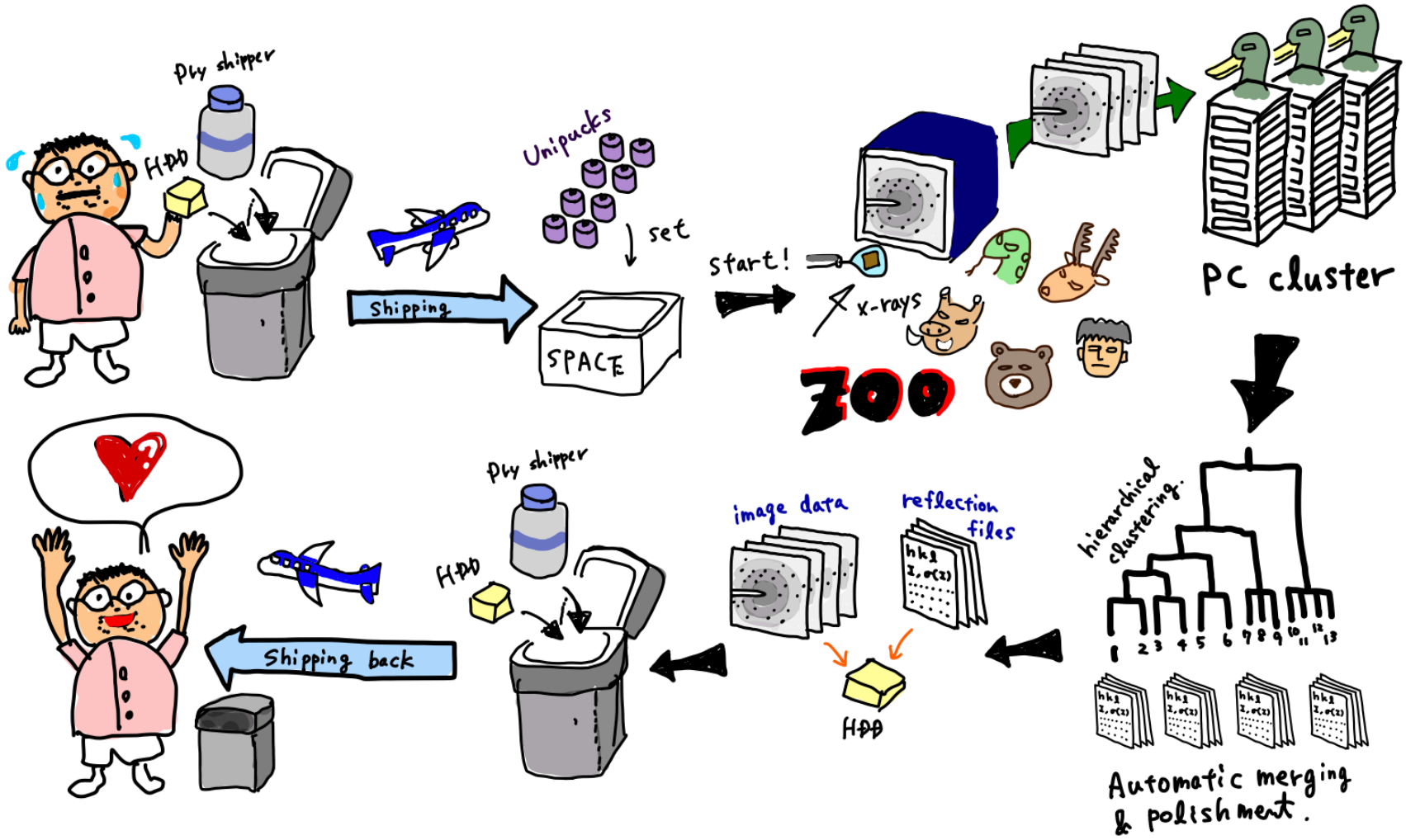
Micro-focus beamline BL32XU



BL45XU
Fully automated beamline



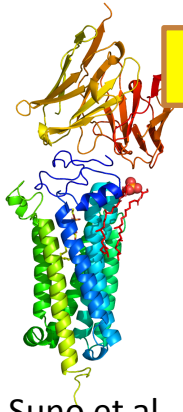
How to use ZOO system



Just send us UniPucks and data collection sheet.

ZOO solved many structures with merging

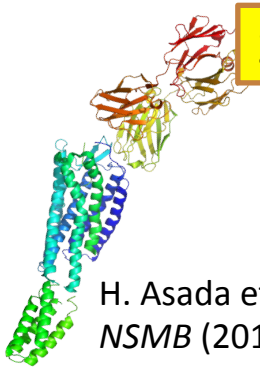
Orexin 2 receptor
2.0 Å



251

R. Suno et al.
Structure(2018).

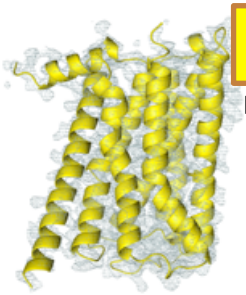
Angiotensin II receptor
3.2 Å



241

H. Asada et al.
NSMB (2018).

M2 muscarinic receptor
2.3Å

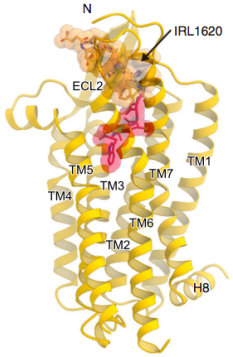


598

Hg-SAD

R. Suno *et al*.
Nat. Chem. Biol.(2018)

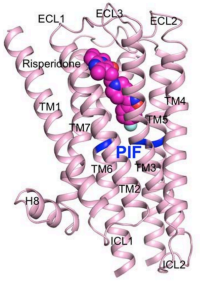
Human ETB receptor
2.0 Å



483

W. Shihoya et al.
Nat. Commun.(2018)

5-HT2A Receptor
2.7 Å

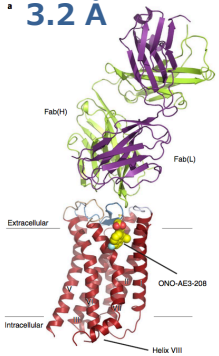


265

K. Kimura *et al*.
Nat. Chem. Biol.
(*2019*)

# of datasets

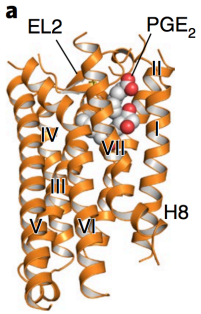
Prostaglandin E receptor(EP4)
3.2 Å



203

Y. Toyoda *et al*.
Nat. Chem. Biol. (2018)

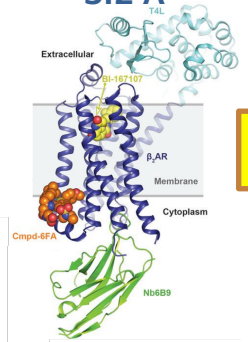
Prostaglandin E subtype EP3
3.2 Å



683

K. Morimoto *et al*.
Nat. Chem. Biol. (2018)

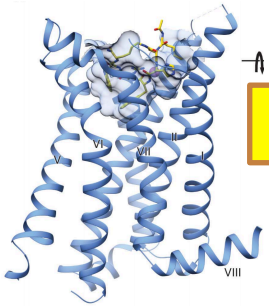
β2AR regulation
3.2 Å



125

X. Liu et al.
Science(2019)

Melanocortin-4 receptor
2.8 Å



83

J. Yu et al.
Science(2020)

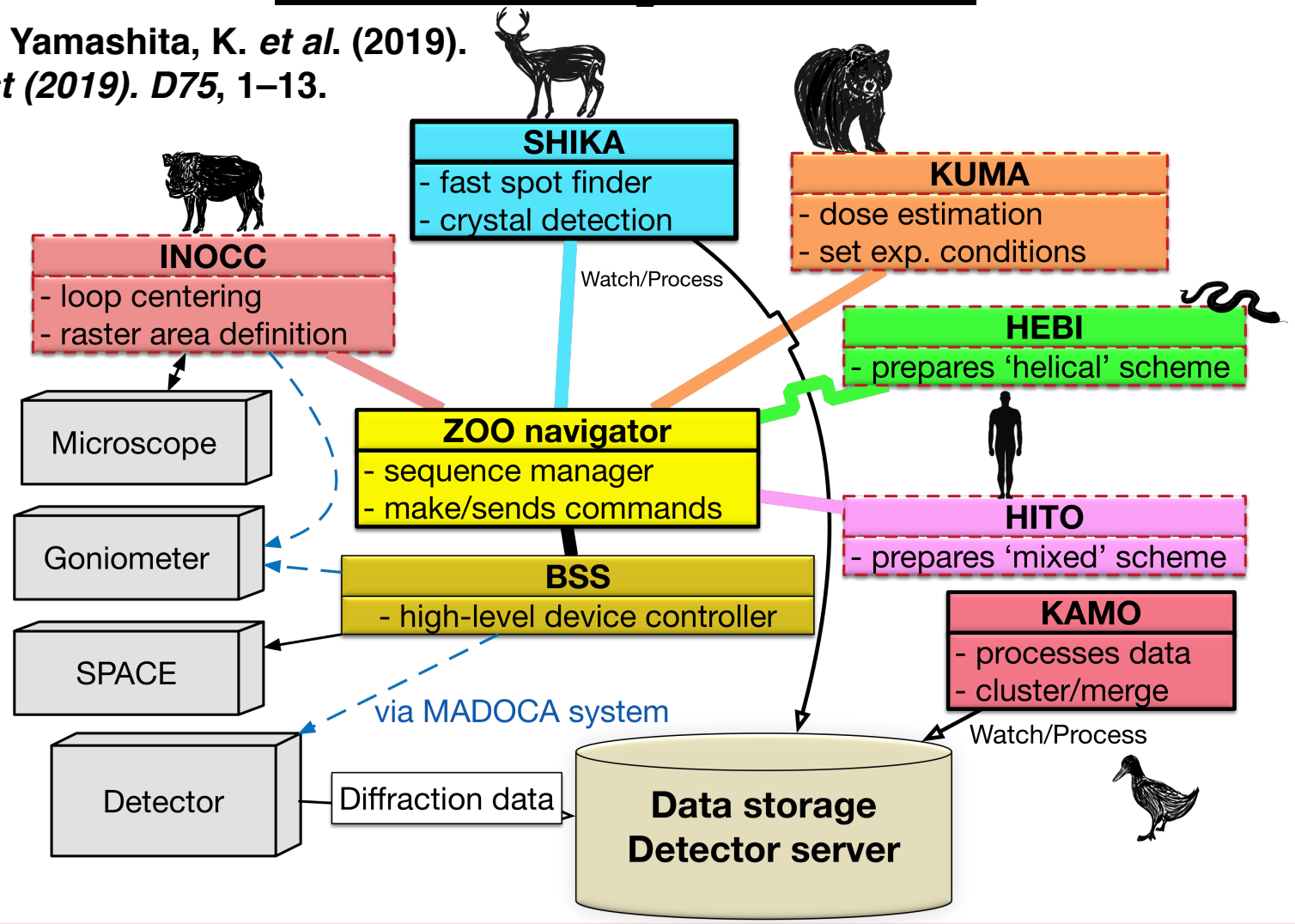
"Quantitative changes" make "Qualitative change"

Merits

- **Un-attended measurements**
  - Users can sleep
  - Users can do other experiments
- **Fast data collection**
  - ZOO shortens data collection time
  - No human errors
- **No prior knowledges/expertise**
  - **ZOO enabled 'technically difficult experiments'**
  - **under dose control**
- **Automatic filter enhancing data quality**
  - KUMA : controls dose
  - SHIKA : rejects bad crystals via the raster scan
  - KAMO : selects better frames/datasets => merge

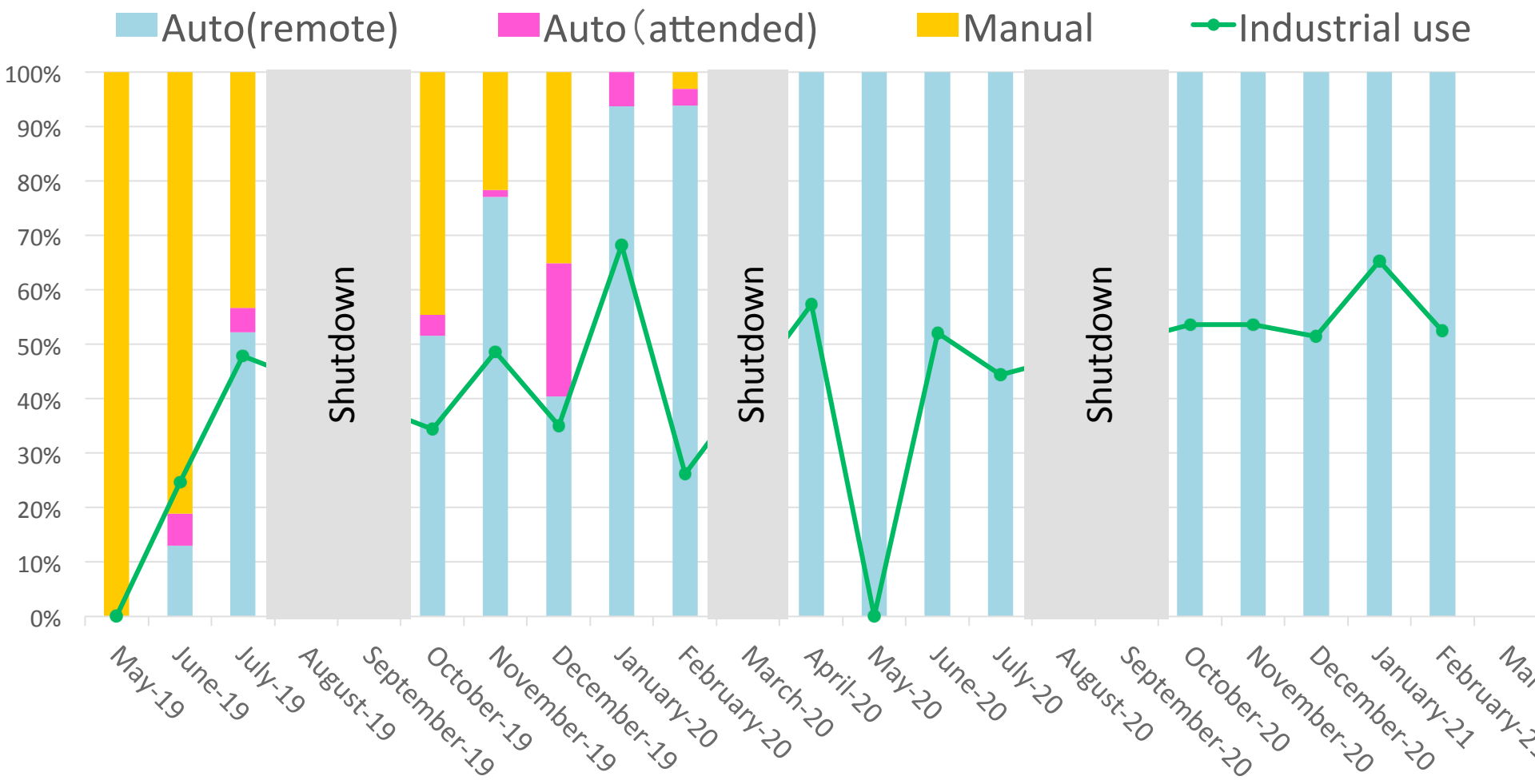
ZOO system

Hirata, K., Yamashita, K. *et al*. (2019).
Acta Cryst (2019). D75, 1–13.



Program package with 'animal names' in Japanese

Current status of user operation at BL45XU



Specifications of MX ID beamlines at SPring-8

- Beam size **5~30 μm**

(1 μm beam is available for SSROX@BL32XU)

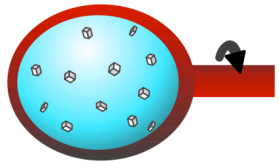
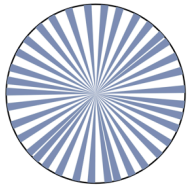
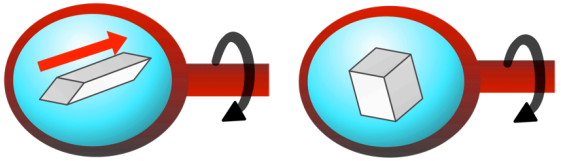
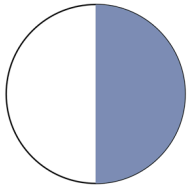
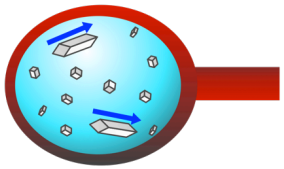
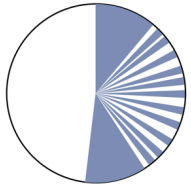
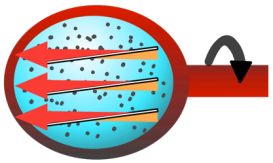
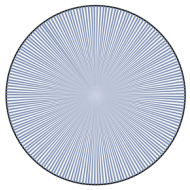
- Photon flux **10^{12}~10^{13} phs./s.**

- Wavlength **0.8 ~ 1.9 Å**

(0.35~0.65 Å available at BL41XU)

ZOO is available at MX ID beamlines at SPring-8
BL32XU/BL41XU/BL45XU.

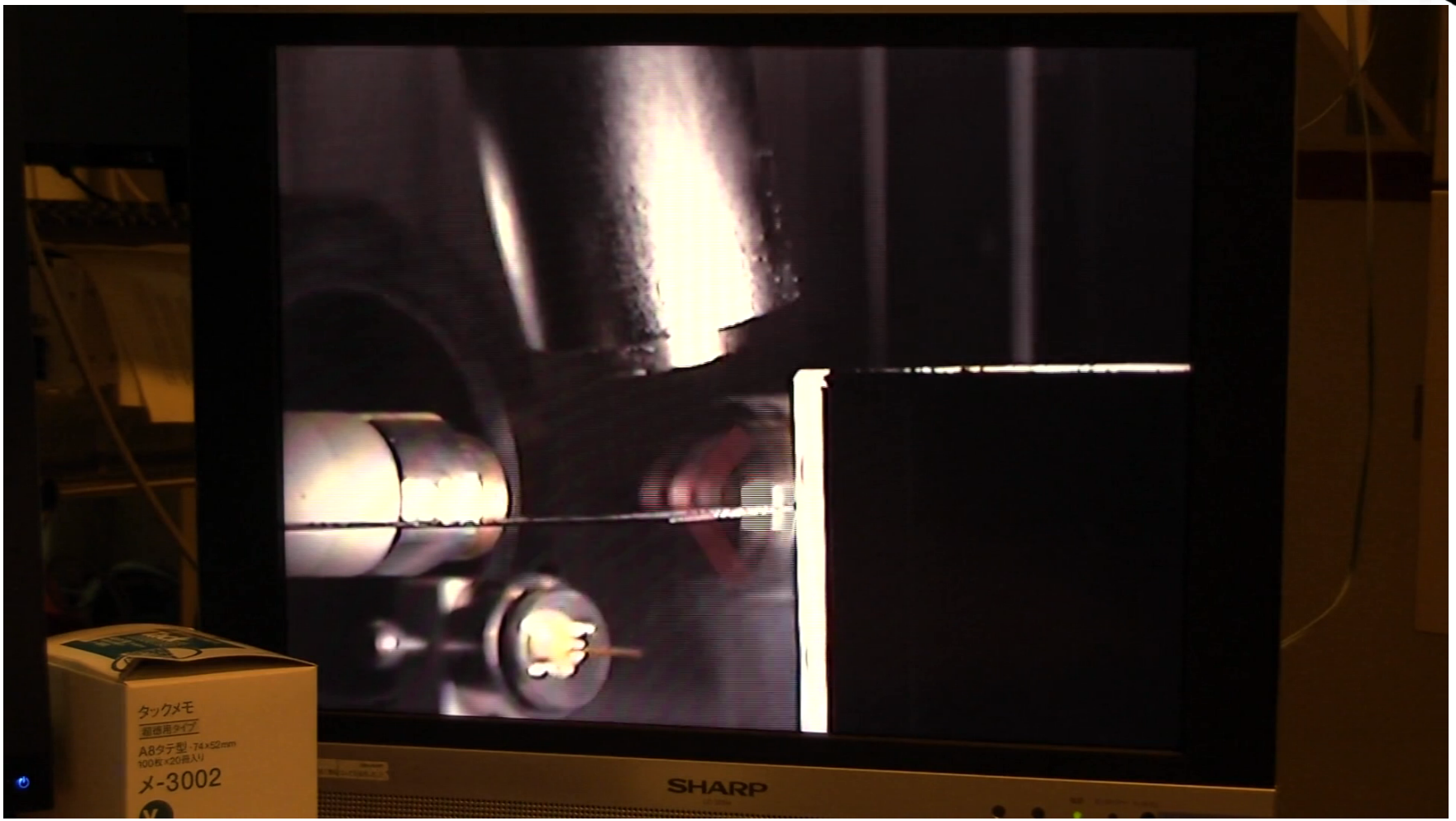
Available schemes in ZOO

Scheme		Rot. (/xtal)
"Small wedge" (SWSX)	 10–300 crystals	 5-10 deg.
"helical" or "single"	 single or a few crystals	 90-360 deg.
"mixed"	 small & large crystals	 5-10 deg. + 30-360 deg.
"SSROX"	 300-10,000 crystals	 < 1 deg.

Users should choose 'scheme' for each cryo-pin.

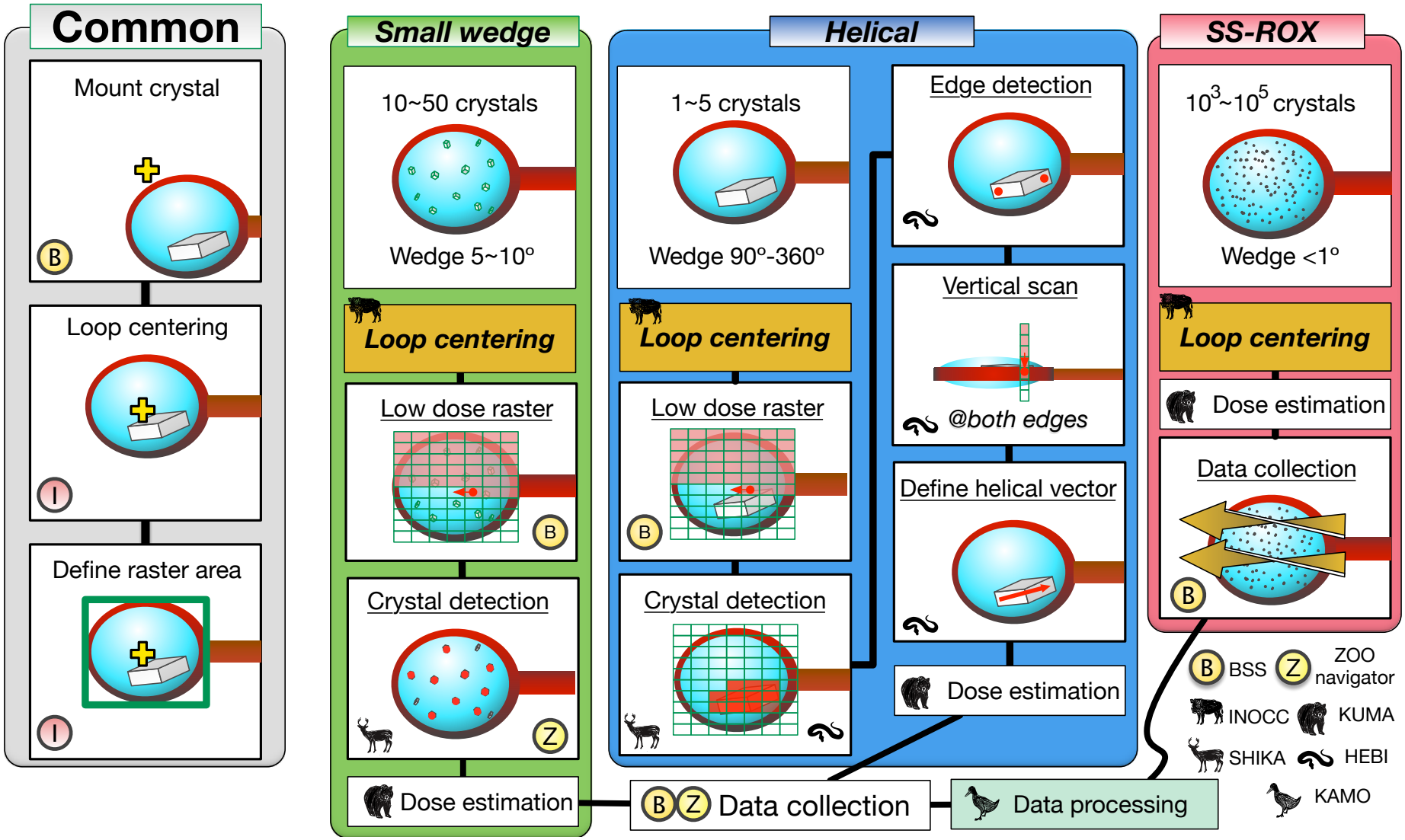
How ZOO works

(automates helical scheme)



HEBI eliminates ‘expertise’ for 3D centering of helical vector.

Protocols in ZOO

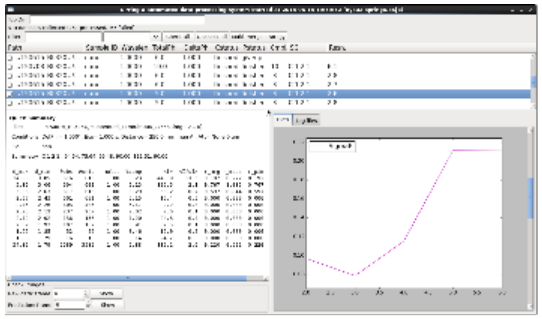


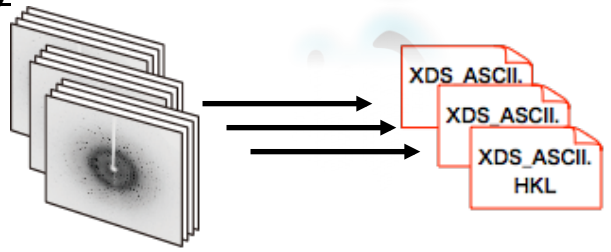
Keitaro Yamashita

KAMO

(Automatic data processing)

- Index/Integrate each dataset
- Grouping datasets (cells)
- Determination of space group
- Clustering (cell/intensity CC)
- Selecting good cluster (Completenes, Redundancy)
- Merging datasets
- Outlier detection/rejection





Data processing

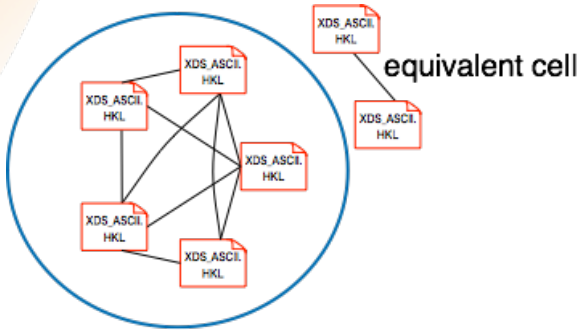
XDS (or DIALS)

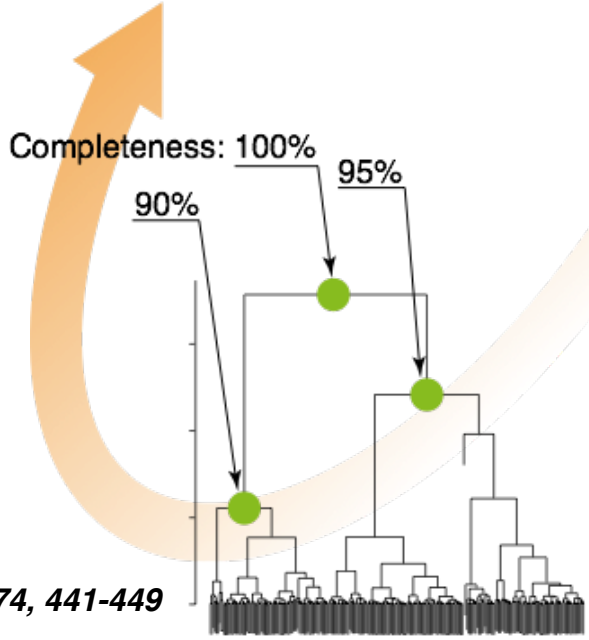
Merge & polish

selected clusters

Grouping

crystals with equivalent cells





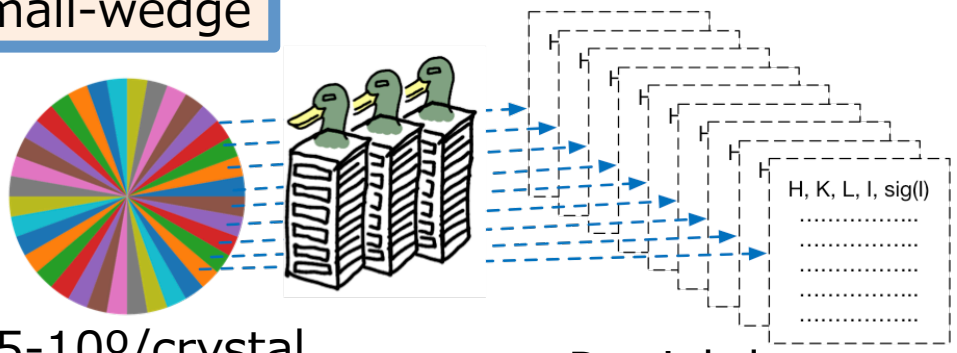
Clustering

- BLEND (cell parameters)
- CC of intensities

Yamashita, K., (2018). *Acta Cryst (2018). D74, 441-449*

Data back to users

Small-wedge



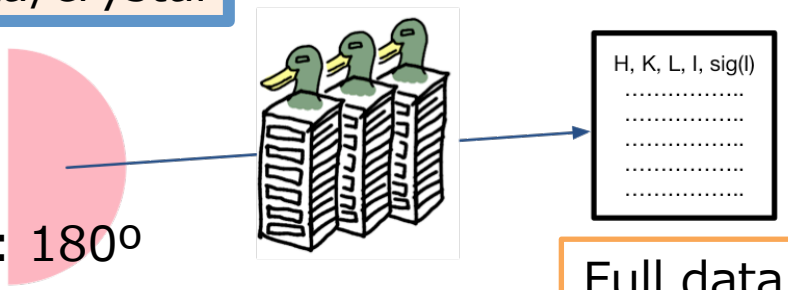
5-10º/crystal

Partial data

Clustering

Full datasets

Full data/crystal

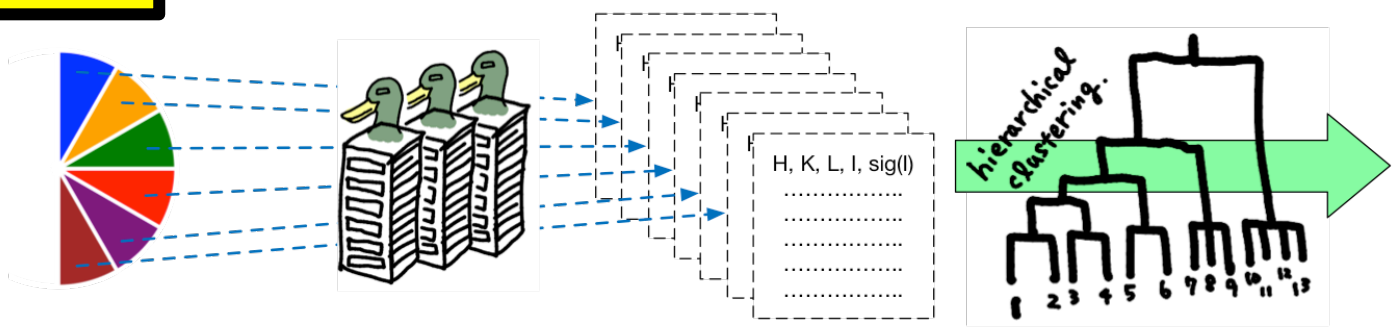


Ex: 180º

Full data

BUTTAGIRI

Clustering

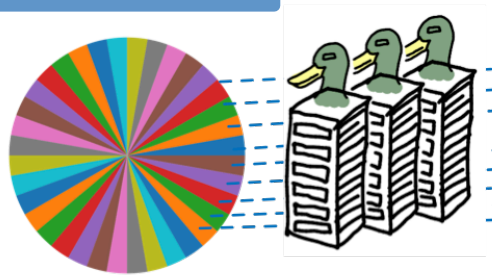


Partial data

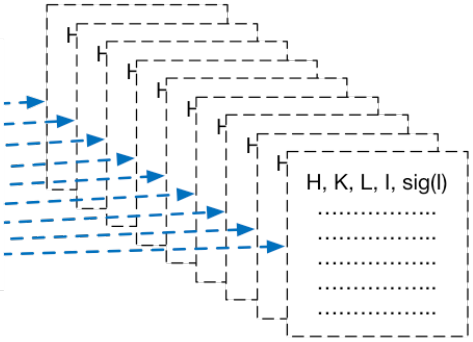
Full datasets

Data back to users

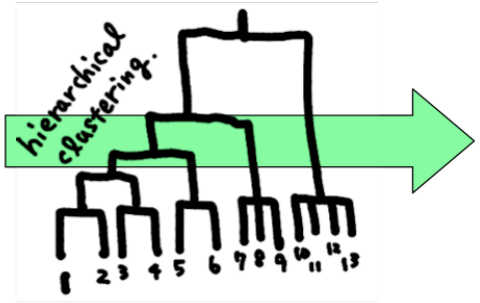
Small-wedge



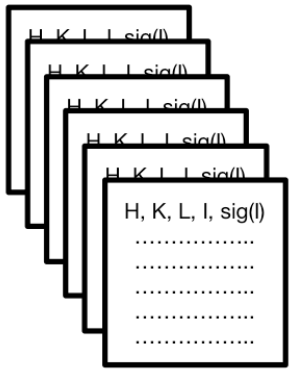
5-10º/crystal



Partial data

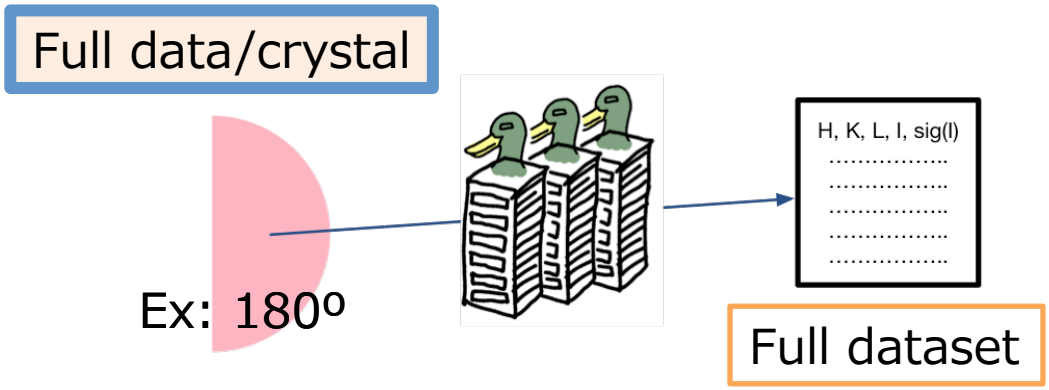


Clustering



Full datasets

Data back to users



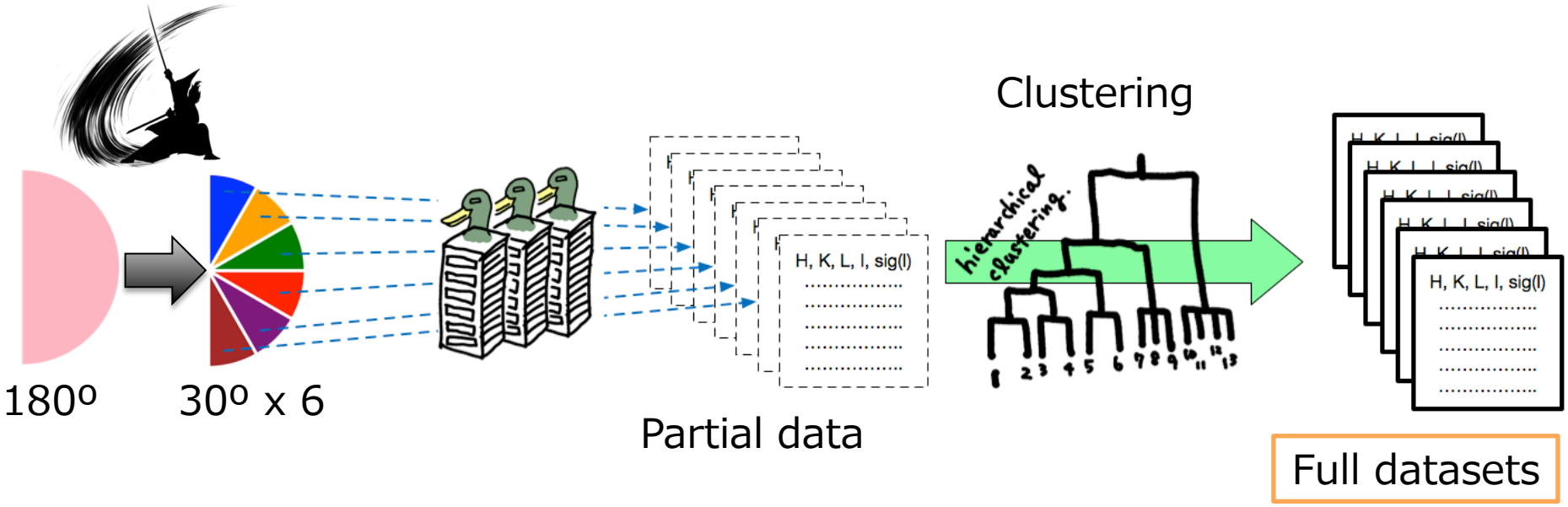
BUTTAGIRI process

divides ‘complete data’ to ‘small wedge data’

(Ex. : Full data 180º = 30º x 6 wedges)

→Hierarchical clustering is applied to them

(Select better data in ‘merging process’)



Merits in ‘BUTTAGIRI’ process

- extracts/merges better crystal volumes

- is very powerful when it works with ‘helical data collection’
  - especially for ‘inhomogenous’ crystals

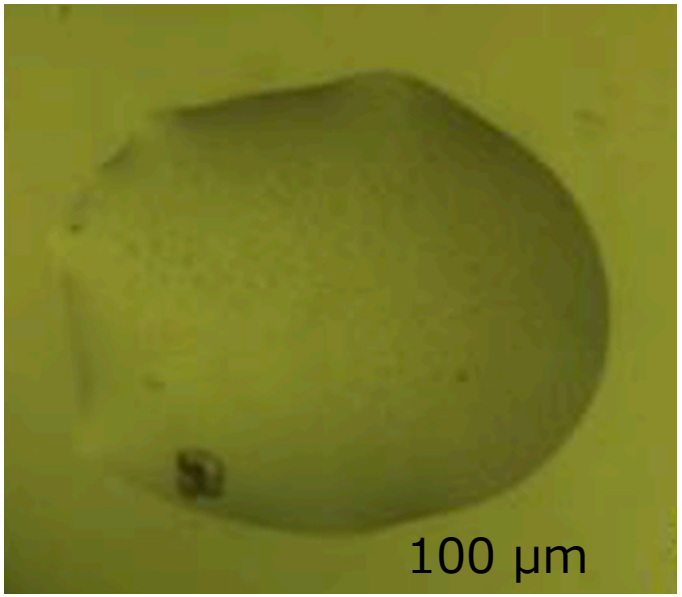
Problem solving by ZOO : case1

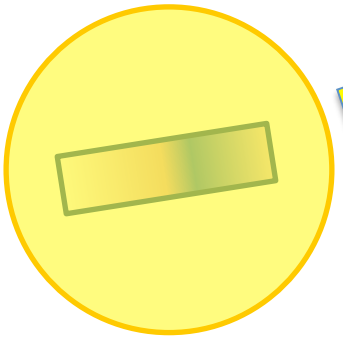
Courtesy of Dr. Sengoku

Data collection from ‘inhomogeneous crystal’

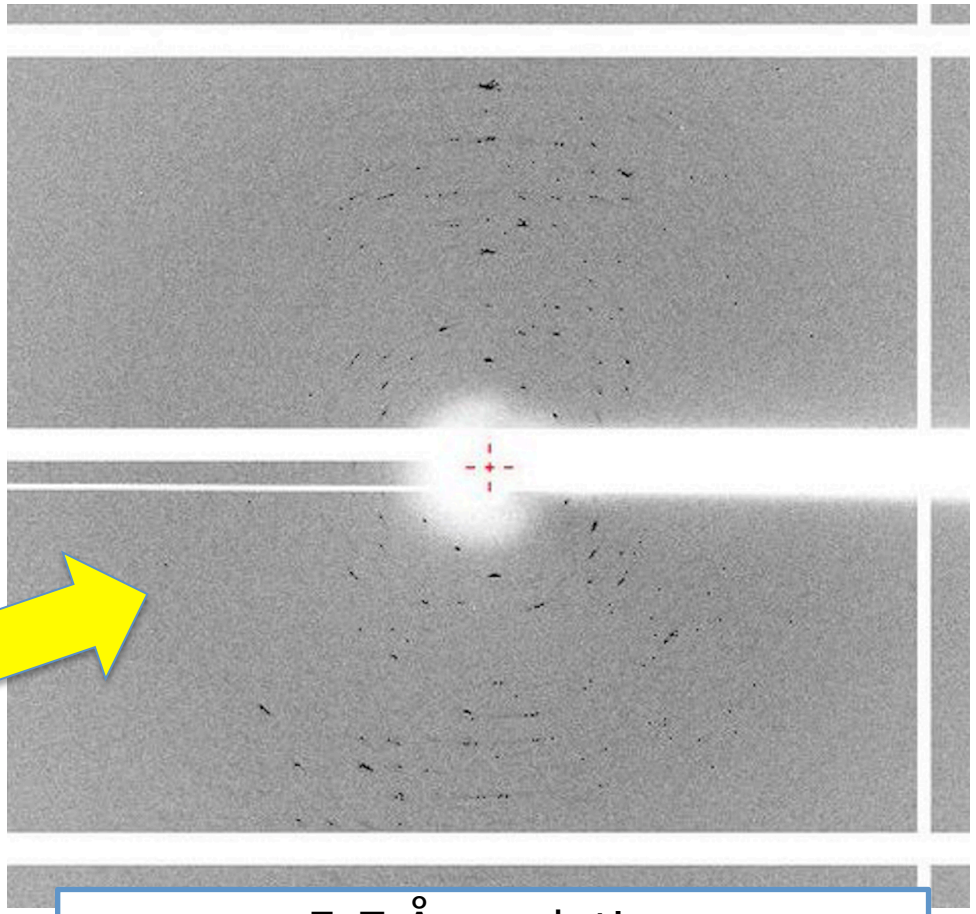
Is a human superior to ‘automatic data collection’, especially for ‘inhomogenous crystal’?

This case is one of the answers to this question.





Expose 200 μm beam



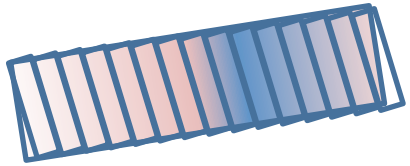
5-7 Å resolution
Diffraction shows ‘elongated’ peaks

Problem solving by ZOO : case1

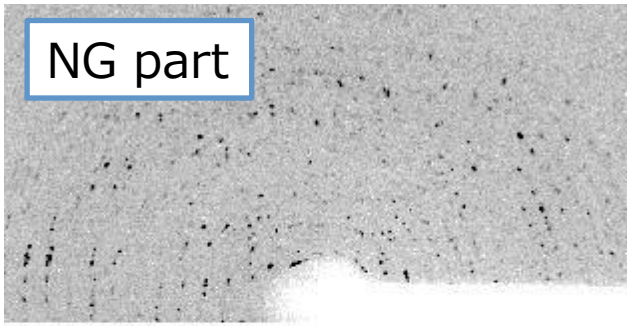
Courtesy of Dr. Sengoku

Data collection from 'inhomogeneous crystal'

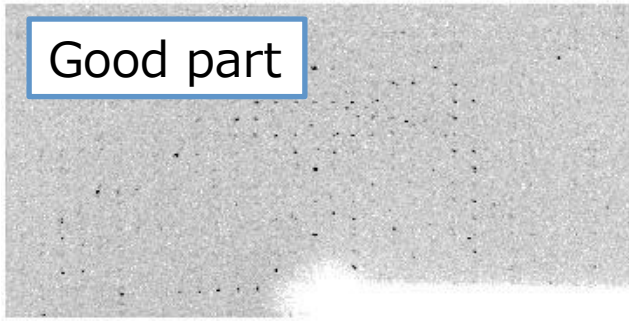
1. Helical data collection x 11 crystals



2. 720 deg/crystal (0.1deg oscillation)



3. Data processing
   - KAMO: 10º BUTTAGIRI processing
   - 720º / 10º = 72 wedges/crystal
   - 72 x 11 crystals = 792 datasets
   - KAMO: Hierarchical clustering
   - Merge → **2.8 Å resolution**



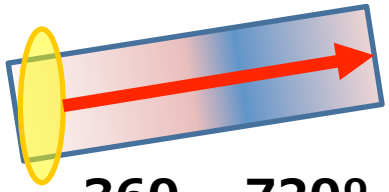
Succeeded data collection from 'inhomogeneous' crystals

T. Kato et al. Nat. Chem. 12, 1081-1088(2020)

Problem solving by ZOO : case1

Data collection from ‘inhomogeneous crystal’

- **The best way: Helical data collection with high redundancy**
  - Data collection from ‘entire crystal volume’
  - 360-720 deg/crystal
  - Data collection in different orientation/volume from each crystal volume



- **BUTTAGIRI process**
  - To select better volumes in data processing

- **Does ‘redundant data collection’ deteriorate resolution limit?**
  - Redundant measurements can recover accumulated signals.
  - (25% x 4 times → 100%) in the ideal case

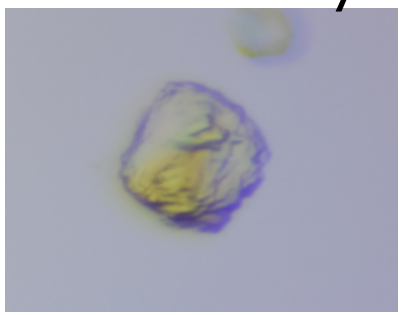
- **Multiple crystal strategy is effective for data collection if ‘better’ volumes in a crystal are small**
  - Only 10%/crystal is good → Merging 10 crystals

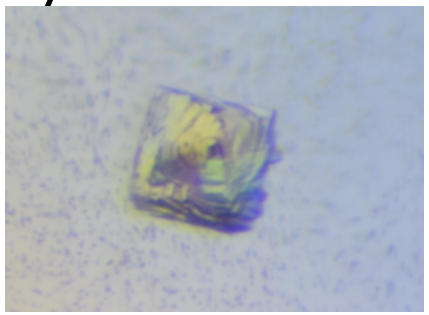
The protocol would be superior to the manual measurement.

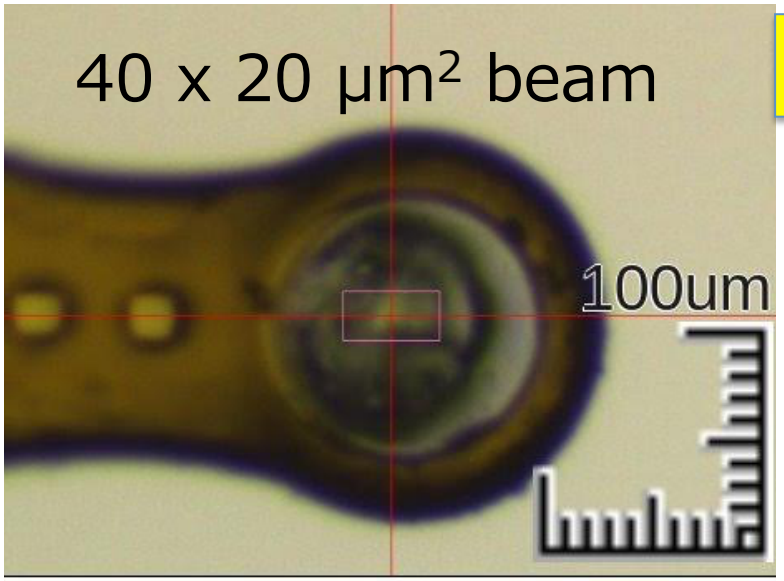
From clustered ‘micro‘ crystals

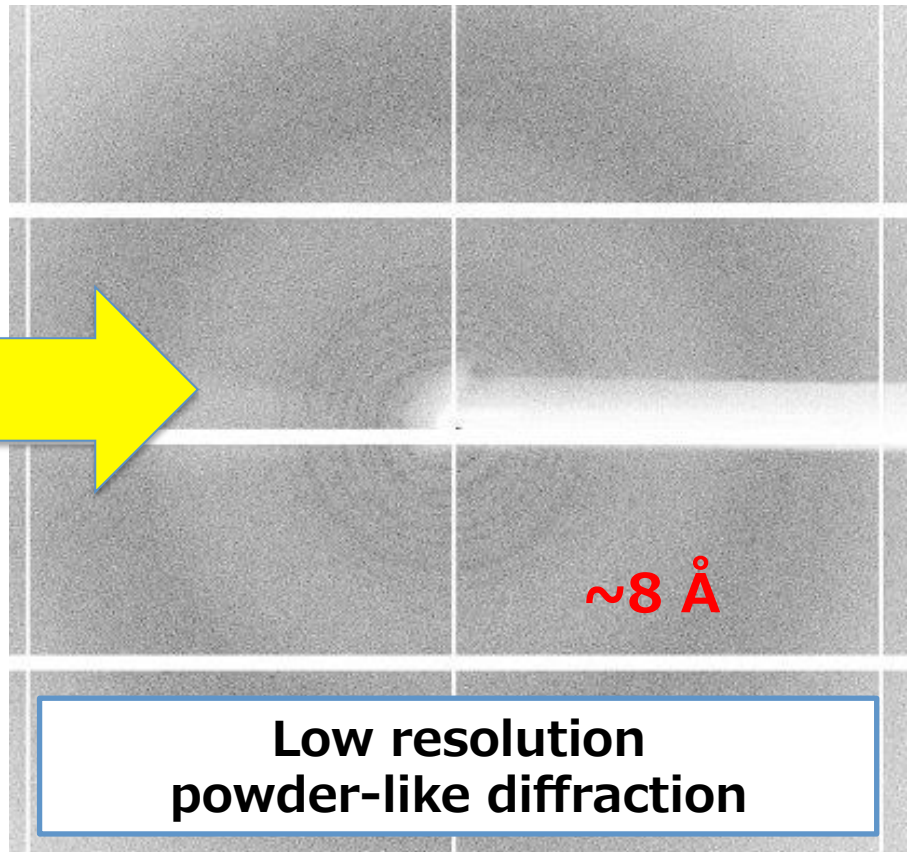
Courtesy of Dr. Numoto

Are they crystals??









Consultation:
Could we collect ‘full dataset’ from them?

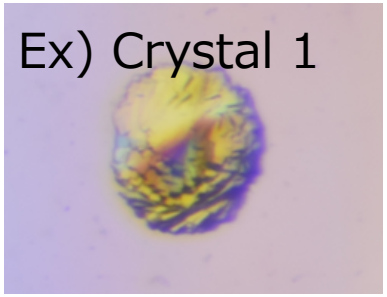
Problem solving by ZOO : case2

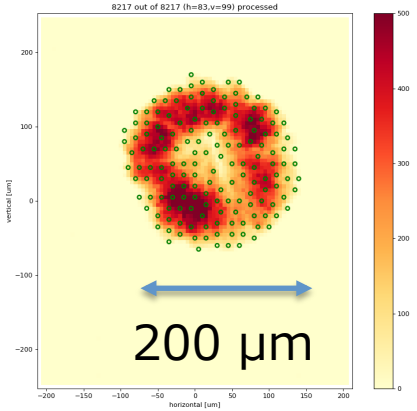
From clustered ‘micro‘ crystals

Data collection with ZOO

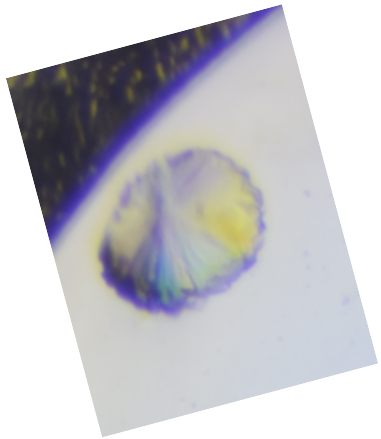
Courtesy of Dr. Numoto

5 μm beam (scan & data collection)

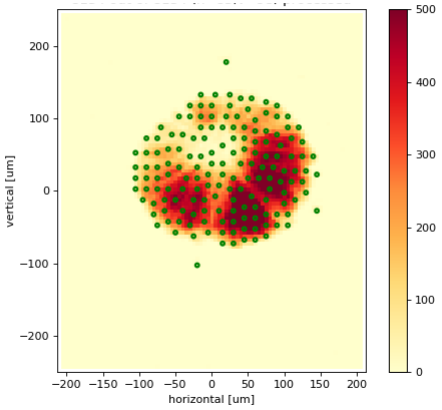




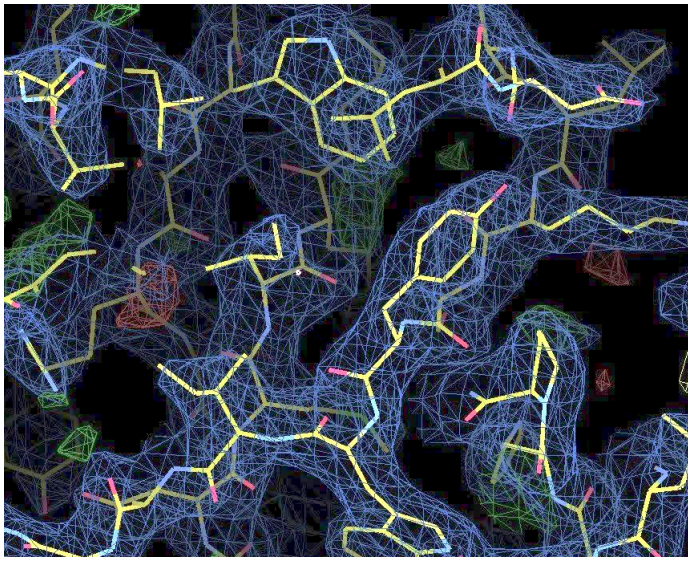
- Oscillation data : 5 deg/point
- **~1,400 wedges** from 16 loops
- **670 datasets**: equivalent cell
- Merging gave **2.5 Å dataset**
- Measurement time: **3 hours**



Ex) Crystal 2



Scan results



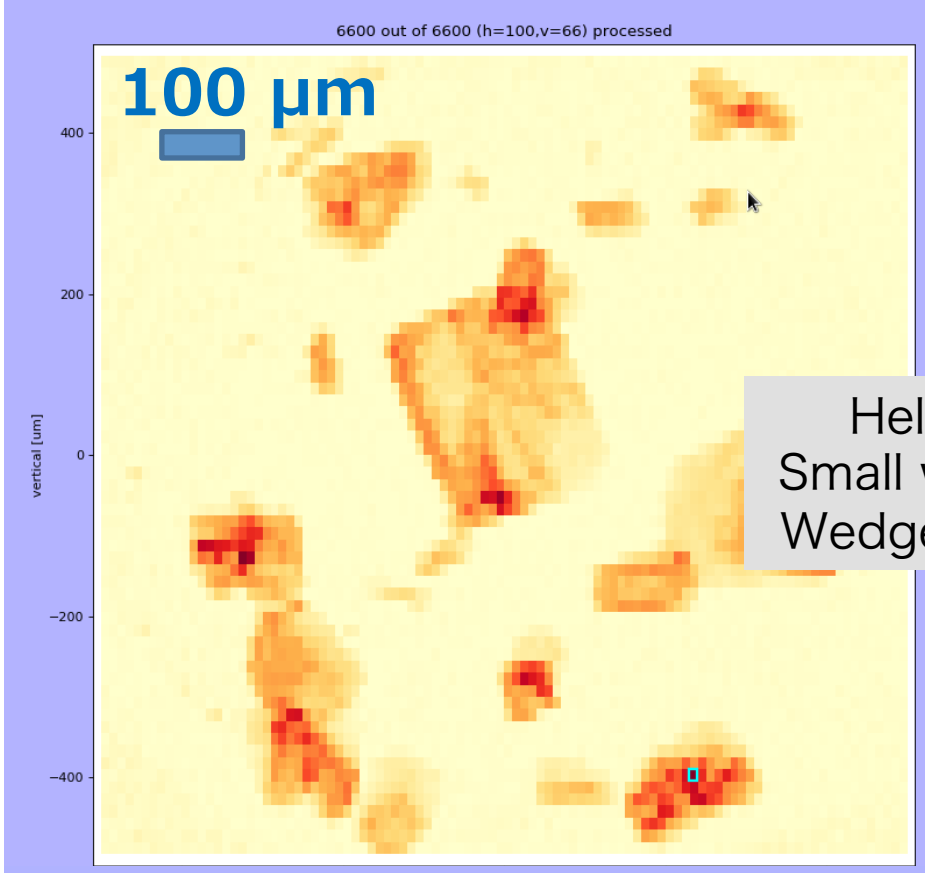
Structural analysis from a crystal that doesn't look good at first glance!

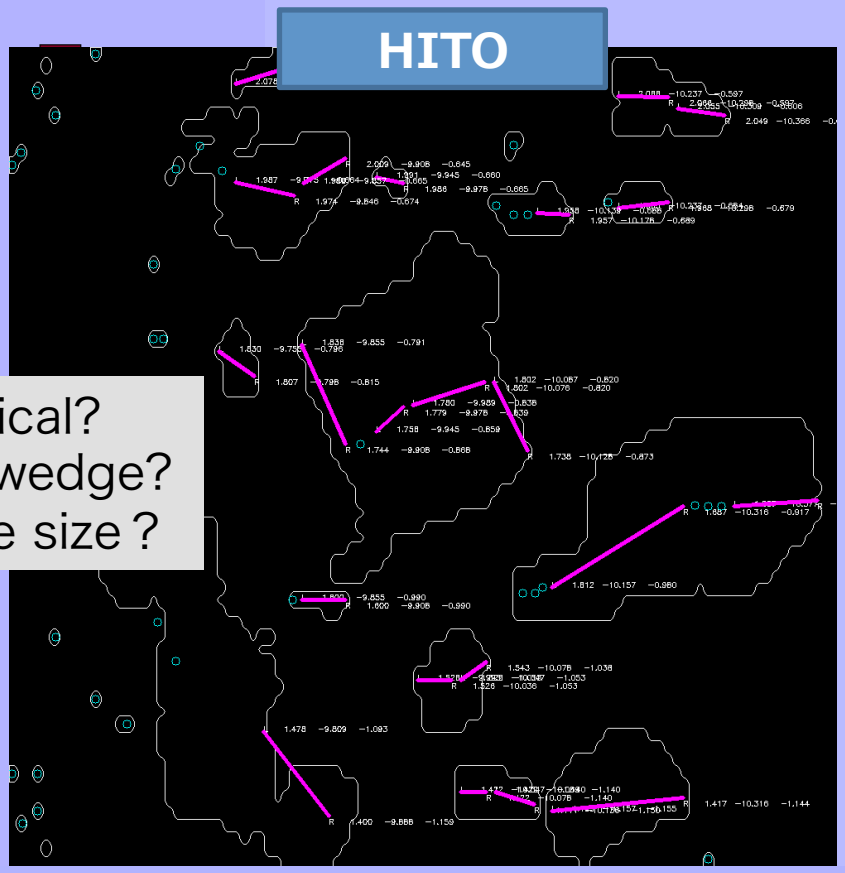
Future perspectives

- **Towards ”Fully” automatic**
  - Minimal prior information from users
- **Feedback**
  - Results of data processing
    - to direct residual measurements
- **Fully automated exchanging pucks**
  - “Endless” automatic data collection

HITO (mixed mode)

Automatically selects ‘data collection scheme’.





Helical?
Small wedge?
Wedge size ?

合計 1,270 deg / 1mm loop 25 min.→ 1.3 Å resolution

(c.f. small wedge 1,200 deg./24 min.)

Future perspectives

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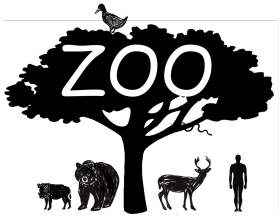

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- **KAMO is open source programs**

https://github.com/keitaroyam/yamtbx