

For preparation of ZOO sample sheet

as of 2020-12-15

<Reference of automatic data collection and automatic data processing>

If you publish your structures from data collected using ZOO and analyzed with KAMO, please cite the following papers as references. The citations are exceedingly important for us to continue developing more advanced techniques at synchrotron radiation technology.

Thank you for your cooperation.

All staff

1. K. Hirata, K. Yamashita, G. Ueno, Y. Kawano, K. Hasegawa, T. Kumasaka, and M. Yamamoto
ZOO: an automatic data-collection system for high-throughput structure analysis in protein microcrystallography
*Acta Cryst. D*75, 1–13, (2019).
[doi: 10.1107 / S2059798318017795]
2. K. Yamashita, K. Hirata, and M. Yamamoto
KAMO: towards automated data processing for microcrystals
*Acta Cryst. D*74, 441-449, (2018).
[doi: 10.1107 / S2059798318004576]

<Automated data collection sample sheet>

When executing automated data collection, **the measurement conditions must be described on the automated data collection sample sheet (hereafter "sample sheet")**.

Download and edit the sample sheet and send it to the contact address (mail-in@spring8.or.jp) by the specified date.

If you have any questions or concerns, please feel free to ask us via the contact address.

<Naming rule for the sample sheet>

Please follow the following naming rules.

File name should be

[ZOOPREP_YYMMDD_NAME_BLNAME.xlsx \(valid as of 2020-09-28\)](#)

YYMMDD:

Date (entry date of the sample sheet) 2019-10-02 -> 191002

NAME:

Enter the name of the experimenter. Please capitalize the first letter of your first name and that of your last name with no space between. The name of your institution/organization is also acceptable. Do not include "_" in your name.

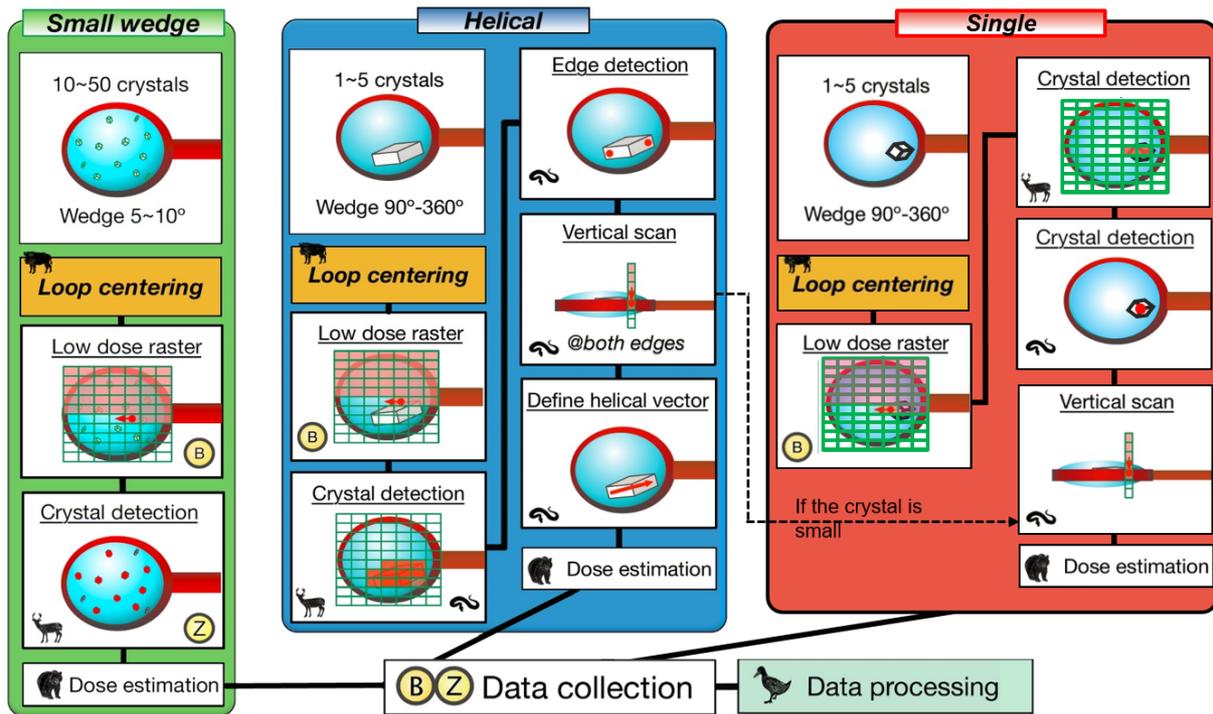
Good example: ZOOprep_191002_KunioHirata_BL32XU.xlsx
 Bad example: ZOOprep_191002_Kunio_Hirata_BL32XU.xlsx

BLNAME:

BL32XU or BL45XU or BL41XU

<Outline of ZOO measurement>

For each measurement scheme shown below, ZOO can be conducted automatically.



B BSS
 Z ZOO navigator
 🐃 INOCC
 🐻 KUMA
 🦌 SHIKA
 🐍 HEBI
 🦅 KAMO
 (small wedge = multi mode)

<Keep in mind>

Automated data collection is executed under the conditions described in the sample sheet from top to bottom.

Please consider your preferred priorities and input them on the sheet.

ZOO cannot currently do the following (but are being considered for implementation).

- ✓ Executing data collection when the resolution is higher than given criteria at raster scan.
- ✓ Intelligent skipping: Skip residual sample pins when it can acquire one or two good datasets.
- ✓ Snapshot at 0° and 90°

<Parameters of the automatic measurement sample sheet>

The following is an explanation of each parameter.

- **PuckID:**
 - ID of UniPuck
 - Enter the ID stamped on the UniPuck lid (SPring-8 definition: 'lid' has more metal, thus heavier, than the bottom "Sample enclosure")
 - When BL staff set the UniPucks into the sample changer, this parameter will be checked against the IDs on their lids.
 - **DO NOT use hyphens for this parameter, 'PuckID', on the sample sheet**

- **PinID:**
 - The position (number) of the sample pin in the UniPuck.
 - It is also possible to enter a group of pins (described later)
 - If there is a duplicate PuckID and PinID, the "Confirmation required" line displays a [Warning]. Please eliminate the duplication.

- **SampleName:**
 - Datasets with the same 'SampleName' are automatically merged with KAMO (basically, merging is applied to multi mode)
 - Merging is also applicable for a full data set from a single crystal (single/helical)
 - ◆ Data sets are first processed individually with KAMO
 - ◆ KAMO merges datasets with the same 'SampleName'.
 - ◆ Please let us know if you need to merge multiple full datasets obtained with single/helical modes
 - ◆ "KAMO_buttagiri processing" (described in detail later) also merges multiple data sets with the same SampleName.
 - **DO NOT use characters that cannot be used for directory names on Linux. SampleName is used as the name of the directory when merging. (e.g. Space, parentheses, etc.)**
 - ◆ Please name them with alphanumeric characters or “_” and “-”.

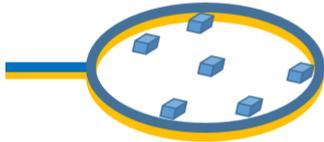
- **Objective:**
 - Select the purpose of the data collection from the pull-down menu
 - According to this selection, the program sets experimental conditions for raster scan and data collection.
 - Please see further details in the table below. If you have any questions, please feel free to contact us.

	Objective	Recommended Beam size	Raster scan (with a salt scan cover@BL45XU)	Dataset (Typical parameters are described as examples.)
1	normal	10x10 - 50x50 @BL45XU 10(H)x15(V) @BL32XU	Performed at 2E10 photons/frame. Max res. 5Å using ROI. 10x10@1A:132kGy, 20x20@1A:33kGy	<ul style="list-style-type: none"> • Single crystal: 360deg (Helical or Single mode is switched automatically) • Multi crystals: 10deg x crystals 10MGy
2	scan_only	10x10 - 50x50 @BL45XU 10(H)x15(V) @BL32XU	Performed at 2E10 photons/frame. Max res. 2.5Å without a cover & full image(no ROI). 10x10@1A:132kGy, 20x20@1A:33kGy	None 0MGy
3	high_dose_scan	10x10 - 50x50 @BL45XU 10(H)x15(V) @BL32XU	Performed at Dose=300kGy. Max res. 5Å using ROI. 5x5@1A:1.15E10 photons/frame 10x10@1A:4.5E10 photons/frame	<ul style="list-style-type: none"> • Single crystal: 360deg (Helical or Single mode is switched automatically) • Multi crystals: 10deg x crystals 10MGy
4	ultra_high_dose_scan	5x5 - 20x20 @BL45XU 10(H)x15(V) @BL32XU	Performed at Dose=1MGy. Max res. 5Å using ROI. 5x5@1A:3.8E10 photons/frame 10x10@1A:1.5E11 photons/frame	<ul style="list-style-type: none"> • Multi crystals: 10deg x crystals 9MGy
5	phasing	10x10 - 50x50 @BL45XU 10(H)x15(V) @BL32XU	Performed at 2E10 photons/frame. Max res. 5Å using ROI. 10x10@1A:132kGy, 20x20@1A:33kGy 10x10@1.5A:324kGy, 20x20@1.5A:81kGy	<ul style="list-style-type: none"> • Single crystal: 720deg (Helical or Single mode is switched automatically) • Multi crystals: 10deg x crystals 5MGy
6	rapid	20x20 - 50x50 @BL45XU 10(H)x15(V) @BL32XU	Performed at 2E10 photons/frame. Max res. 5Å using ROI. 10x10@1A:132kGy, 20x20@1A:33kGy	<ul style="list-style-type: none"> • Single crystal: 180deg (Use a single mode to save time,) 10MGy

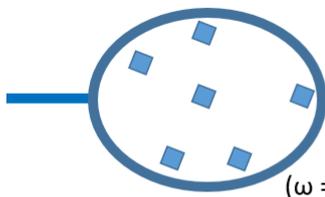
- **Modes (see also following figures):**
 - multi
 - ◆ Collect multiple datasets from multiple microcrystals mounted on one loop.
 - ◆ Typically, a 5-10° wedge is collected from each crystal.
 - ◆ Each dataset is acquired from the angle where the largest surface of a loop is the most visible against X-ray with ±2.5–5.0°.
 - ◆ Collected datasets are merged into the final dataset with KAMO.

Multi mode

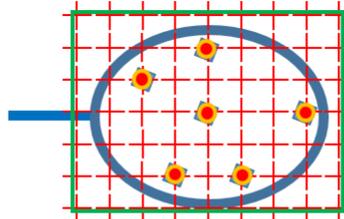
Multiple microcrystals mounted on the cryo-loop.



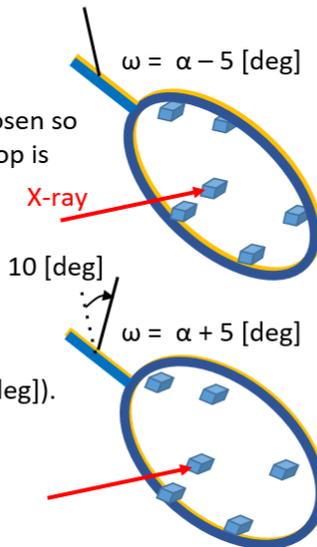
1. Loop centering with *INOCC*. A goniometer rotation axis is chosen so that the largest surface of the loop is perpendicular to the the beam.



Recommended "Total osc" is ω (total) = 10 [deg]
then rotation range is $\omega = \alpha - 5 \sim \alpha + 5$ [deg]



2. Raster scan area \square determined by *INOCC* (width is set by "Hor. scan length"). Each images are scored by spot-findings managed by *SHIKA*.



3. Rotation range is from $\alpha - 5$ [deg] to $\alpha + 5$ [deg].

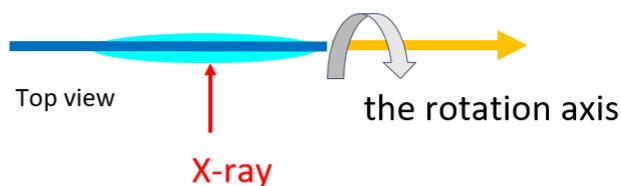
The number of data sets is defined by "# of crystals / loop" .

The order of measurement is in the order of scoring by *SHIKA* (the number of found diffraction spots).

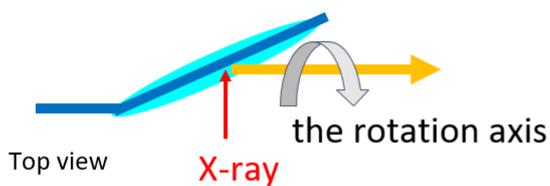
Precautions for sample preparation in multi mode

Use a straight loop

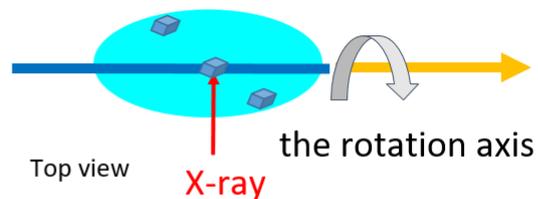
Optimal shape



In the case of a bent shape, the left and right sides of the loop will be off from the center of rotation.

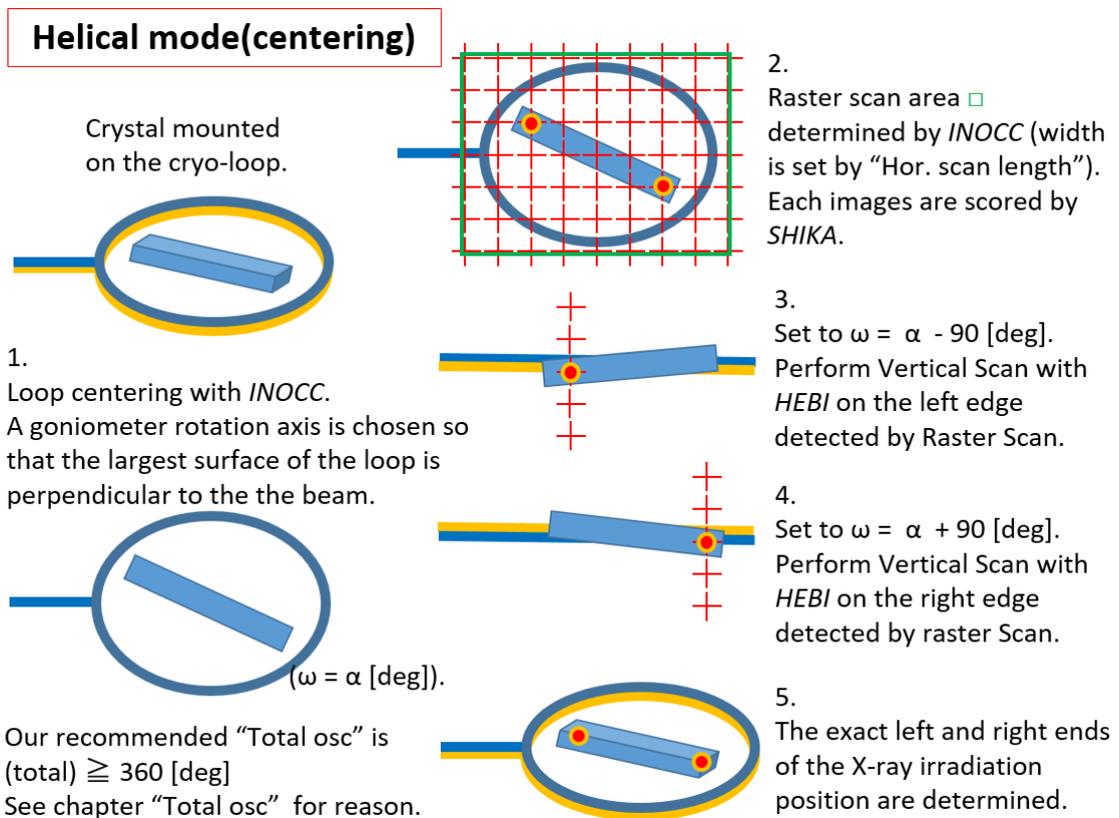


If there is a lot of solvent in the loop, the crystal will be misaligned from the center of rotation due to the thickness of the solvent.



✘ 3-D centering is not applied to this mode

- helical
 - ◆ Data collection with the rotation method while moving the X-ray irradiation point from the left to the right edge of the crystal.
 - ◆ This mode is suitable when the crystal is larger than the beam size.
 - ◆ A full data set is collected from one crystal.
 - ◆ Crystal size and shape are automatically recognized from the result of the 2D raster scan. If the crystal size is less than twice the beam size (e.g., 40 μm or less for 20- μm beam at BL45XU), the mode will be automatically switched to single mode (if you are not sure whether the crystal size is for single or helical modes, please choose helical)

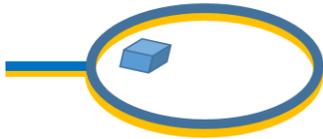


- single
 - Single-point irradiation (full data set from single crystal)
 - We recommend that you do not select this option unless you would like to increase data-collection speed, such as for proprietary beamtime

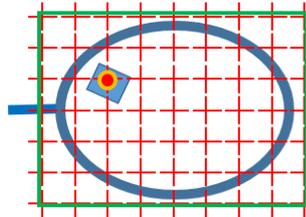
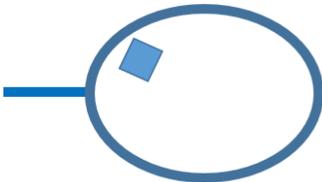
Single mode(centering)

If "Helical" is selected but a crystal is not large enough, HEBI switches the mode to "Single" automatically.

Crystal mounted on the cryo-loop.

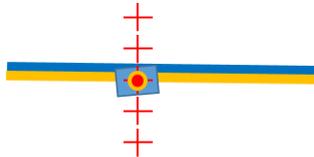


1. Loop centering with *INOCC*. A goniometer rotation axis is chosen so that the largest surface of the loop is perpendicular to the the beam. ($\omega = \alpha$ [deg]).

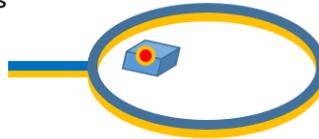


2. Raster scan area determined by *INOCC* (width is set by "Hor. scan length"). Each images are scored by *SHIKA*.

If the number of good grids in a heatmap is smaller than $2 \times$ "horizontal beam size", *HEBI* switches the mode to "Single".



3. Set to $\omega = \alpha - 90$ [deg]. Perform a vertical scan for 3-D centering.



4. The exact X-ray irradiation position is determined.

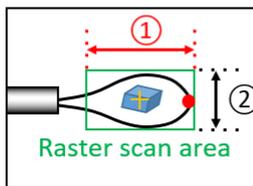
- **HA:**
 - Select this option if the crystal contains heavy atoms and you need to use anomalous scattering for phasing
 - Select "no" if the crystal does not contain heavy atoms or you do not need to use anomalous scattering for phasing
 - On the sample sheet, when you select heavy atom from the pull-down menu, the "Wavelength" field shows the wavelength that is about 0.001 Å shorter than the literature edge wavelength of the heavy atom (so-called 'peak wavelength'). This is used as a suggested value for SAD measurement. However, we are not sure that the value is suitable for your objective. Please input your preferred value in the column of 'wavelength' if you like (see also 'Wavelength' section)
 - If "no" is selected, 1.000 Å will be entered.

- **Wavelength:**
 - Wavelength for raster scan and data collection.
 - Please be informed that when HA is selected, it will be automatically changed to the wavelength near the peak one. **If you input the wavelength manually here, the wavelength derived from HA selection will be overridden.**
 - Available wavelength range depends on beamlines; please refer the link below. If you have further questions, please use the contact address. http://bioxtal.spring8.or.jp/ja/BL/SPring8_PX_beamlines.pdf
- **Hor. scan length [μm]:**

(Please read the document below carefully with figures.)

 - This parameter is used for defining the 2D raster scan area after loop centering by INOCC.
 - ◆ INOCC defines the 2D raster scan area after loop centering.
 - ◆ This parameter defines the length of the raster scan area in the horizontal direction (parallel to the rotation axis of the goniometer)
 - ◆ **ZOO scans from the tip of the loop by this value (unit in μm)**
 - ◆ It is recommended to enter the longitudinal length of the loop with some margin.
 - ◆ The length of the vertical direction is automatically determined by the shape of the loop with INOCC.

Notes on "Hor. scan length" setting required for optimal Raster scan



- + Beam center
- Tip

Centering operation by INOCC



1. Set the horizontal width with "Hor. scan length" specified in the sample sheet (Excel file).
2. Automatically detected and set by INOCC.

The number of grids in 2D raster scan is determined by dividing the length of ① and ② by beam size respectively.

Notes on input depending on the type of loop
Please enter the size of ①.

Circle type loop

Nylon loop

* : Catalog size

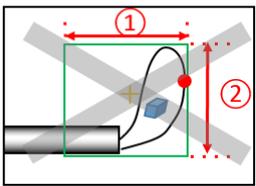
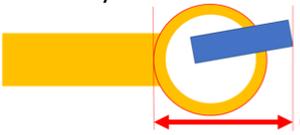
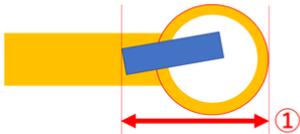
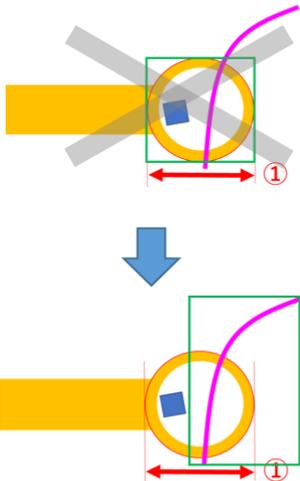
When the catalog size (*) of Nylon loop is set to "Hor. scan length".

Set * to ①

Crystals may not enter the scan area !!

You can set a longer value if your crystals are possibly on the "neck part" of loop.

Other notes on setting "Hor. scan length".

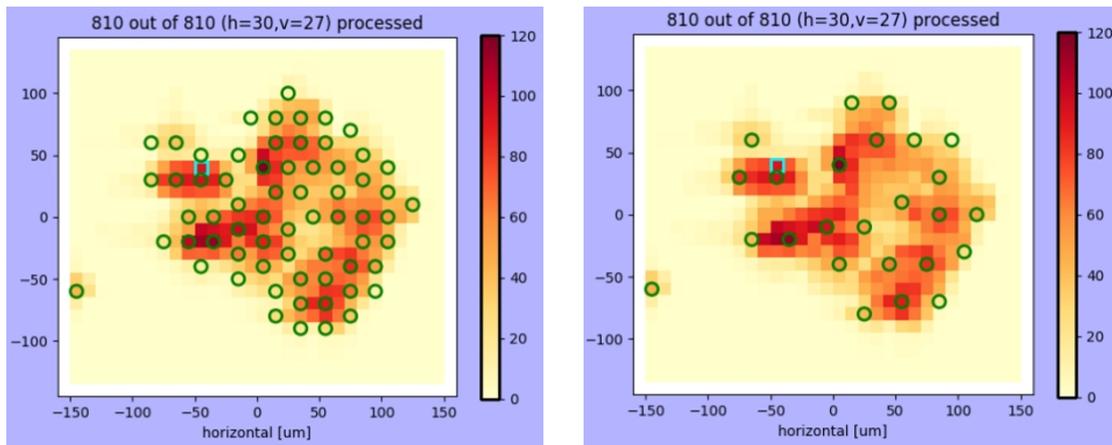
The loop is bent	Crystal is protruding	Dust is attached
<p>If X-rays hit the metal part of the sample pin, strong diffraction rays will be generated and the detector will be damaged.</p>  <p># Old nylon loops are not suitable for automatic measurement, so do not use ones that are extremely bent if possible.</p>	<p>Please set the value carefully.</p>   <p>In such a case, there is no need to increase the value.</p> 	<p>Small dust is recognized as part of the loop by INOCC. X-ray irradiation cannot be performed on the assumed loop area (currently).</p> 

- **Resolution limit [Å]:**
 - The maximum resolution available based on the "Beamline" and "Wavelength" is displayed. Please override these values by referring to the instructions below.
 - Resolution at the detector edge for data collection.
 - This parameter defines the camera length.
 - Enter the resolution of "this is more than enough".
 - Of course, you can also enter "3" if it is the first irradiation for the crystal and is not expected to diffract to "3 Å".
 - If the longest cell parameter is quite long (e.g., >300 Å), you should consider this value carefully. In this case, please let us know.
 - If not specified, we assume this value to be **2.0 Å**.
- **Beam size [um] (Order: HxV, horizontal x vertical):**
 - Reference
http://bioxtal.spring8.or.jp/ja/BL/SPring8_PX_beamlines.pdf
 - Available beam sizes depend on each beamline; please refer to the above. If you have further questions, please use the contact address.
 - For the beam size "HxV", set the center letter to lowercase "x". DO NOT include "space" between characters.
 - ◆ Example: 10x15

- **Crystal size [μm]:**

- crystal size
- This parameter is important in multi mode because it will be the shortest distance between the exposure points
 - ◆ (e.g.) If one crystal is larger than this parameter, more than two datasets will be collected from one crystal (the exposure point is of course different) (see figure below).
 - ◆ If the crystals are needle-shaped or columnar, please input the shortest length of the crystal shape.
 - ◆ if it is set to the value less than "Beam size", the "Confirmation required" line displays a [Warning].
- In the case of helical/single mode data collection, crystal shape is automatically determined from the results of the raster scan, so please enter the approximate value for reference.

Example of crystal size and interval between X-ray irradiation positions



Crystal size = 20 [μm]

Crystal size = 30 [μm]

Beam size : 10 x 10 [μm] ○ : X-ray irradiation positions

- **# of crystals/loop:**

- Number of crystals in the loop (rough estimation is okay)
 - ◆ In multi mode, this number defines the maximum number of datasets per loop.
 - ◆ In multi mode, if the value is set to "19 or less", the "Confirmation required" line displays a [Warning]. It is recommended to set the value to "20 or higher".
 - ◆ If you are not sure, please enter the possible number of crystals.
 - ◆ If you are not sure AT ALL, you can use "50" or "100".
 - ◇ Please keep in mind that the measurement time is affected by this parameter.

✧ (Note)

A catastrophic case with a large number:

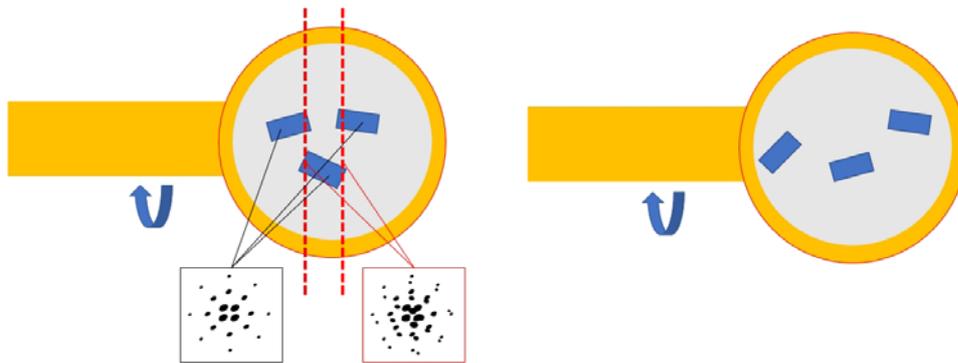
There were a lot of substances that produced a strange diffraction pattern. It was incorrectly determined that there were many crystals, and totally meaningless datasets were collected for that particular number. In the end, much machine time was consumed, so it could not be completed data collection on time. (Countermeasure)

It is better not to enter large values for crystals that are not well characterized.

- ◆ In helical mode, if the value is set to “2 or higher”, the "Confirmation required" line displays a [Attention]. The condition is only feasible if two or more crystals do not enter the path of the X-rays.

In this case, it cannot be measured.
Overlap to rotation axis

In this case, we can measure it.

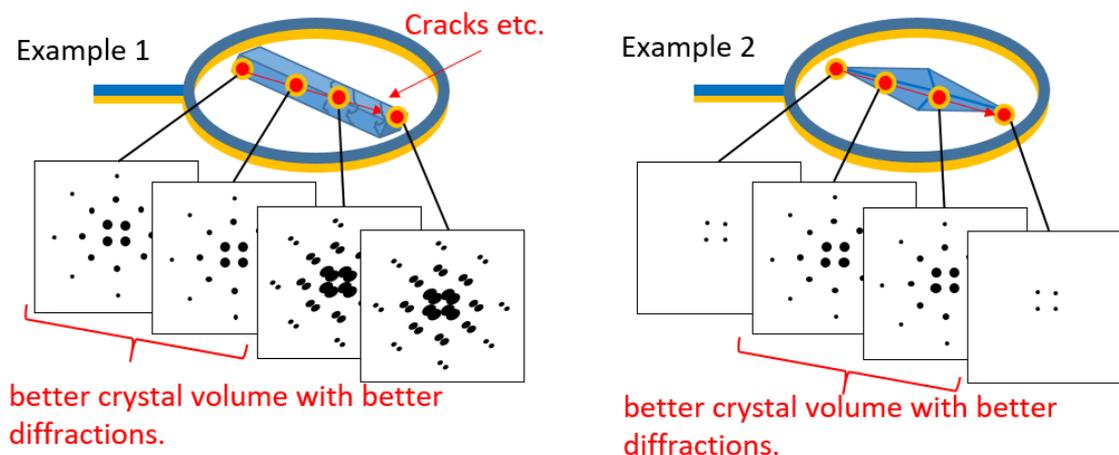


- ◆ In single mode, if the value is set to “2 or higher”, the "Confirmation required" line displays a [Warning]. The condition is not enforceable.

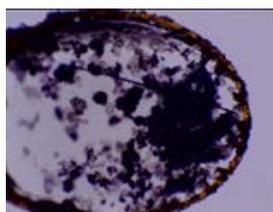
- **Total osc. [deg.]:**

- Oscillation range for data collection (deg./crystal).
- In multi mode, the maximum value should be 15°/crystal (recommended 10°/crystal). If the value is set to “16 or higher”, the "Confirmation required" line displays a [Attention].
- In helical mode, it is recommended to collect at least 360° (if the value is set to “359 or less”, the "Confirmation required" line displays a [Attention]). Currently, ZOO does not automatically evaluate the quality or volume of the crystals. Thus, it is recommended to collect data with a large total rotation angle. Therefore, it may be possible to obtain complete data, even excluding the diffraction images from a bad region.
- ◆ In helical mode data collection, the irradiation point is translated. Therefore, if there are crystal volumes with poor quality in the crystal (e.g. crystals are cracked, or stacked), there is a risk of incomplete data collection because we should throw away the data from such crystal volumes after data processing.

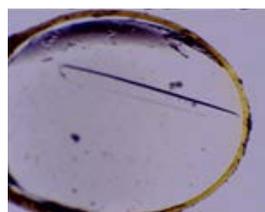
- ◆ If you collect a large amount of data, it may be possible to exclude some of the data during data processing.



- ◆ For the data collected in helical mode, "data split processing" (known as "buttajiri processing") can be applied in addition to normal data processing.
 - ◆ Data split processing involves selecting only the better data, as described above.
 - ◆ The data set is split into 30° (standard setting), processed as independent data, then merged after clustering.
 - ◆ You can compare the results with those from normal processing and data split processing. Please choose the best results for structural analysis. Normal processing results are saved in the "_kamoproc/" directory, and the data split processing results are saved in the "_kamo_30deg/" directory.
- **Osc. Width:**
 - Oscillation width per frame (deg.) for data collection. Recommended value is 0.1°
 - **Liquid N₂:**
 - After the sample pin is mounted on the goniometer, wash off any frost on the loop by using liquid N₂. This can prevent or reduce the effect of the ice ring. This takes about 15 sec. per sample.



Before dropping LN2



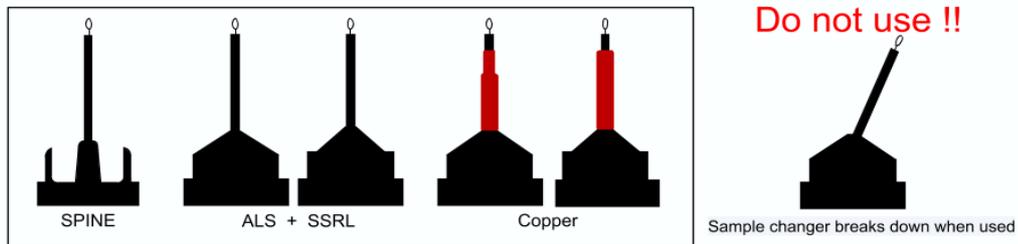
After dropping LN2

Acta Cryst. F76(12):616-622 (2020).

- **Pin Type:**

- Select a sample pin type (SPINE, ALS+SSRL, Copper, or No-wait).
- It has been determined that the sample pins extend to a max. of 50 μm in about 5 min after being mounted on the goniometer. You can define the waiting time for letting the pin extend (please refer the CAUTION section at the end of this document)
- Recommendation: SPINE: 10 sec., ALS+SSRL: 20 sec., Copper: 60 sec, No-wait: 0 sec.
- If you would like to define a different waiting time, please use the contact address.

The degree of elongation depends on the type of sample pin; the Copper type seems to be the worst. The SPINE type is recommended because it is less elongated.



- **Zoom Capture:**

- In addition to the standard magnification, the crystal images are also taken at high magnification, which takes about 5 sec.

<Pin ID setting method>

- 1) Specify the range with a hyphen
 - 1-16
 - 2-5
 - 14-16

- 2) In case of skipping

Continue the number with "." "+" ";" (Period, plus, semicolon).

(Prohibited character ",". Because it is a CSV file, insertion of comma destroys the setting of sample sheet.)

Examples:

1 + 5 + 10 + 15 (Measures 1 and 5, 10 and 15)

1.5.10.15 (measures 1, 5, 10, and 15)

1; 5; 10; 15 (measures 1, 5, 10, and 15)

1-5 + 10-15 (measures 1 to 5 and 10 to 15)

1-5; 10-15 (measures 1 to 5 and 10 to 15)

1-5.10-15 (measures 1 to 5 and 10 to 15)

As software, the character string of "column of pin ID" is first separated by a "." "+" ";", followed by the range-specification character hyphen. The pin ID cannot be set beyond the pack.

<The following information is for those who want to know more detail>

**Setting the scanning conditions for difficult crystals
→ under an assumption that they are not salt crystals**

As of Jul 19, 2019

(Set the dose of raster scan to 1 MGy and the dose of data collection to 9 MGy, etc.)

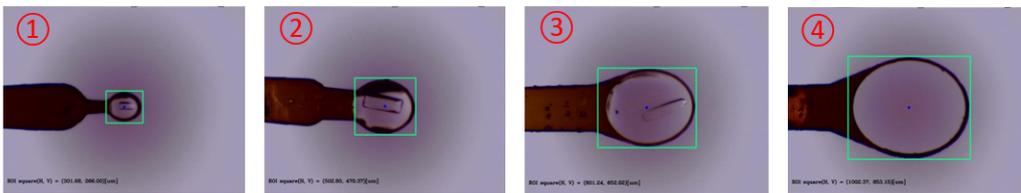
- For example, if we use a 10 x 10 μm beam, let us say that Flux uses a $9.7\text{E}12$ phs/sec beamline.
- Flux density is $9.7\text{E}12/10.0/10.0 = 9.7\text{E}10$ photons/ μm^2
- For a wavelength of 1 \AA , 10 MGy is approximately equivalent to $2\text{E}10$ photons/ μm^2
- This does is reached in $2\text{E}10/9.7\text{E}10 = 0.206$ sec.
- If you want to scan 1 MGy, this 1/10 exposure time is necessary.
 - Exposure time of 0.02 sec.
- In this case, the scanning time is about a 400- μm loop.
 - 400 $\mu\text{m}/10 \mu\text{m}$ step = 40 points/line
 - 40 points * 0.02 sec/points = 0.8 sec/line
 - The number of lines is 400 $\mu\text{m}/10 \mu\text{m}$ = 40 lines with about 2 sec of wrapping.
 - 40 lines * (2 + 0.8) sec/line = 112 sec
 - The number of crystals found in this will take a few minutes of data-collection time.
 - 30 crystals * 10 sec = 300 sec
 - Therefore, if there are 30 crystals in the 400- μm loop, the required calculation is 112 sec + 300 sec = 412 sec/loop
 - It is about 7 min/loop, so it is about 2 hrs per pack.

<FAQ>

“Litho loop catalog size” and “Hor. scan length” (input value in Excel file)

※ Hor. Scan length setting should be slightly larger than catalog size for **Litho loop**.

	①	②	③	④
Litho loop catalog size [um]	200	400	600	800
Hor. scan length [um]	300	500	800	1000



※ □ : Raster scan area

※ For nylon loops, Hor.scan length needs to be longer

Average measurement time of automatic measurement with BL45XU (example of actual measurement value)

Measurement time per sample pin (min.)

[Hor. scan length (μm) Vs. Beam size (μm)]

Single mode

	200	400	600	1000
50x50	2.7	3.4	4.1	6.4
20x20	4.2	4.8	5.8	6.3
10x10	4.8	6.2	8.0	10.0
5x5	6.8	11.5	18.1	25.2
10x50	3.8	4.1	5.4	6.9

Helical mode

	200	400	600	1000
50x50	4.4	4.8	5.5	no data
20x20	4.6	5.3	6.1	7.1
10x10	6.0	7.1	8.8	10.6
5x5	8.1	13.4	18.9	27.2
10x50	3.9	4.6	6.0	8.0

Multi mode

	200	400	600	1000
50x50	5.9	6.5	7.1	7.4
20x20	6.1	6.5	7.6	7.9
10x10	6.7	7.9	10.2	12.4
5x5	11.1	14.5	19.4	24.7
10x50	6.2	7.0	8.4	9.6

Exposure time : 0.02 (sec.) [0.1(sec.) for 50x50 only]

Oscillation width : 0.1 (deg.)

@Single, Helical

Total Oscillation : 180 (deg.)

Max_hits : 1 (point)

※Max_hits = Number of beam exposure point

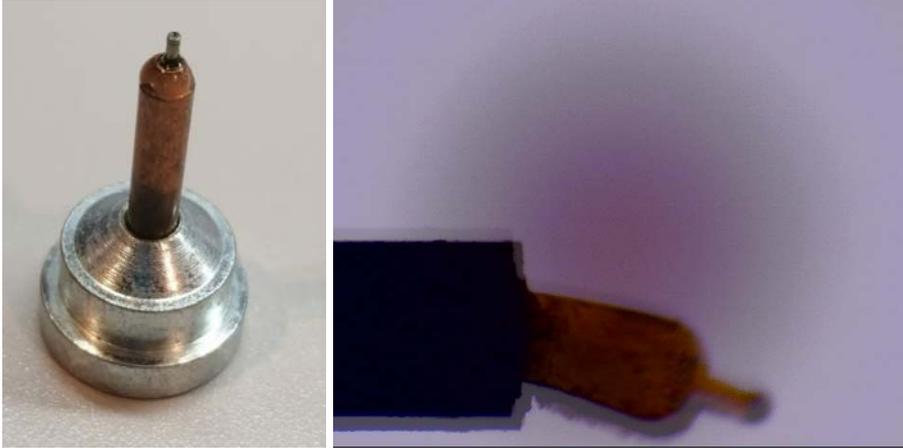
@Small wedge (multi)

Total Oscillation : 10 (deg.)

Max_hit : 30 (points)

<CAUTION>

It is not recommended to use the sample pin with copper (shown in the figure below) for automated data collection.



Right)

A composite photo of the sample loop mounted on the pin with copper after 0 and 5 min.

The loop shifted 50 μm horizontally and 36 μm vertically within 5 min. Such significant elongation occurs in a pin with copper. We are analyzing other types of pins as well.