Automatic data collection at SPring-8/Japan

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RIKEN/SPring-8

Macromolecular Crystallography (MX) Beamlines at SPring-8

Bending magnet beamlines BL26B1/B2

Developed for structural genomic research in 2000s

Automation at SP8 MX started from here

- Flux ~10^{11} (ph/s)
- Data collection from well diffracting crystal

Undulator BL, BL45XU

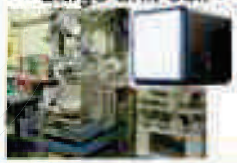
Automated data collection, High flux beam

- High flux 2 x 10^{13} ph/s @ 20 x 20 μm
- Fully automated data collection, ZOO system

Undulator BL, BL44XU

Low divergence beam

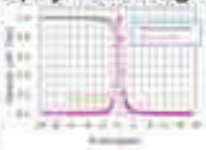
- Macromolecular complex
- Large unit cell > 500 Å

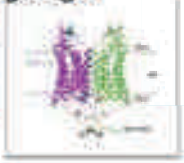


Undulator BL, BL32XU

Micro-beam minimum 1μm

- Micro-focus beam (1 μm; 6 x 10^{10} ph/s)
- Micro-crystallography / challenging target





Undulator BL, BL41XU

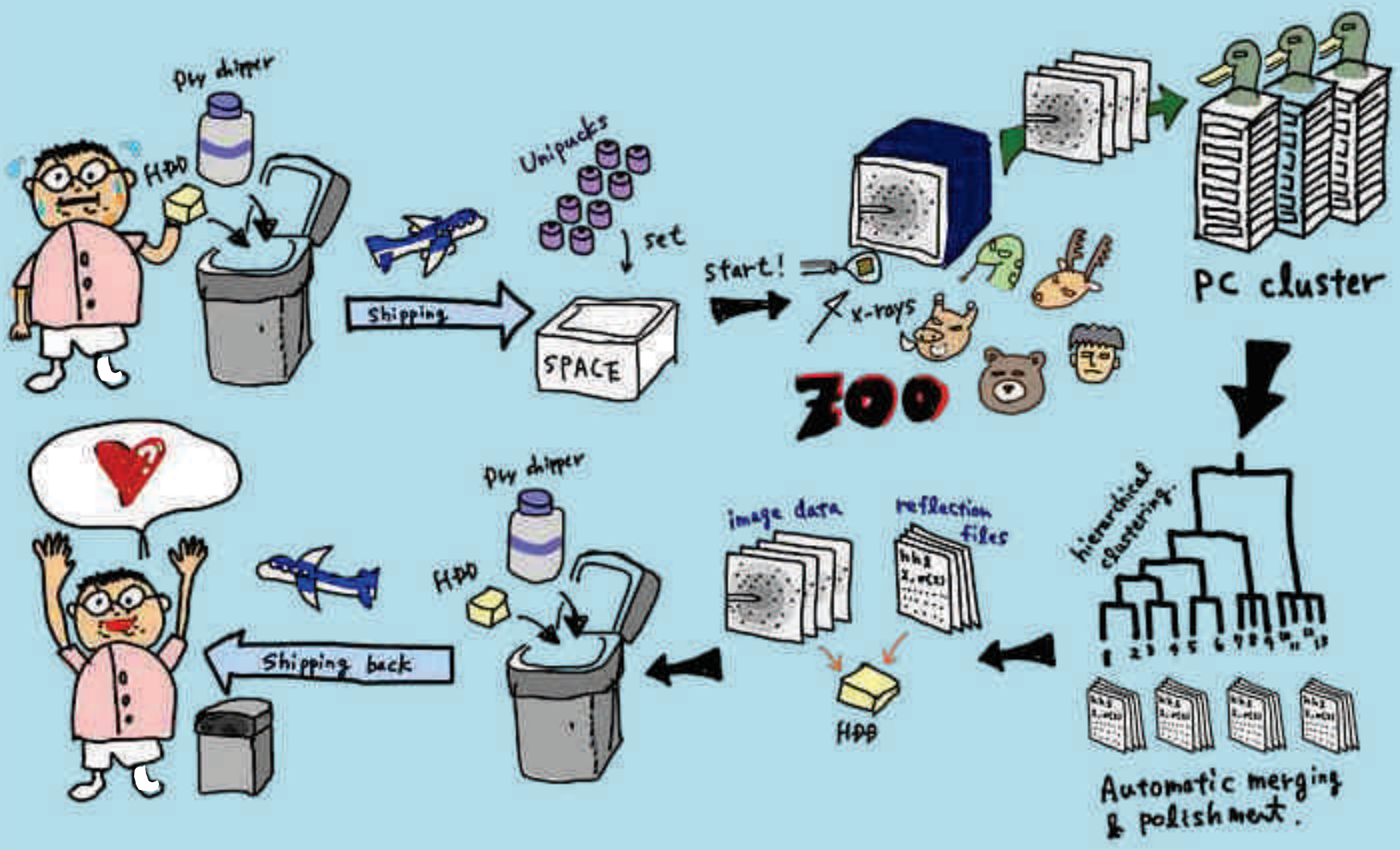
High flux beam

- High flux (4 x 10^{13} ph/s @ 2 x 20 μm)
- Ultra high resolution (λ ~ 0.5 Å, ID 3rd H)

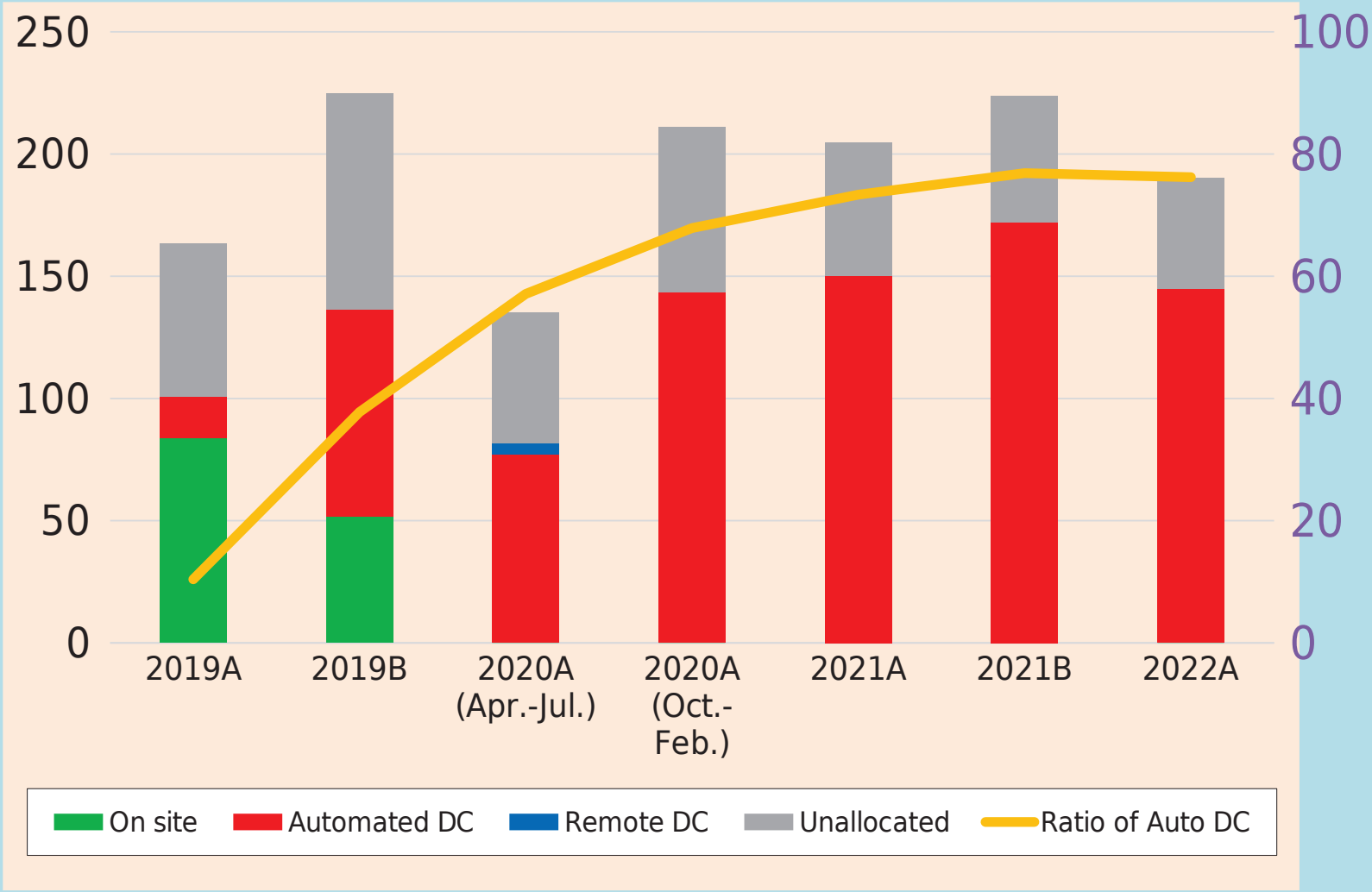
Operated by three organizations, but with common data collection environment, data collection software, sample changer etc.

ZOO system

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



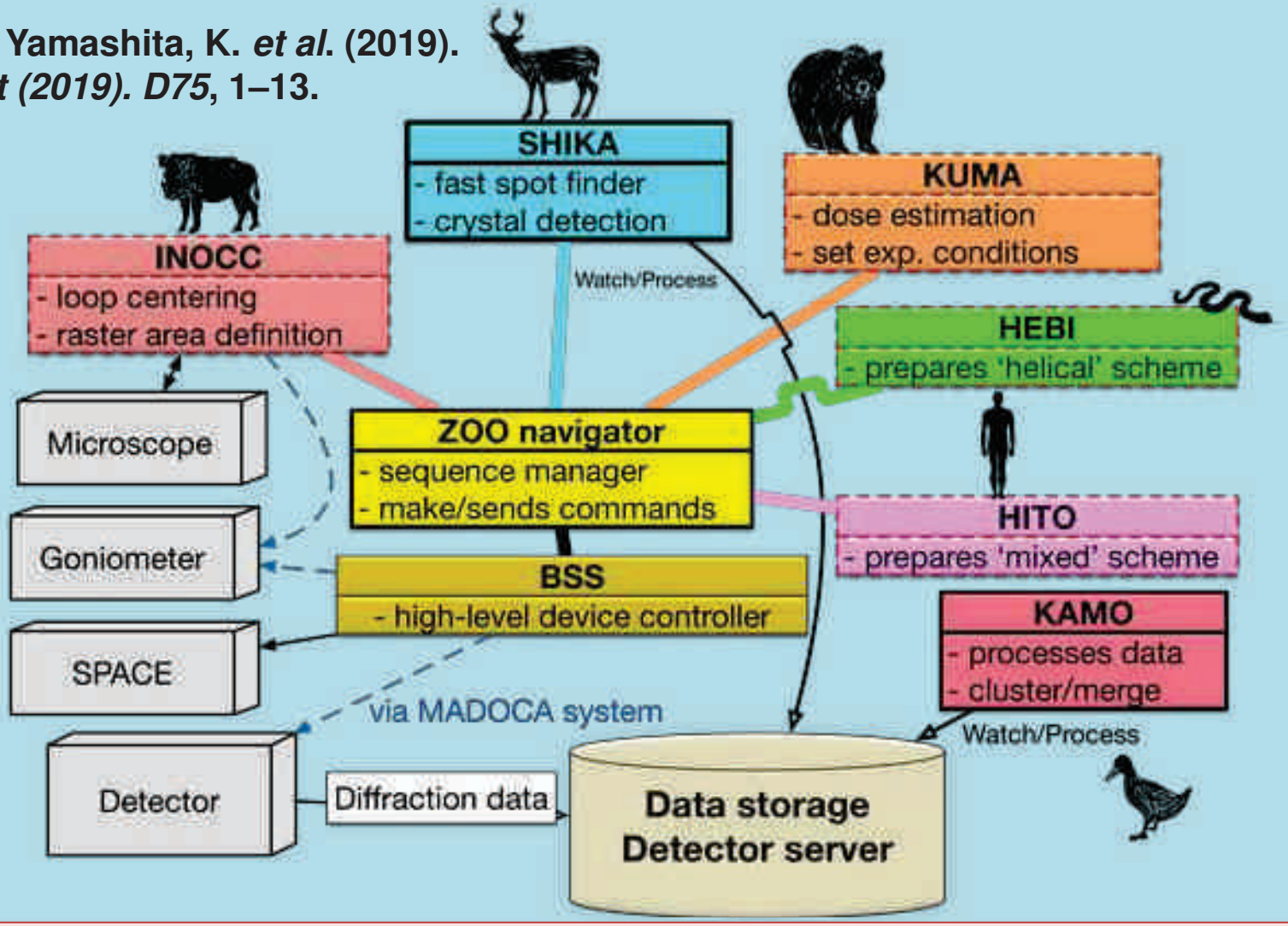
Transition to ‘unattended experiments’



80% public users conduct automatic data collection

ZOO system

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



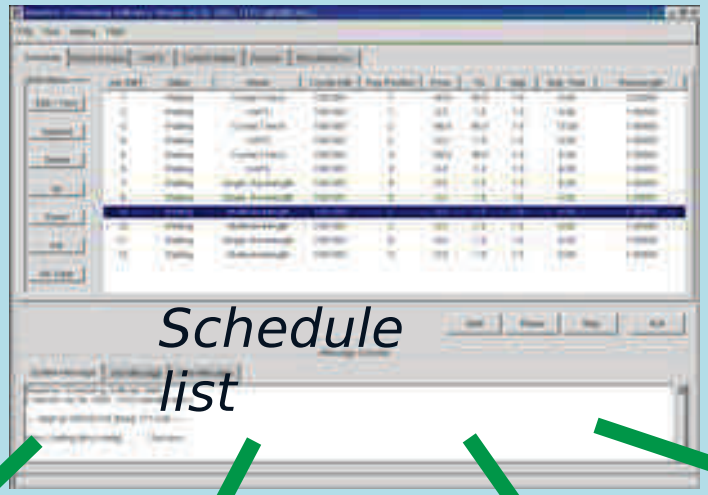
Program package with 'animal names' in

BSS (Beamline Scheduling Software)

Standard GUI for all MX beamlines at SPring-8

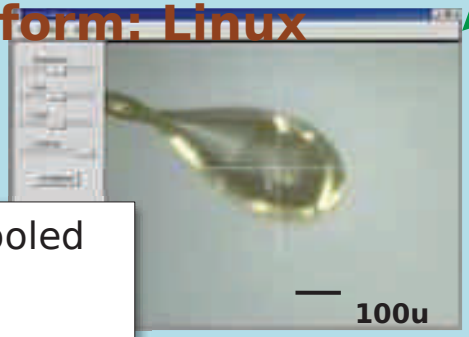
- **All-in-one control**
- **Job list for multiple conditions**
- **Load text BL configuration file**
- **Language & library: C, GTK+2, OpenGL, V4L2**
- **Platform: Linux**

Like MXCuBe?



Schedule list

Diffraction measurement



Cryo-cooled protein crystal

Sample Exchange, Centering

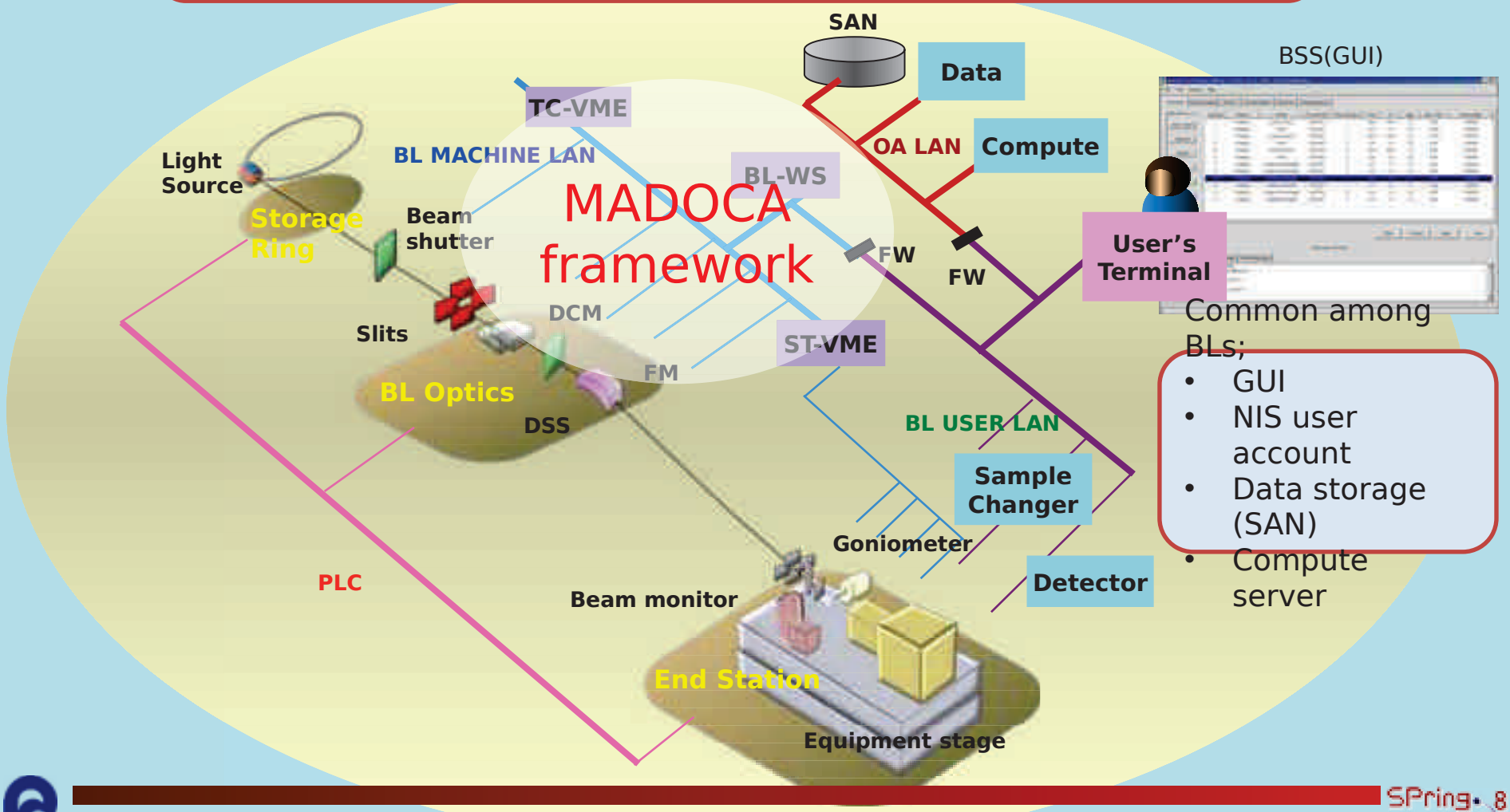
Exp. Conditions

XAFS measurement

G. Ueno et al., J. Synchrotron Rad. (2005). 12, 380-384

Control system for SPring-8 MX beamlines

- Unified beamline control with a GUI under C/S architecture
- Common storage & compute server on the network
- SR, BL, end station are seamlessly controlled via MADOCA

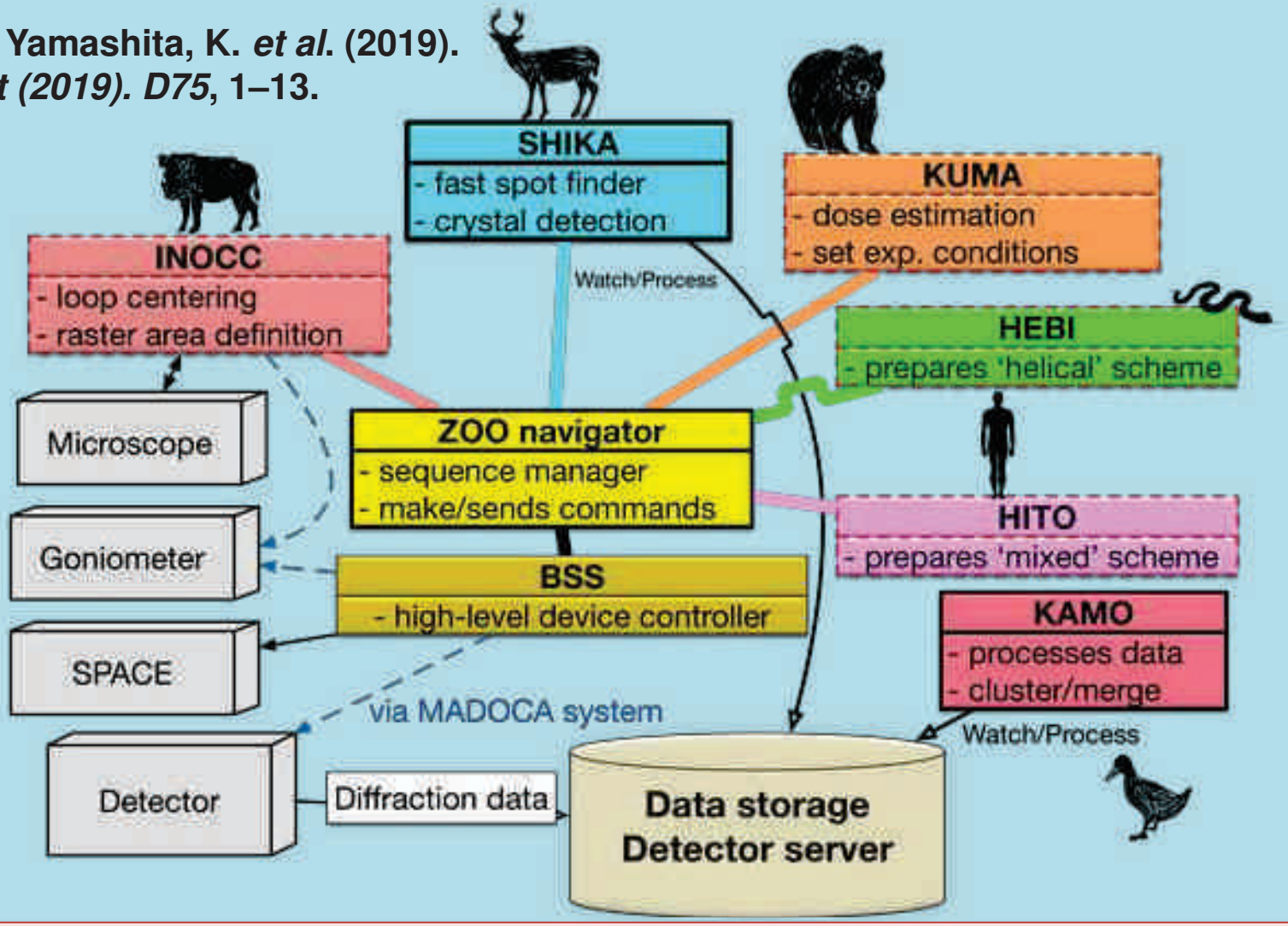


Common among BLs;

- GUI
- NIS user account
- Data storage (SAN)
- Compute server

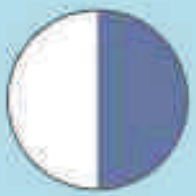
ZOO system

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



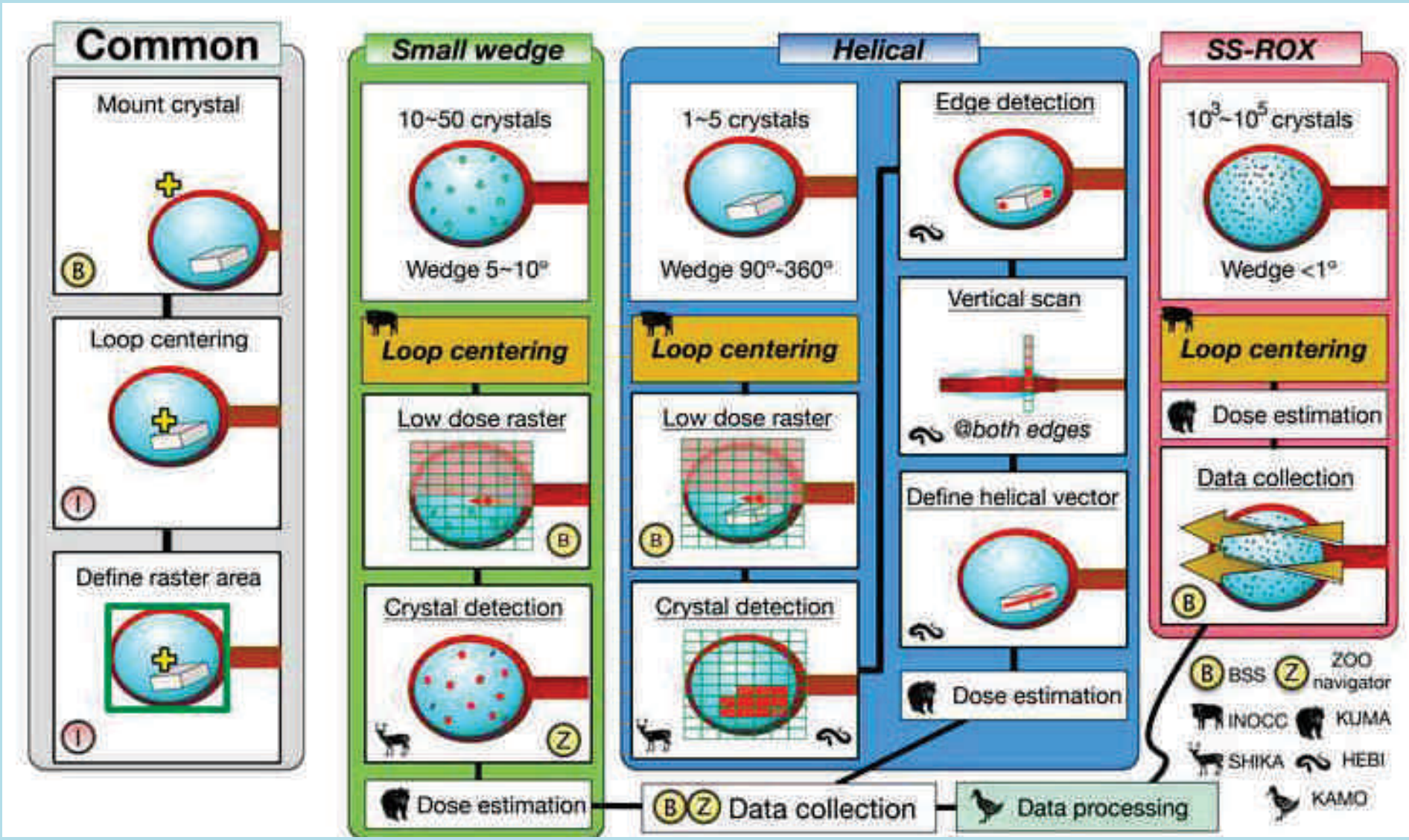
Program package with 'animal names' in

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.

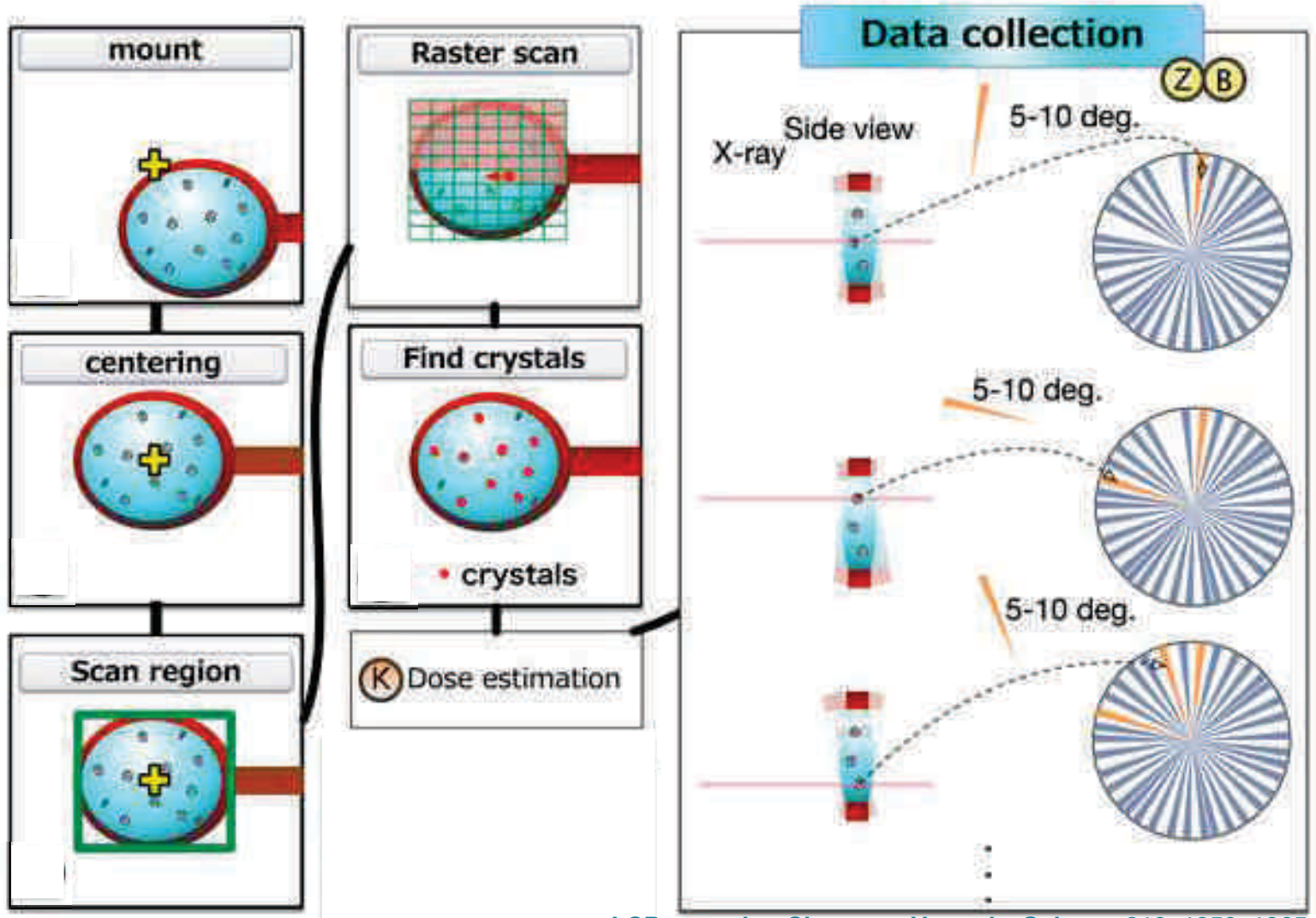
Users should choose ‘scheme’ for each cryo-pin.

Available schemes in ZOO



“Small Wedge Synchrotron Crystallography”

Many randomly oriented crystals on each loop



LCP crystals : Cherezov, V. *et al.* : Science 318, 1258–1265 (2007).

Many sub-datasets are merged into the final one in SWSX

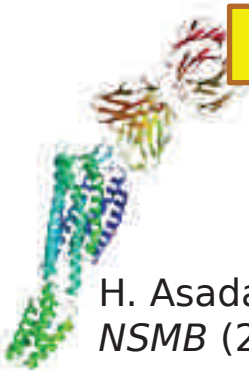
Membrane protein structure analysis with ZOO

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

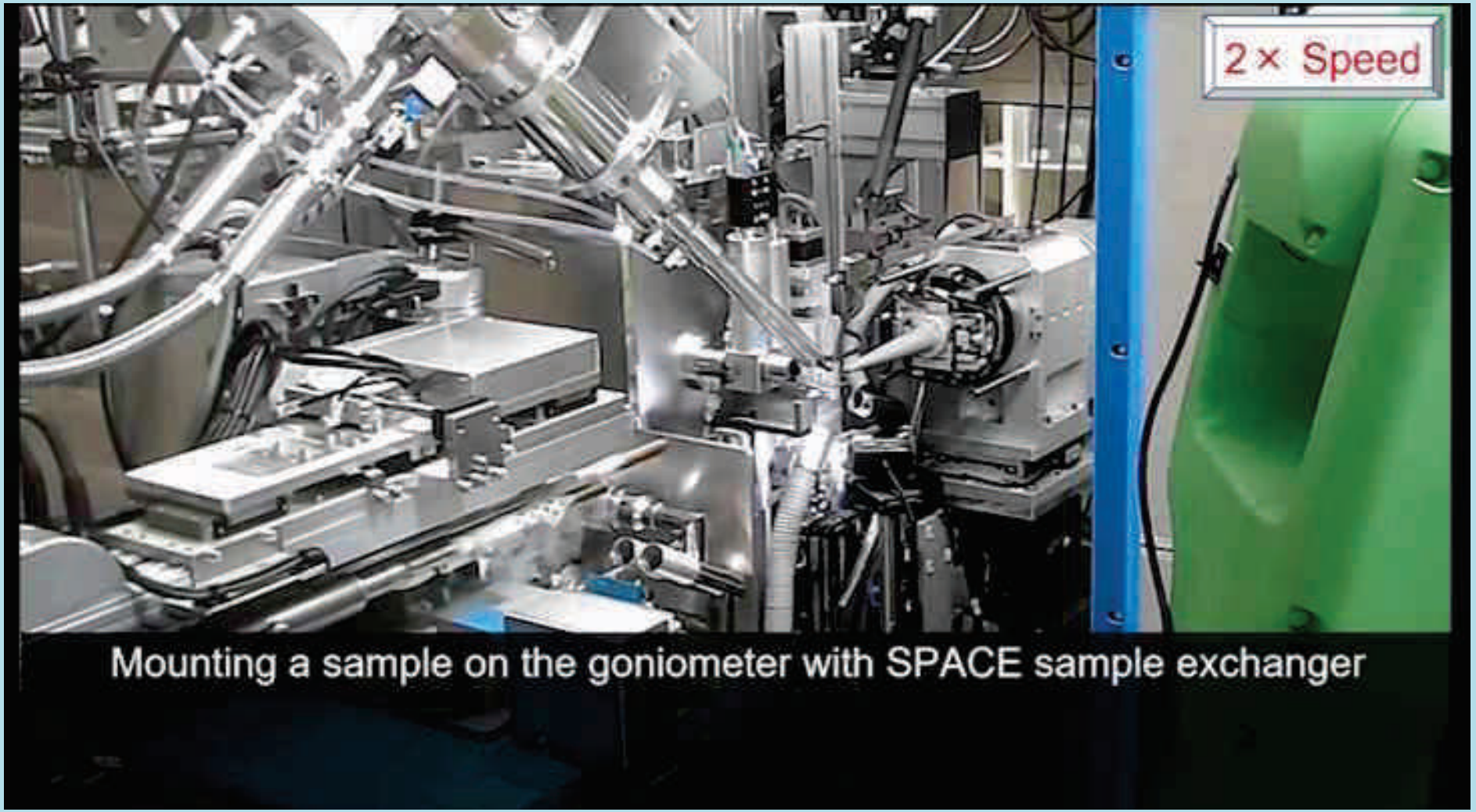
83

J. Yu et al.
Science(2020)

“Quantitative changes” make “Qualitative

Helical data collection

Movie at BL45XU



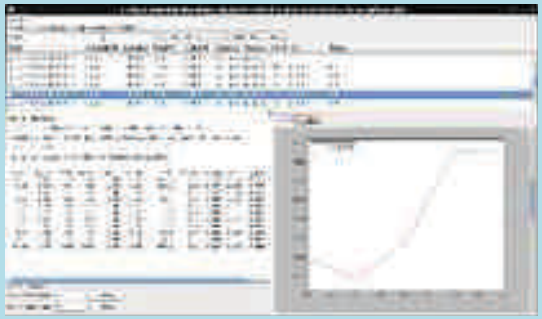
Keitaro Yamashita

KAMO

(Automatic data processing)

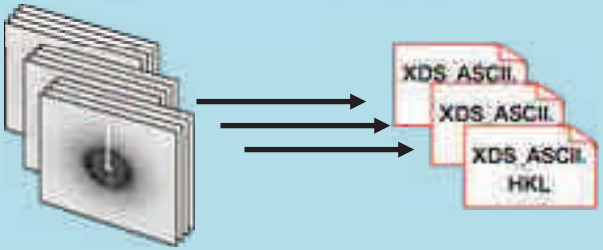
鴨

- 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



Merge & polish

selected clusters

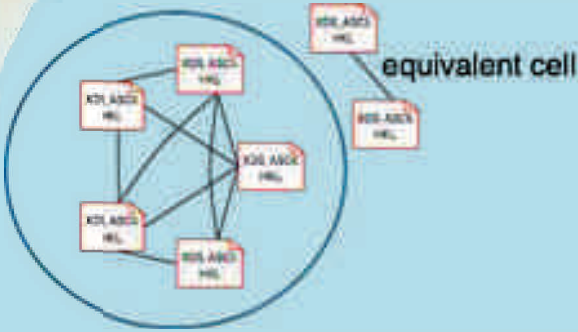


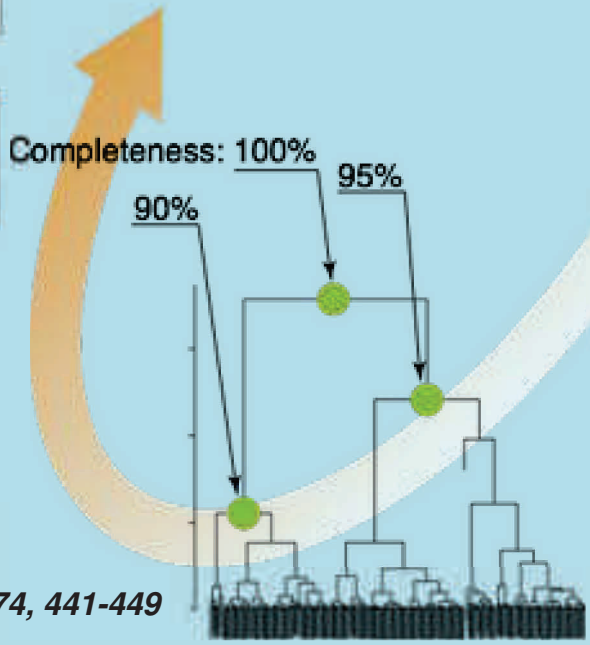
Data processing

XDS (or DIALS)

Grouping

crystals with equivalent cells





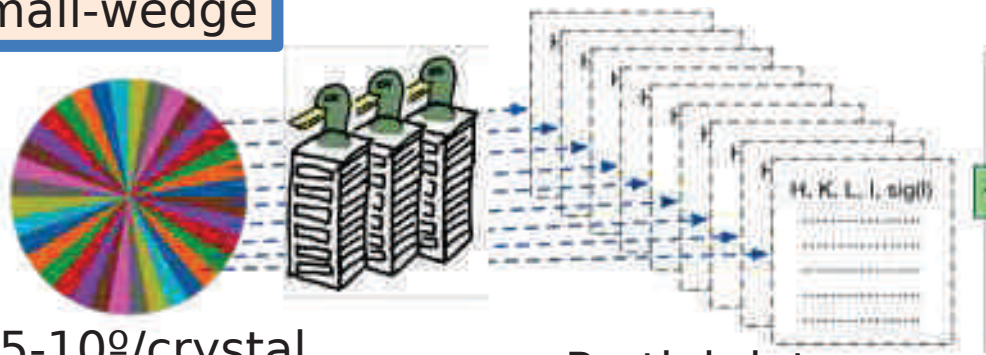
Clustering

- BLEND (cell parameters)
- CC of intensities

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

Data back to users

Small-wedge



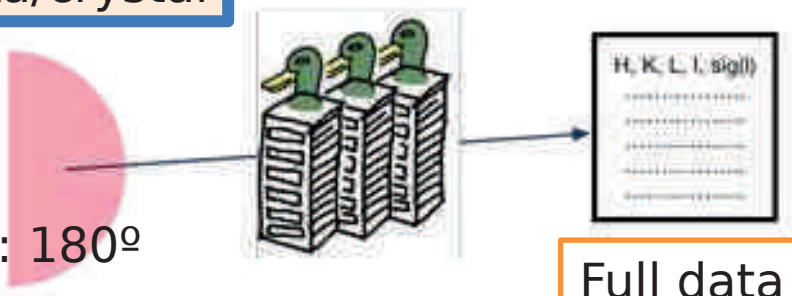
5-10º/crystal

Partial data

Clustering

Full datasets

Full data/crystal

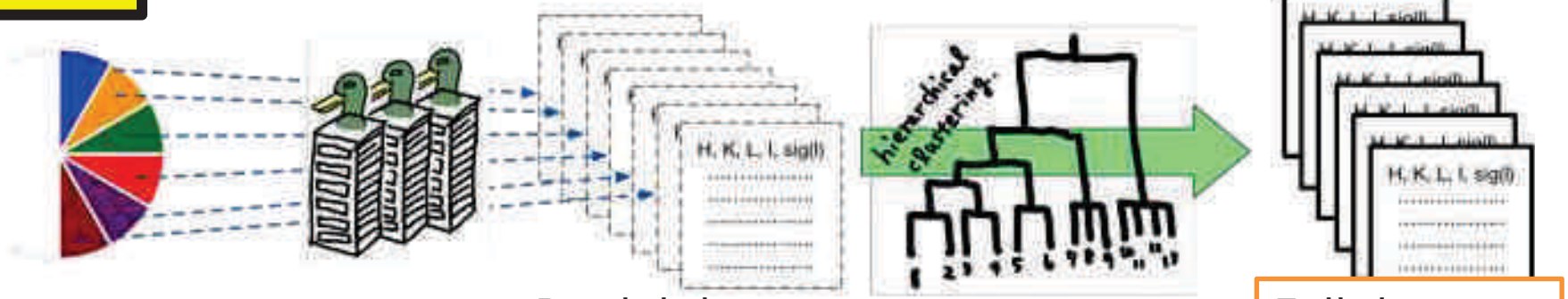


Ex: 180º

Full data

BUTTAGIRI

Clustering

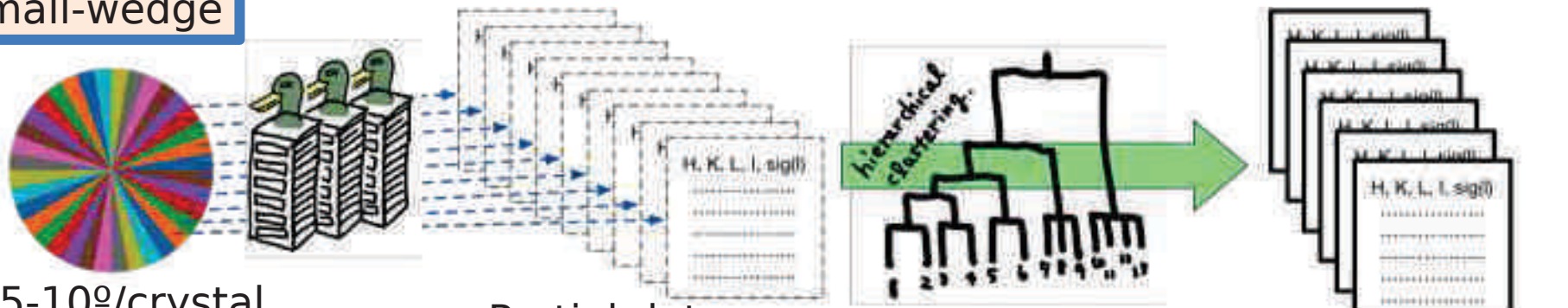


Partial data

Full datasets

Data back to users

Small-wedge



5-10º/crystal

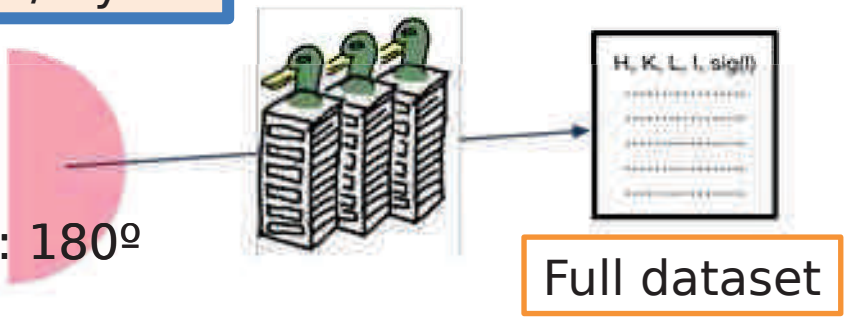
Partial data

Clustering

Full datasets

Data back to users

Full data/crystal



Ex: 180º

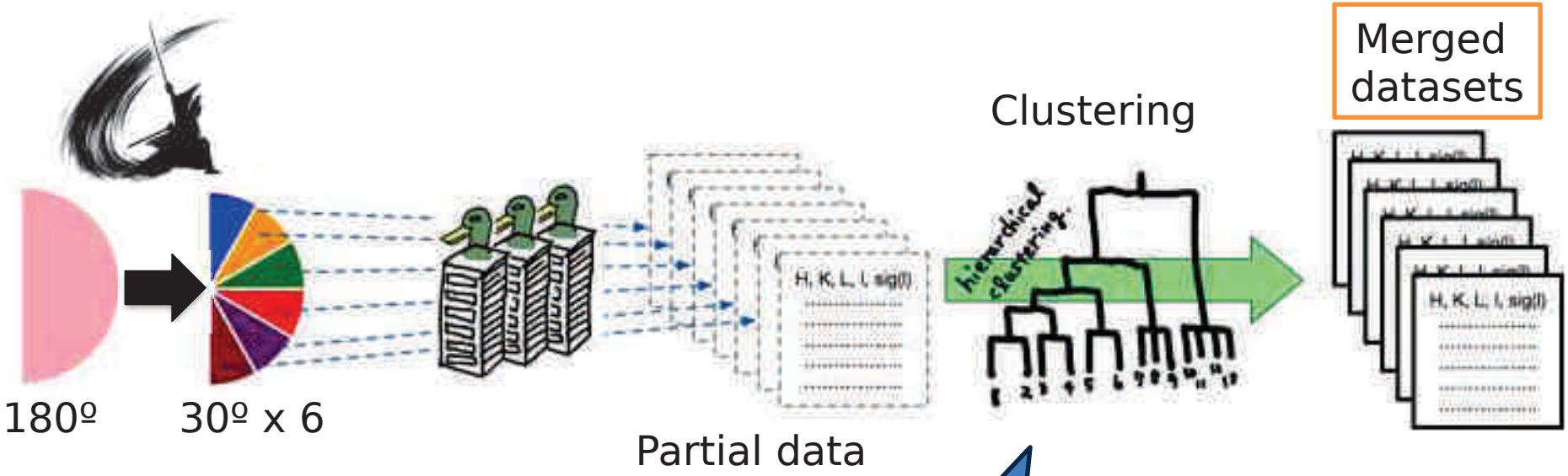
Full dataset

BUTTAGIRI process

(Chunk data processing?)

divides ‘complete data’ to ‘small wedge data’

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



Hierarchical clustering

(Select better data in ‘merging process’)

All types of processed/merged datasets is shipped back

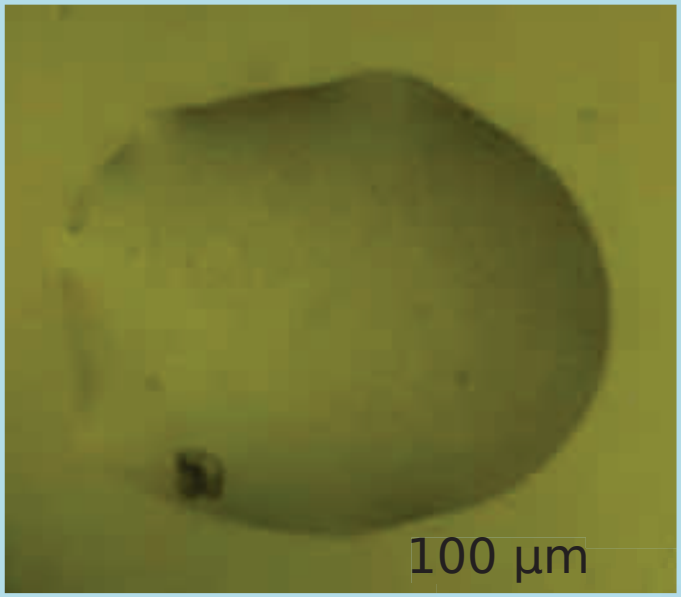
problem solved by ZOO

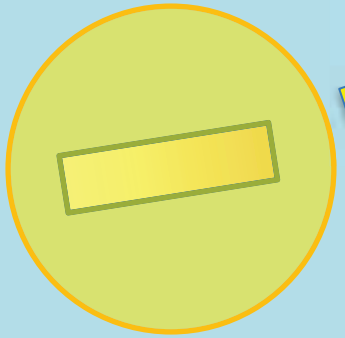
Courtesy of Prof. Sengoku

Data collection from ‘inhomogeneous crystal’

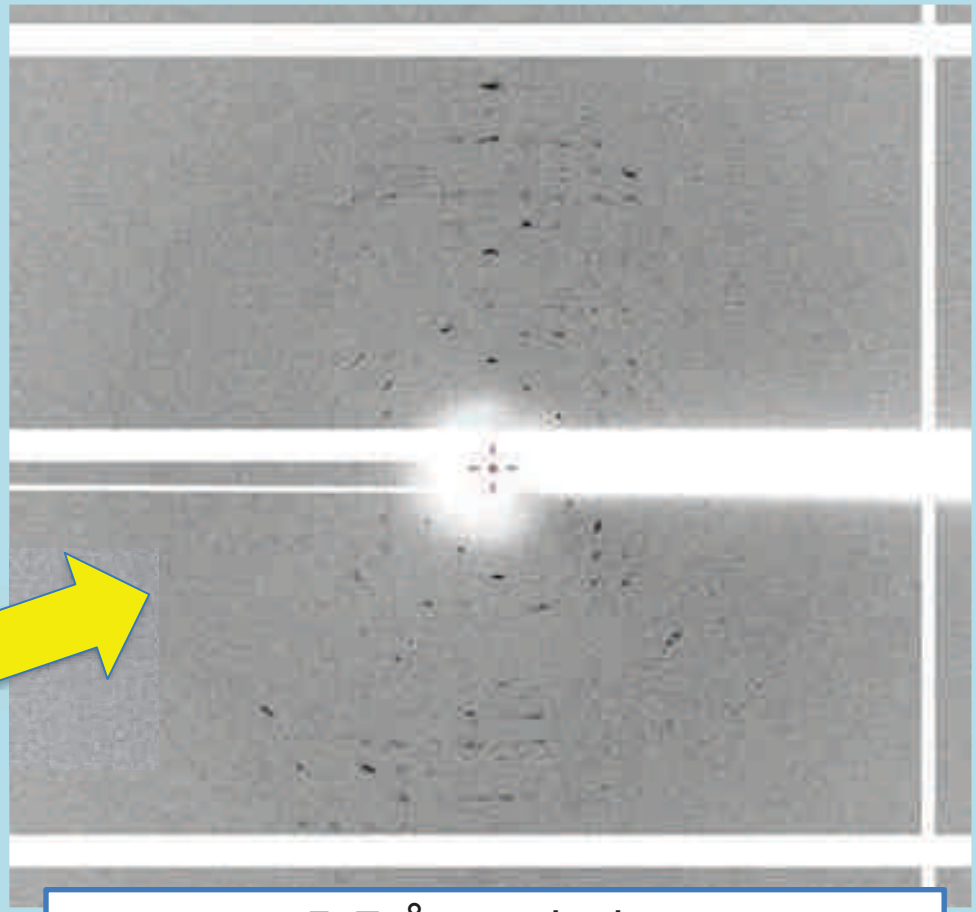
Is a human superior to ‘automatic data collection’, especially for ‘inhomogenous crystal’?

This case is one of the answers to this question.





Expose 200 µm beam



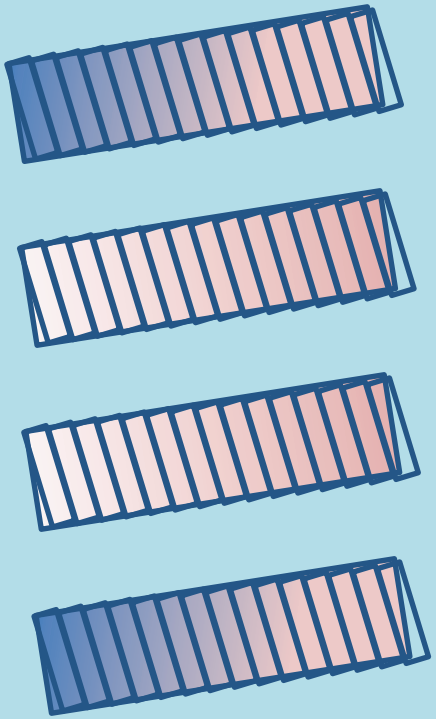
5-7 Å resolution
Diffraction shows ‘elongated’ peaks

problem solved by ZOO

Courtesy of Prof. Sengoku

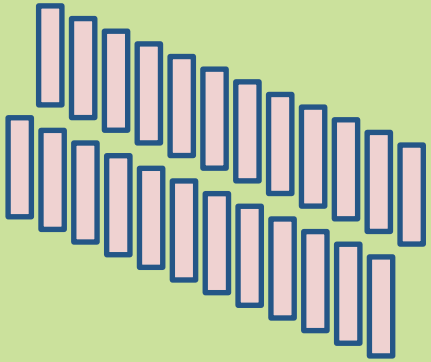
ta collection from ‘inhomogeneous cryst

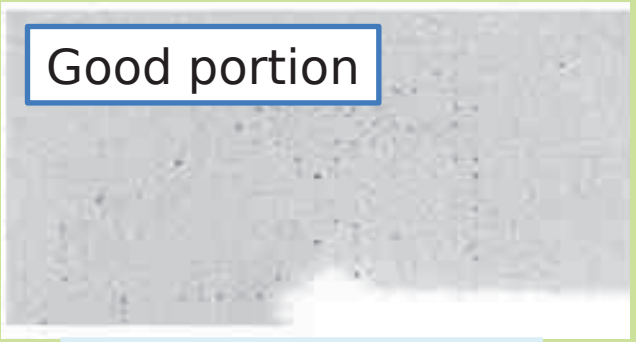
Helical 720º
→ BUTTAGIRI



Clustering

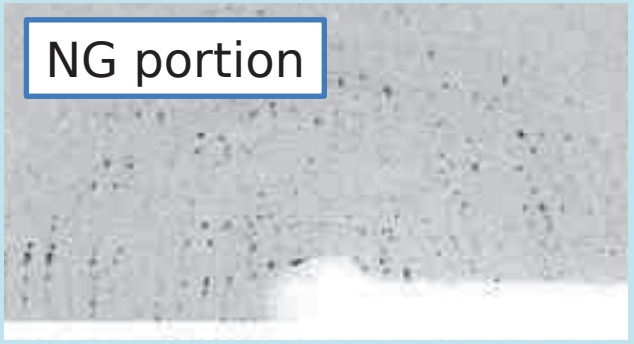
Better datasets





2.8 Å resolution

Worse datasets



11 crystals

Automation can solve structure using ‘inhomogeneous’ crystals

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

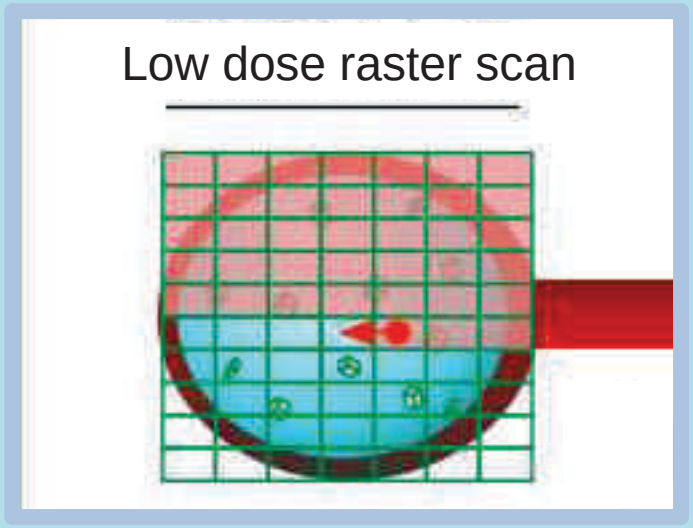
Mixed scheme(HITO)

objective :

To apply optimal scheme for each crystal with different size and configuration

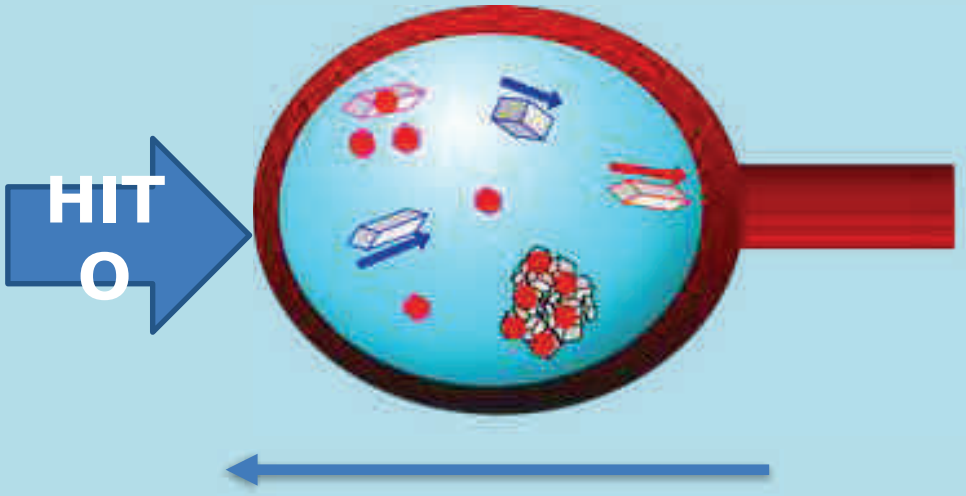
Small crystal → “SWSX”
Large crystal → “helical”

ZOO always does



SHIKA makes a crystal map

HITO recognizes crystal size/configuration



Rotation axis

HITO can choose ‘suitable scheme’ for each crystal

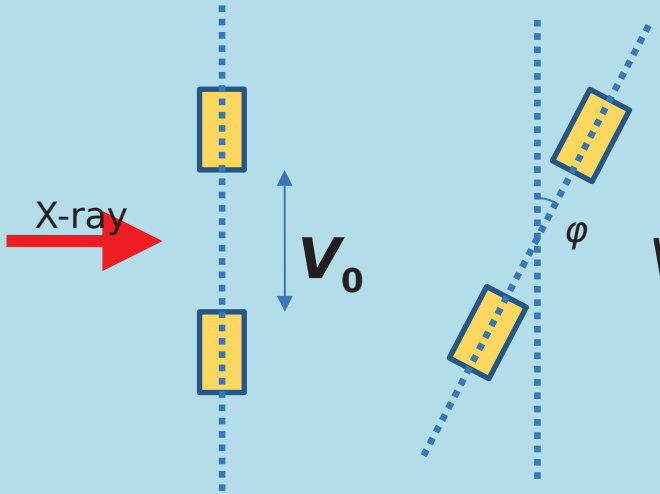
What HITO does?

Rotation axis

Crystal map from raster scan (from SHIKA)

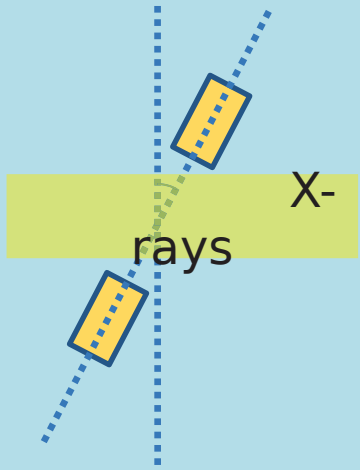
Find ‘overlapped crystals’ along with a rotation axis and calculate gaps between them.

How to calculate ‘oscillation range’



inclination φ *[deg.]*

$$V_x = V_0 \cdot \cos\varphi$$



(Current)

$$V_0 \cdot \cos\varphi \quad > 2V_{beam}$$

(beam size)

Ex) BL32XU

V beam size **15 µm**

$V_0 \cdot \cos\varphi = 2 \times 15$

V_0 is from a crystal map from raster

Ex) $V_0 = 50$ µm

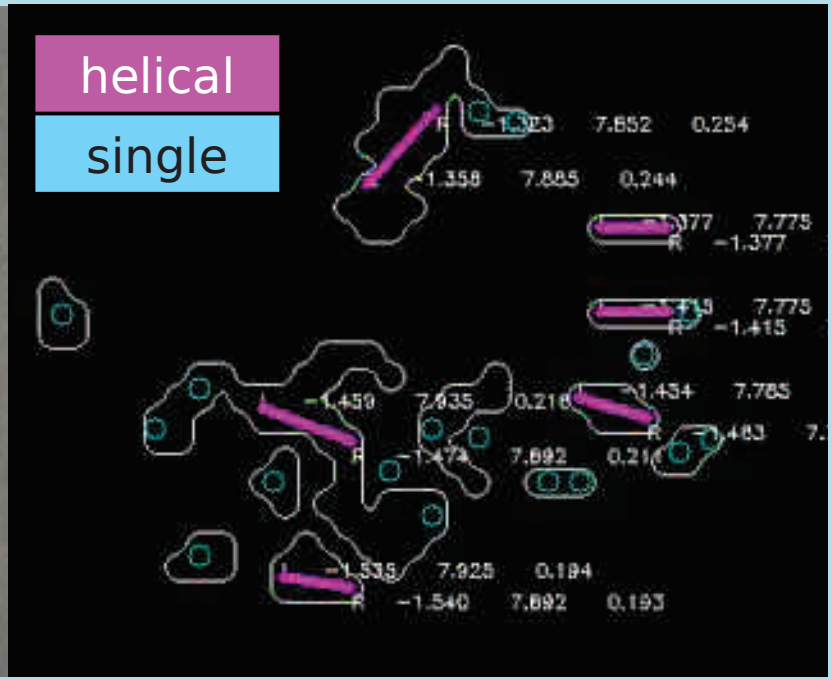
Φ ~ ±53º

※Crystal thickness -> X

HITO .vs. LCP crystals

Company users @ BL45XU (Oct 2021）

LCP crystals in a plate



HITO scheme map

Rotation data from mixed mode

PIN	Osc. range
Helical(no-align)	40° x 5
Helical part(align)	50° x 1
Multi	10° x 6
Single(full)	30° x 4
Single(no-align)	20° x 3
Single(align)	70° x 1, 50° x 1

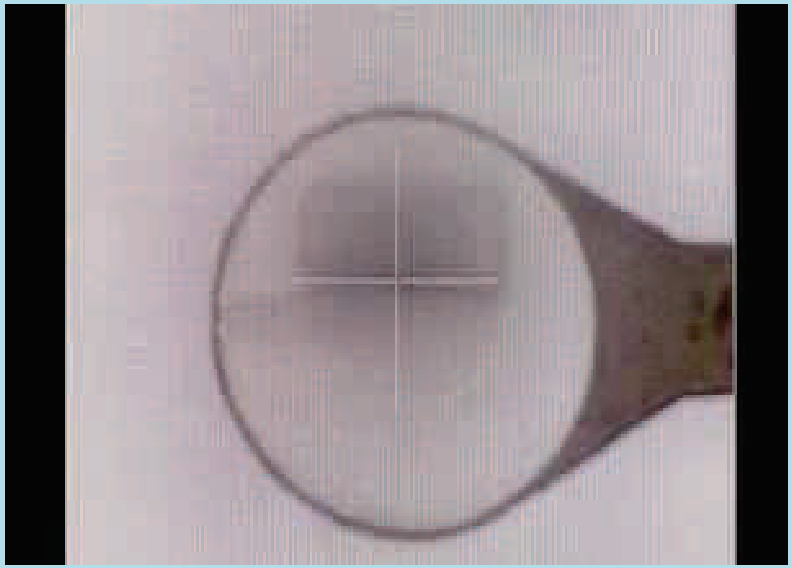
Rotation data of 610º was collected from 1 loop.

User's feedback

8 times more redundancy (cf. SWSX)

Serial Synchrotron Rotation crystallography (SSROX)

2D raster scan with goniometer rotation



- Micro focused beam (1~5 μm)
- Translation+Rotation
  - ±45 deg./line
- High dose expose
  - 10 MGy/pts

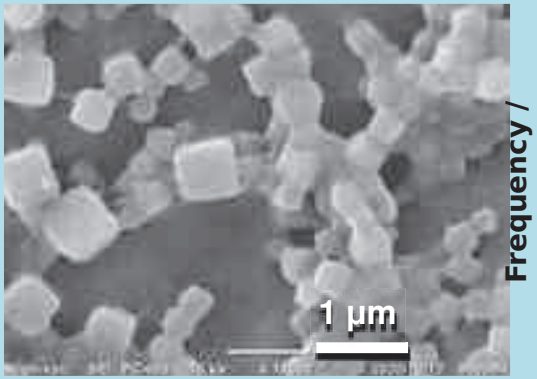
J. Synchrotron Rad. (2017). 24, 29–41

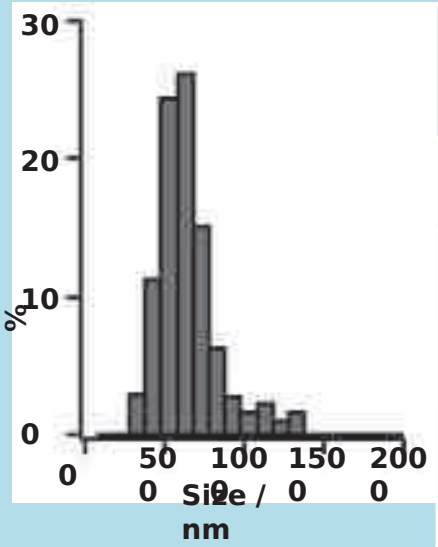
- **Still measurement requires exceedingly more crystals with the ‘monochromatic’ beam.**

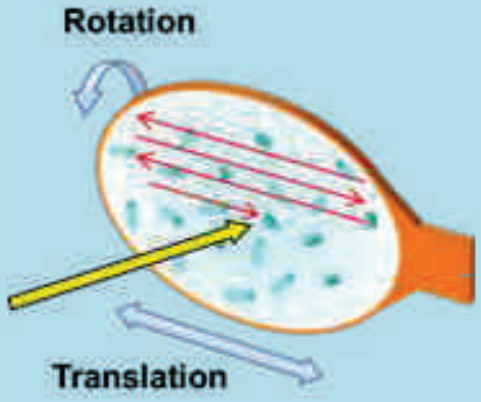
A single snapshot from a single crystal

Nano-sized PhC with SSROX

580 ± 230 nm





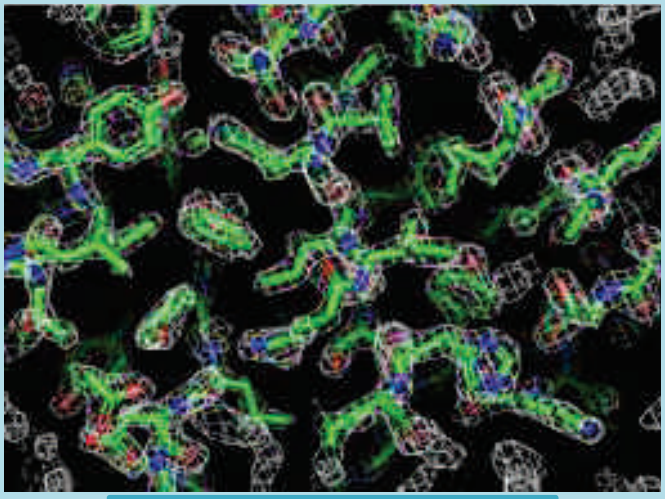


BL32XU	SSROX params.
Beam size	1 μm
Dose/point	10 MGy
Translation	360 μm
Rotation	-45 deg. ~ +45 deg.
# of frames	360
Translation / frame	1.0 μm
Oscillation width	0.25 deg. / frame
Exposure time	0.01 sec / frame
Attenuation	None.

Automated data collection with ZOO under cryo-condition

SSROX data using 600 nm crystals

# of loops	4
# of collected frames	330,480
# of hits	35,204
# of indexed	8,494
Resolution	1.95 Å
<I/σI> (1.96-1.95Å)	7.3 (1.23)
CC(1/2) (1.96-1.95Å)	0.9908 (0.6707)



1.95 Å resolution

Cell dimensions a~105Å (*I*23)

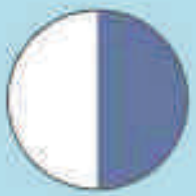
Crystal size 600 nm = 6000 Å ~ 57 copies

57 copies ^ 3 = **1.9×10^5 copies**

8,494 crystals : **1.6×10^9 copies**

Scientific Reports **volume 12**, Article number: 16031 (20

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.

Possible ‘goniometer based’ experiments are available

Summary

- After COVID
  - Ratio of automatic data collection rapidly increases
- After CryoEM appears
  - The number of users is getting smaller
  - Especially for membrane proteins
- Nano-crystallography
  - 600 nm crystals at atomic resolution
  - Each crystal: unit cells 2 x 10^5 copies
  - Total 1.6 x 10^9 copies
- Near future
  - **Towards** “No spread sheet” experiments

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