

# Manual for the Program SAngler

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## 1. Introduction

- ① This manual is compatible with the version 1.1.13 as of Nov 12<sup>th</sup>, 2015. Please be sure that the operation method may change after each version upgrade. Please check the latest manual.
- ② In fact, the following type of data is not still completely compatible with the program.
  - Wide-angle X-ray diffraction data with no direct beam inside the data (we are currently working for the solution)
- ③ As SAngler is not compatible with high DPI density of the display setting, it can be displayed correctly only when the display setting is [small-100%]. Please be reminded that in some PAD-type PCs, the default setting is [Medium (M) - 125%] (Adjust by [right-click the screen] – [Screen resolution] – [Make text and other items larger or smaller]).  
※ In VAIO (SONY), the default setting may be “medium” even in laptop-type computers.

## 2. Preparation

- ① The program is compatible only with Windows. The current program has been tested with Windows 7 and 8.1, under the 64-bit environment. It also operates in the virtual environment of Mac.
- ② The program is released in the form of a single compressed file. Please download and extract it anywhere you like inside the hardware.
- ③ When you extract the compressed file, two executable files, one dll file, five xml files as well as system and gnuplot folders will be created. In principle, if you move these files together, you can use the program on any computer.

## 3. Method

### ① Check the basic operation setting of the software


- i. Launch SAngler (.exe) by double-clicking the icon.

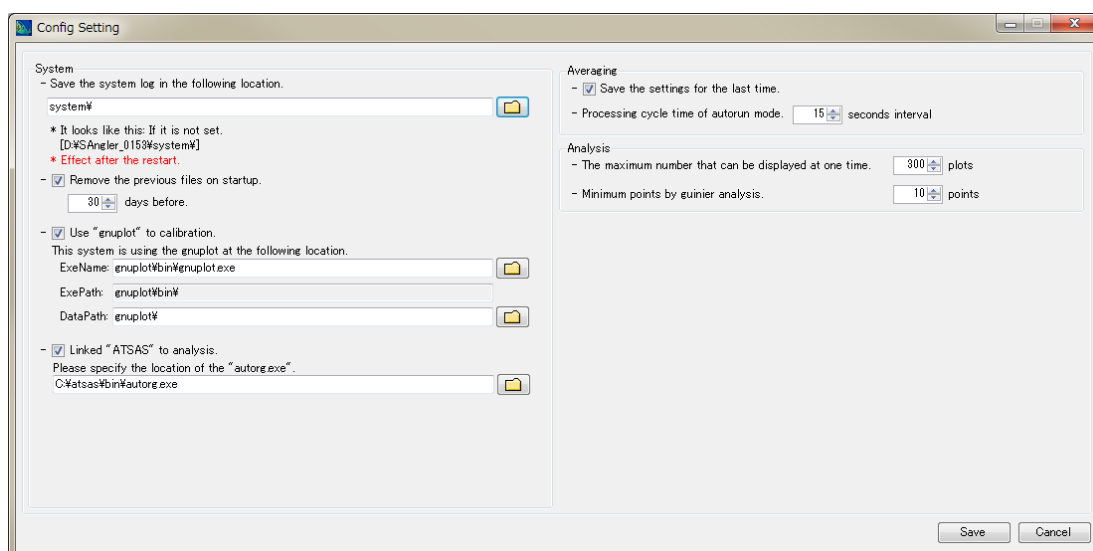
※ The following error message may appear when you launch the program.

Γ”autorg.exe” was not found. Please set up the place of “autorg.exe” at [Option] – [ConfigSetting] if you need.]

In Guinier analysis, the autorg of ATSAS is available. This message will be displayed if you have not installed ATSAS version 2.6.0 or above. If you have installed the version

2.5.2 or lower, this message will be displayed because 2.6.0 and autorg.exe are located in different directories. Indicate the folder where autorg.exe is located by selecting [Option]-[ConfigSetting] as asked by the message above.

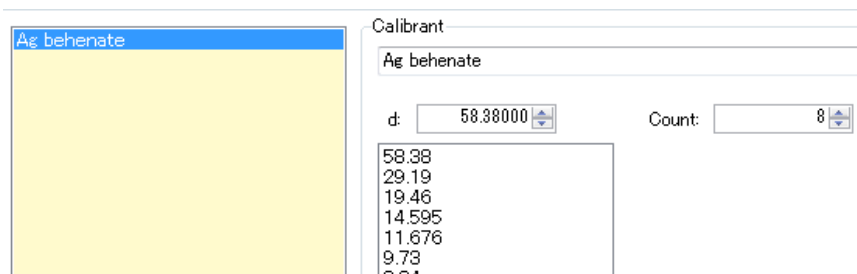
- ii. Execute [ConfigSetting] from the [Option] button at the upper-left of the window.
- iii. In the [System] field, select the folder where you want to save the system log. Click  and select the [system] folder located in the folder launching SAngler (this folder is selected under the default setting). This folder change will become valid after you reboot SAngler.
- iv. Although log files are set to be deleted after 30 days under the default configuration, this value can be changed.
- v. In the original configuration, the [Use “gnuplot” to calibration] icon is checked. Gnuplot is used when correcting the camera length or the beam center using the Debye-Scherrer ring pattern. It is in the “gnuplot” folder inside the startup folder that its program path is set. Although it is possible to carry out processing without using gnuplot if you uncheck this icon, be careful as some detectors may not be able to process data correctly.



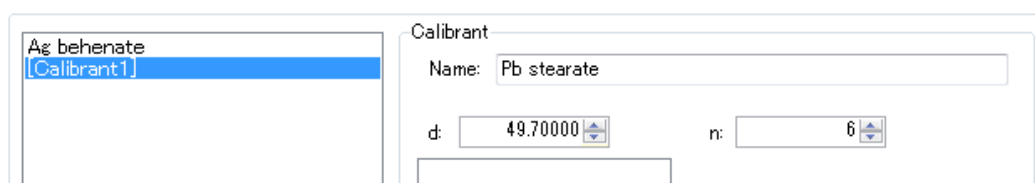
- vi. The [Linked “ATSAS” to analysis] box is checked in the default setting. The install path for ATSAS autorg, available for Guinier analysis, is displayed. However, you need to install ATSAS independently by yourself. The current version of ATSAS is 2.6.1. Please be reminded that the executable file for the version 2.6.0 is installed at a different place from the one for earlier versions. Moreover, **although ATSAS is free for academic use, licensing fee occurs for commercial use. Please see the following site for details about ATSAS.**  
**<http://www.embl-hamburg.de/biosaxs/software.html>**
- vii. The [Averaging] box is checked so that the settings upon exit can be saved. Please uncheck if you want to change this setting.
- viii. When you use the folder automatic monitoring mode (Autorun) to perform circular averaging,

processing is set to be executed every fifteen minutes in the default setting. Please modify this timing as adequate according to the measuring or processing speed. In addition, please note that it will take one second per image for circular averaging in case of PILATUS 2M images.

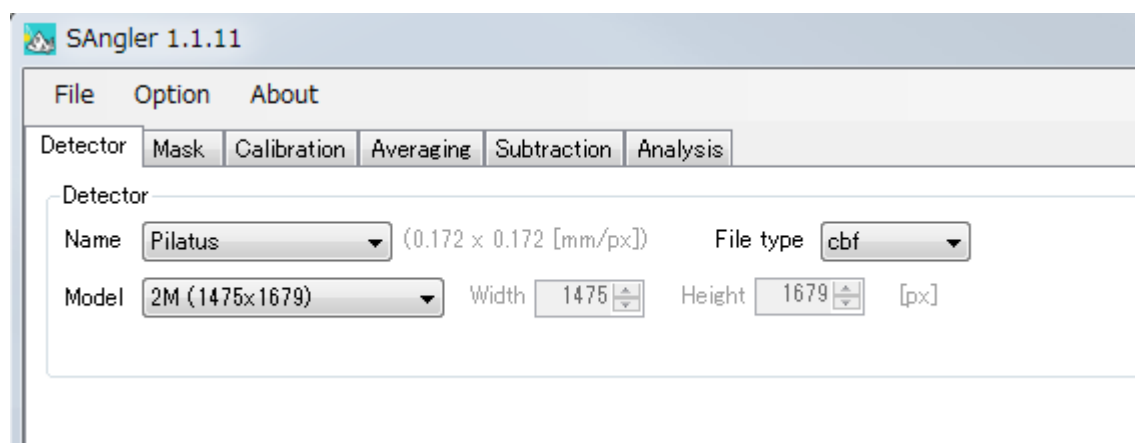
- ix. The maximal number of data sets that can be processed at once with the Analysis tab is 300. Reduce this number if the operation is unstable.
- x. The minimal number of measurement points to be used for linear approximation is set for Guinier analysis. The default value is 10 points. If the value is below 10 points, you will encounter an error message.
- xi. When setting is complete, click [Save] to close.
- xii. Next, execute [Option] - [ConfigCustom]. Here, there are a total of three tabs. First, the [Facility] tab works to register the specification of the counter file necessary for the normalization of measured scattering intensity by means of incident (integrated) intensity. Next, the [Detector] tab can be used to set the information of available detectors. Finally, the [Calibrant] tab is available for the registration of information including silver behenate for calibration. First, we explain how to register a new standard sample in [Calibrant].
- xiii. In the original setting, Ag behenate (silver behenate) is registered. In case data processing is possible with silver behenate for eight peaks or less, this setting is OK. Meanwhile, if you use other samples including lead stearate or in case the camera length is short even if you use silver behenate, then you can have images of eight to twelve peaks. New registration is necessary if you use such data for calibration.



- xiv. Moving to the [Calibrant] tab, only [Ag behenate] is available. If you click [Ag behenate], you will see the spacing value of d: 58.38Å, the number of peaks is n: 8, and below that the values for first to eighth peaks. Click [Add] if you add some new elements.
- xv. The new item [Calibrant 1] has been added. Edit Name (sample name) in the Calibrant tab in the right, and enter the value of first-order spacing in the d box. In the n box, show until which peaks you will use the ring and click [Entry] in the lower right.



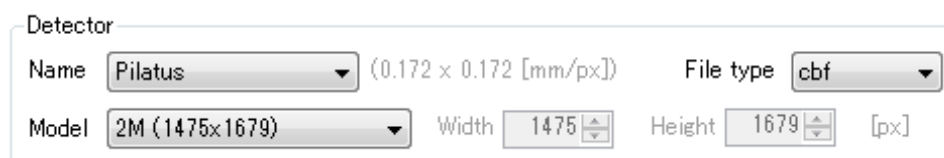
- xvi. By clicking [Entry], newly-entered items are added. By clicking such items, you will see that the d values from first-peak spacing to the maximal entered number of peaks have been automatically calculated.
- xvii. When you have finished, click the [Save] button in the upper right. The saved file should be overwritten with the [calibrant.xml] in the same folder. When this process is complete, close the window by clicking [x] in the upper right. The information that you have newly registered here will become valid when you click [Load] in the “Calibration” tab.
- xviii. In the [ConfigCustom], there are also [Facility] and [Detector] tabs. The [Facility] tab sets counter files necessary for the normalization of scattering intensity by means of the incident X-ray intensity in the PF beamline and SPring-8 BL45XU. Compatible detectors are set in the [Detector] tab. Currently, SAngler is compatible with file formats of PILATUS series (tif file) and R-AXIS series. Detectors of the same types can be made compatible by carrying out new setting here.
- xix. There are six tabs in the main window of SAngler. By moving forward in the order of [Detector]→[Mask]→[Calibration]→[Averaging]→[Subtraction]→[Analysis], you can process data one by one.



## ② Setting of File Type

Define the detector model and the format of an image data. The format set is fixed as a default in the analysis after Mask tab. Currently, PILATUS is compatible with the TIF (32bit TIF) and CBF (Crystallography binary format), and R-AXIS is compatible with the standard Rigaku format. Each detector chooses the size (R-Axis is compatible with IV, IV++ and VII).


- i. Choose the detector and model name from the pull-down menu.
- ii. Choose the file type (format). Pilatus: cbf or tif, R-AXIS: stl or osc.



- iii. Click [Set] to fix them.
- iv. Move on to the Mask tab.

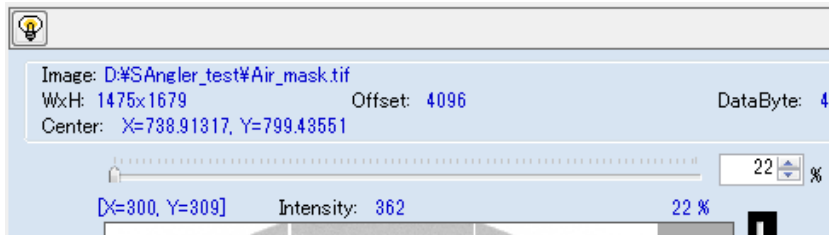
## ③ Creating a Mask file


PILATUS has succeeded in making its area large by arranging 100K basic modules in a mosaic pattern. For this purpose, GAPS of three to five millimeters exist between modules. By masking and processing these GAPS, it is possible to conduct analysis as if you were analyzing seamless data. In addition, there are bad pixels, which should be masked too. With SAngler, you can easily create mask files.

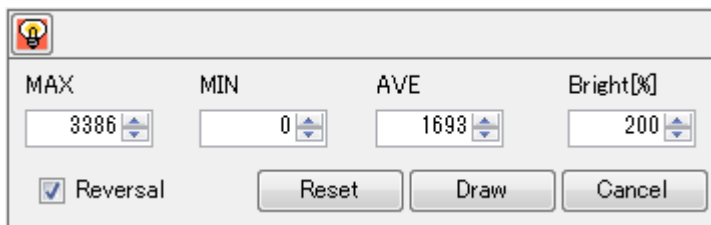
- i. First, load an exposed image to create a Mask file. It is recommended to obtain data of air scattering with an exposure of around one minute without samples, but you can proceed without it. If you do not have such data, load a freely-selected image. Select an image from  in the [Image file:] field. You can also directly enter (copy and paste) the directory configuration (character string).



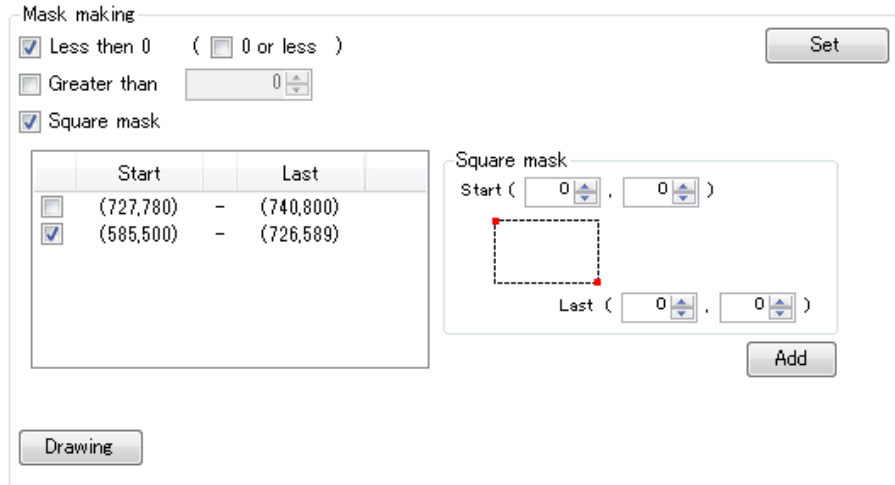
- ii. After having selected the image, click [Draw].
- iii. Data will be displayed in the right of the window. In the upper side of the image, file information, number of pixels, as well as the currently set beam center are displayed. If you move the mouse cursor onto the image, the coordinates of the spot (ex. [X=300, Y=309], Intensity: 362) will be indicated in the upper left of the image and the line profile centered on that spot will be shown in the right and lower side of the image. You can also enlarge or reduce the image by using the scroll button of the mouse on it.



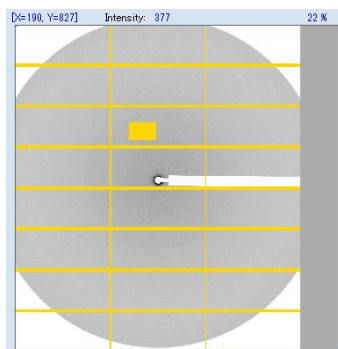
- iv. By clicking the  mark, you can adjust the contrast or brightness of the image. MAX represents the maximal count value inside the image and MIN shows the minimal value (equal or above zero). As the acronym suggests, AVE shows the average value. If it goes down, the display gets darker and allows users to recognize weak scattering, while the display becomes brighter if this value goes up. [Bright] is used to adjust the brightness of the display. Black and white will be reversed if you uncheck the [Reversal] box. If you have modified the values, the display will change after clicking [Draw].



- v. Select the conditions for the Mask in the [Mask making] field. If you check [Less than 0], negative values will be automatically masked. As it is set to output the values of -1 and -2 respectively for PILATUS GAP and Bad pixel, check [Less than 0] if you want to mask them. In addition, do not forget to check [0 or less] too if the 0 value should be included.
- ※Although [bad pixels] are output as [-2] if set in advance, this setting may not be in place to a new bad pixel (A new bad pixel may appear suddenly). If you found such pixels, they can be automatically masked if “0 or less” box is checked.
- vi. Contrary to the [Less than] box, [Greater than] box masks pixels with larger values than set. Some newly created bad pixels may not be dead pixels (always with zero value) but hot pixels (pixels that always output very large values). This is a function aimed at masking such pixels. If you set this value somewhat inferior to the count value of discovered pixels, you can mask them with certainty.




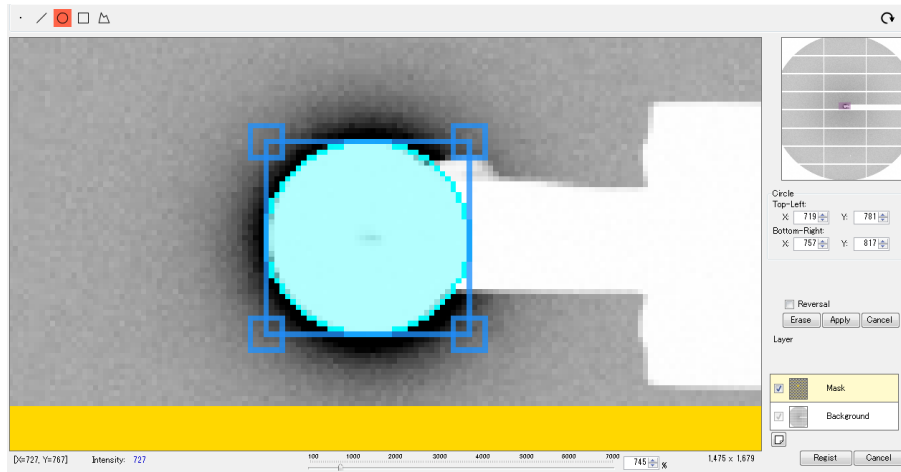
- vii. The [Square mask] function can be used to mask the quadrangular area that you have selected. As shown in the screen, select the pixel of the upper-left side (Start) and the lower-right side (Last) of the quadrangle. If you want to select only one pixel, indicate the same coordinates for [Start] and [Last]. Click [Add] when you have entered these values. What you have input will be moved to the left field. This setting will be applied if the box remains checked, but will not be if unchecked. When you click [Set], the modified setting will be applied to the image and shown in yellow. (Although the figure below has a quadrangular masked area around the center, this is only an example shown as a test result).



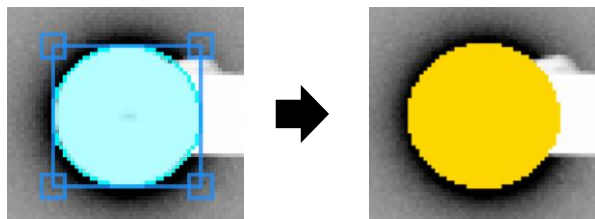
- viii. In order to create the mask of a round shape and a polygon, click [Drawing]. The Mask Drawer window newly opens. Users can create a various-shaped mask by drawing a mask region on this image. Although the image data is displayed as full size, users can change a displayed region by dragging left-clicking a mouse, and can magnify/reduce it by scrolling. It is as follows if a function is summarized.
- When a point, a line, a circle, a quadrangle, and a polygon are drawn, these regions are masked.
  - When [Reversal] is checked after drawing the mask regions, they are reversed, that is, users can mask other than the drawn regions.



The procedure is as follows. Since GAP and bad pixels can be masked independently, this time, the mask of a beam stopper's shadow is created.

- (1) Magnify the area near the beam center by scrolling of a mouse. Click  at the upper left of the window, and draw a circle so that a round shadow may be covered.

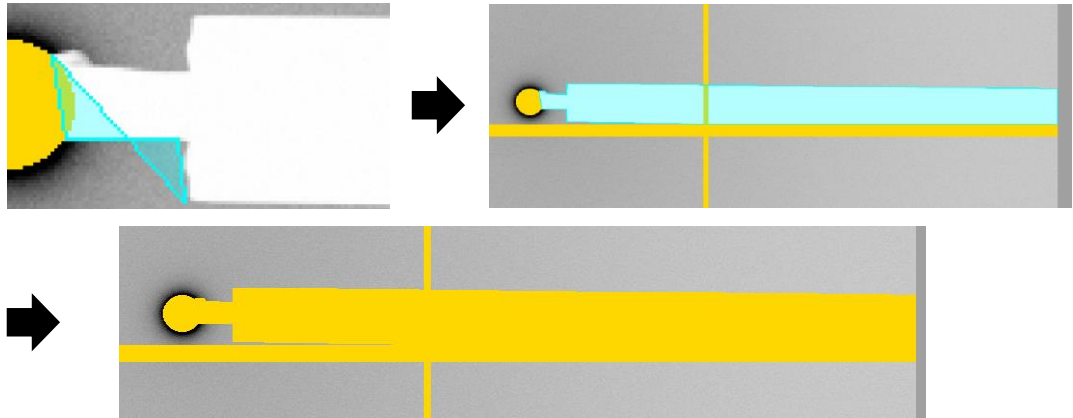


- (2) The shape and size of the circle can be finely adjusted by clicking and moving four corners of the quadrangle which contacts the circle. In the menu "Circle" on the right side of the window, the coordinates of Top-Left (upper-left) and Bottom-Right (lower-right) are modified by an arrow key to adjust the circle size and shape. In the case that users directly input the values, it will be applied when Enter key is pushed. When you have finished working, click the [Apply] button in the right side. The mask color changes to yellow.

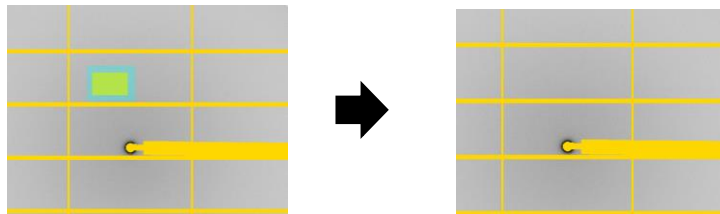


- (3) When  is clicked again, the drawing mode is finished and users can move the image data.
- (4) Next, the shadow of the shaft of the beam stopper is masked. Click  to start making the mask. Click the pixel of a polygonal corner in order. If the image data is magnified, the other domain of a stopper's shaft cannot be seen in the display range. The sub-view of image data is on the upper right of the window, and the displayed range in the main-view is shown by the purple color. During mask drawing, users can change the display range by dragging and moving this purple region. Click [Apply] to complete this work.

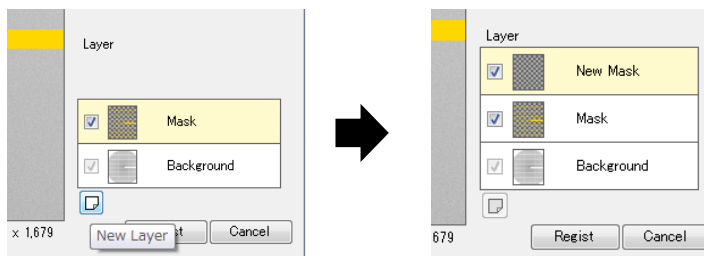




- (5) If you erase the mask region, you overlay the mask region shown in yellow with another figure. For example, in the case that users erase the test region shown in (vii), click  at the upper left of a window, and create a quadrangle so that the test region may be overlaid. Then, when clicking [Erase] on the right side of a window, it will be erased.

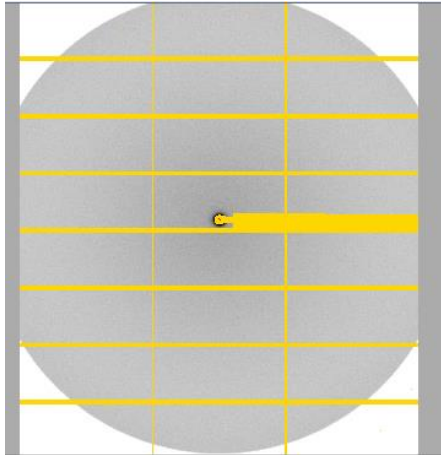


- (6) When erasing the mask region created once, you may also erase the other mask region not to erase together. For example, in the upper figure (Figure at (5)), since the mask region for lattice-like gap of PILATUS is overlapped with the mask region for a beam stopper's shadow, it is quite difficult to erase only the mask of the beam stopper. Now, in this Mask Drawer, users can add one other layer. Therefore, you can draw a new mask region to the other layer so that you may not change the status of the original layer. Click [New Layer] button to add a new layer. If "New Mask" is unchecked, the newly created mask is not applied.

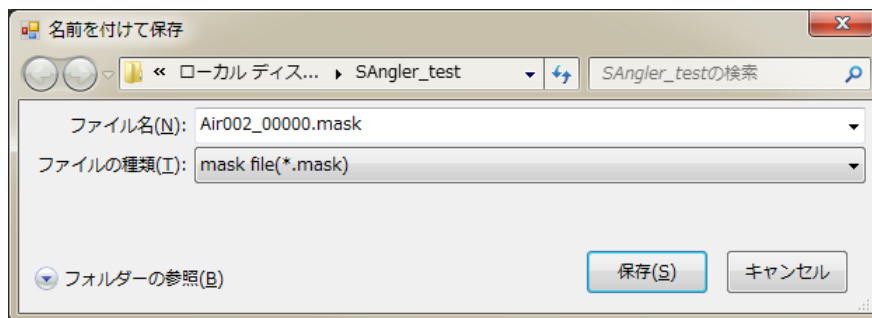


- (7) Click [Regist] button if all the work was completed. All the layer will be combined after clicking [Regist].

- ix. When you finished setting and click [Set], the modified setting will be applied to the image and shown in yellow. In case you have made additional editing, the setting will be updated after you click [Set] again.






- x. When setting is complete, click [Save] and save the Mask file. The extension will be automatically “mask”. When saving is complete, the yellow display will be modified to green. The saved Mask file will be automatically applied to future data processing. Even in case you close the software or process it on another PC, you can perform processing under the same condition by loading the previously saved Mask file.

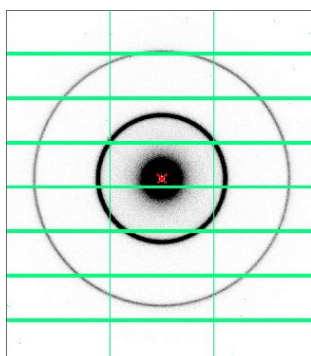


- xi. Now, the Mask file has been successfully created. Move on to the Calibration tab.

#### ④ Correction of the beam center and the camera length

Correct the beam center and the camera length using, for example, the powder diffraction data of silver behenate.

- i. On the [Setting] tab, check if the file you have created earlier is set in [Mask file:] (to set a file that you have saved earlier, click  to load it)
- ii. Select an image file to be used for calibration in the [Image file:] from .
- iii. After having selected the file, click [Draw] to display it.
- iv. When the display is too bright to see, click the  mark so that the ring pattern is visible, by setting AVE to 500 and Bright to 200, for example.



- v. Check the [Refine BeamCenter] box and enter approximate coordinates of the center. The spot is shown with the cross (x) signal in red on the image. If this spot is quite irrelevant, move the mouse cursor to the area around the beam center. The coordinates of this spot will be displayed in the upper left of the image, so enter this value.
- vi. Check the [Refine Wavelength or Camera distance] box. As the measured wavelength and the camera length are corrected in an exclusive way, check either of them and enter an approximate value.

Refine BeamCenter  
X  Y  [px]

Refine Wavelength or Camera distance  
\* Choose the value you want to refine.

Wavelength [Å]

Camera distance [mm]

Display

Calibrant  
Ag behenate

d1=  +/-

d2=  +/-

d3=  +/-

d4=  +/-

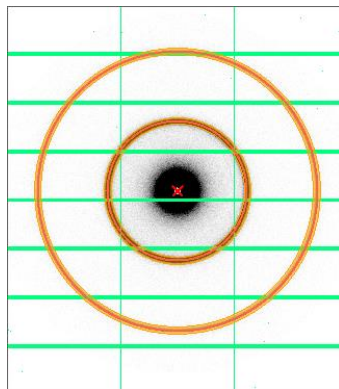
d5=  +/-

Add d=  n=  +/-

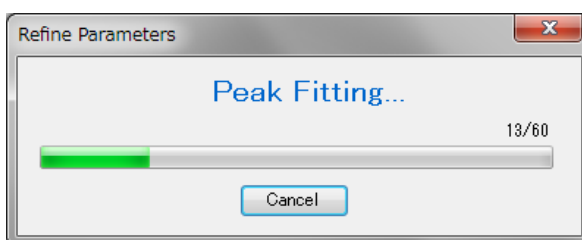
- vii. Select a parameter to be used for the [Calibrant] field. In the [Ag behenate], parameters of up to the eighth peak are in place in the default setting. Here, as the loaded image only reflects rings of up to the second peak, delete d3 to d8 and leave only d1 and d2. On the

other hand, if you have separately set standard samples for correction other than the [Ag behenate] as in the default setting using the [Option] - [ConfigCustom] buttons, information on the pull-down menu will be updated by clicking [Load].

- viii. If you check the [Display] box, the prediction based on the default values will be displayed. Edit the default values so that the prediction ring matches the actual ring. If the [Redraw] button is pushed, the prediction will be calculated again.



- ix. With [Num sectors], select how many sections the entire circumference of the ring pattern should be divided into when calibration is executed. The default value is 60. There is no problem to use this value if the entire circumference of the ring is visible as in the image above. However, analysis points may be insufficient if the entire circumference is divided into 60 sections in case the entire circumference of the ring is not visible, for example when detectors are installed in off-set or PILATUS 100K is used. In such cases, execute calibration by increasing the number, for example from 60 to 240.
- x. When ready, click [Start]. The following dialog will be displayed and the processing will continue. If [Plot Display] is checked, the graph will be displayed during analysis.



- xi. When calibration is complete, corrected values will be shown in red. In principle, this processing should finish in one time. However, if the default value is quite deviant at the beginning of the processing, perform it several times until the predicted ring matches with the actual one.

Refine BeamCenter

X  Y  [px]

Refine Wavelength or Camera distance  
\* Choose the value you want to refine.

Wavelength [A]



Camera distance [mm]

- xii. When calibration is complete, save the result by clicking [Save]. The file extension will be automatically “cal”. Even in case you close the software for the moment or process it on another PC, you can perform processing under the same condition by loading the previously saved cal file.
  
- xiii. Now, calibration has been successfully processed. Move on to the Average tab.


## ⑤ Circular averaging

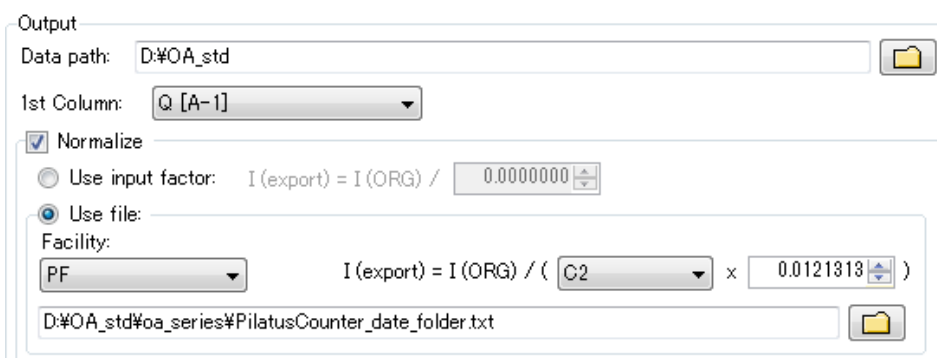
Execute circular averaging based on the calibration result. Two modes are available for circular averaging

### (1) In case you process measured data by selecting them sequentially


- i. In the [Data path] field of the [Input] section, click  and select the directory where the image data is saved. This field can also be edited directly.
- ii. In case of PILATUS data, check if the [Use Mask data] box is checked and the MASK file created earlier is selected. In order to load the MASK file you processed before, select it by clicking . This field can also be edited directly.



- iii. Now, set items in the [Output] field. In the [Data path:] field, select the directory to output the file on which circular averaging has been executed. Click  or edit directly.
- iv. Select which unit to use to output the horizontal axis of the data (1<sup>st</sup> column).  
Q ( $=4\pi\sin\theta/\lambda$ ) [ $\text{\AA}^{-1}$ ], Q [ $\text{nm}^{-1}$ ], S ( $=2\sin\theta/\lambda$ ) [ $\text{\AA}^{-1}$ ], S [ $\text{nm}^{-1}$ ], d [ $\text{\AA}$ ], d [ $\text{nm}$ ],  $2\theta$  [degree],  
r [mm] (distance from the beam center)
- v. Check the [Normalize] box to normalize the scattering intensity with the incident X-ray intensity. If you want to normalize the scattering intensity with a specific value, enter the value using the [Use input factor] box.



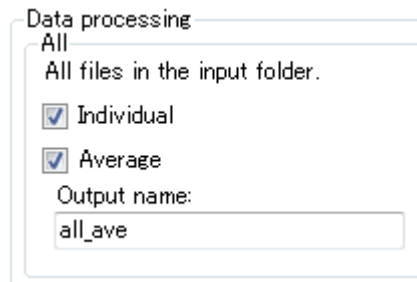
- vi. In order to perform normalization using the incident X-ray intensity value (integrated intensity in PF), select and load the log file of the counter value that is output separately from the image data. First, select the facility. This process is compatible with the formats of the counter files of PF and SPring-8 BL45XU. In addition, select with what value registered in the counter file you will perform normalization. In case of PF, C2 is the current value before the sample, and C3 is the value after the sample. With BL45XU, MIC up is before

and MIC down is after the sample. Moreover, it is possible to perform normalization by using the value input simultaneously with normalization with counter values. For example, this is used to convert the scattering intensity [counts] into the absolute scattering intensity [cm<sup>-1</sup>]. For more information, ask the beamline person. If this is not necessary, you should input [1.000]. Click  and select the counter file.

vii. Next, select the range of files to be processed in the [Data processing] field. SAngler can process several files at the same time. There are roughly two methods to select files, namely [All] and [Range selection].

(1) All

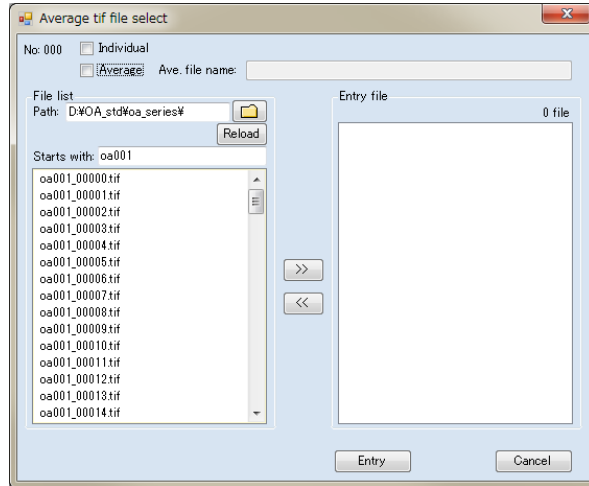
With this method, all image data located in the Input Data Path Directory, as selected earlier, will be processed at one time. If you check [Individual], all image data located in the folder will be subject to circular averaging one by one. The names of the output files will be the same as those of the image files, except that the extension becomes “dat”. Since the [Average] function selects files to average all images in this folder, enter the file name (the extension “dat” will be added automatically). You can execute both [Individual] and [Average] or only either of them.



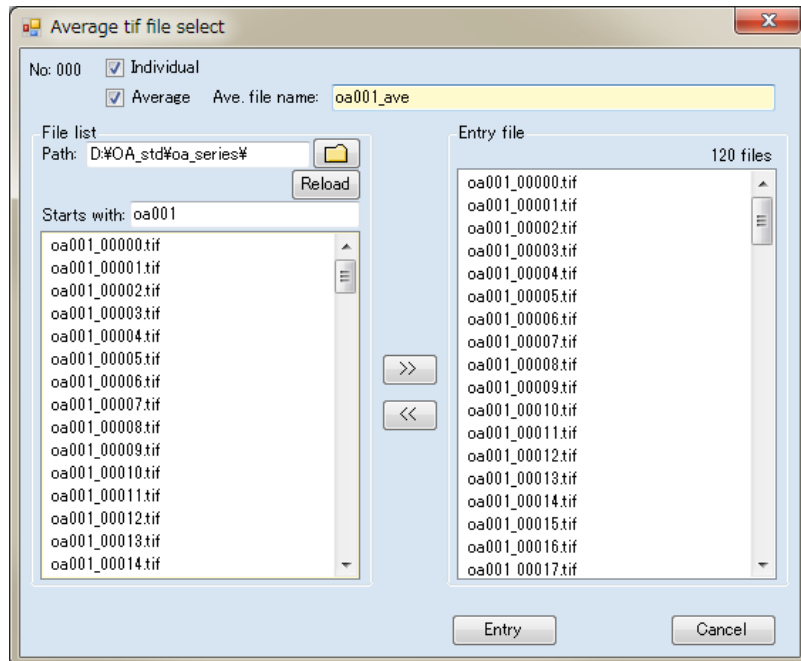
(2) Range selection

Select the range of processing for the data located in the [Input Data Path] directory. For example, select the range of background data and sample data separately and process them individually.

- A) Click [Add]. A new window opens. The current directory and the files in the directory are shown in the [File list].
- B) Select several files that you want to load at the same time (you can also select only one). As the functions of the OS are available, you can for example select several files while pushing the [ctrl] key or just by clicking and dragging them with your mouse. [Starts with:] is a function of the filter and serves to display specific data sets only if you input a string that can be used to eliminate other files from the head of the file name. After that, you can select all by using [ctrl + A].



- C) Confirm the selection of the data by clicking . The selected files will then be moved to the right side. Repeat the same operation for other files if necessary. Select if you want to execute circular averaging separately for each file selected (Individual), average these files together (Average) or execute both of these operations. In case you choose [Individual], the output files retain the same name with the dat extension. On the other hand, if you select [Average], enter the file name after averaging (the extension dat will be automatically added). When this operation is complete, close the window by clicking [Entry].



- D) In the [Range selection] section, the jobs you set just now are in place. Click one to display the list of files to be processed. If you want to process other data sets at the same time, Repeat the procedure from (A) to (C). As each job individually performs



processing, the order will be executed without interference even if the same image data is processed differently with different jobs.

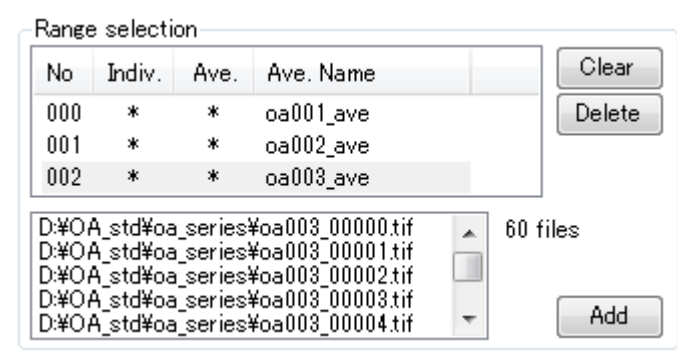
(Example) A total of 100 data sets, from OA001\_00000.tif to OA001\_00099.tif, are subject to following jobs:

(Job 1) Carry out circular averaging for 100 data sets by executing [Individual] and [Average].

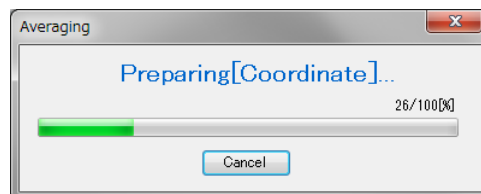
(Job 2) Execute [Average] only for the first 50 data sets, from 00000 to 00049.

(Job 3) Execute [Average] only for the last 50 data sets, from 00050 to 00099.

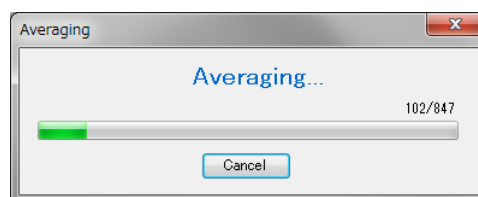
As three jobs are executed in turn, a total of 103 dat files will be output.



- viii. When you finished inputting, execute the operation by clicking the [Run] button. The “Preparing[Coordinate]...” dialog will be displayed only if this is the first time to do this operation since the launch of SAngler. This processing is performed to speed up circular averaging and the dialog will not be displayed from the second time...

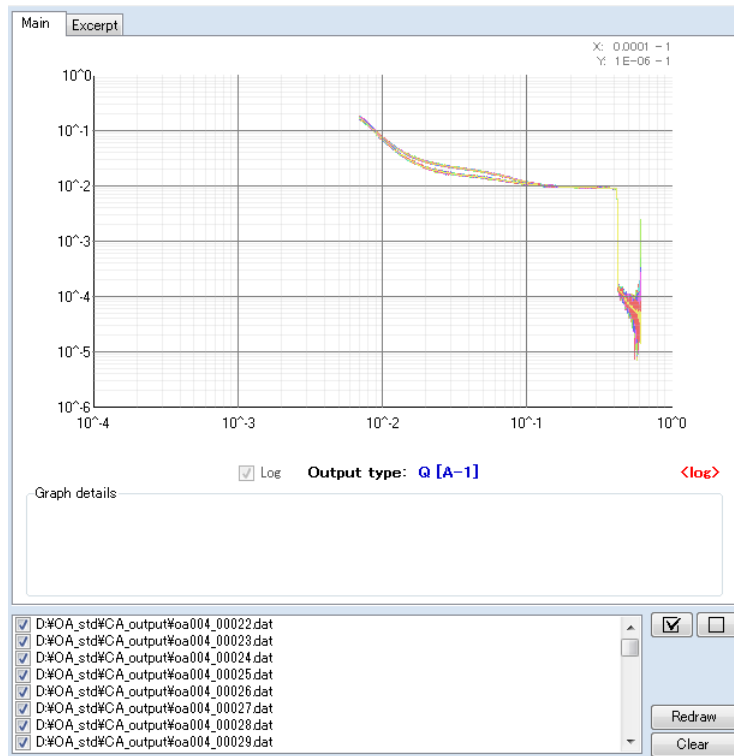


- ix. When “Preparing...” is complete, circular averaging starts (circular averaging starts immediately from the second time). In case of PILATUS3 2M, it takes one second to process one image, and the average time to process several images differs according to the number of images.

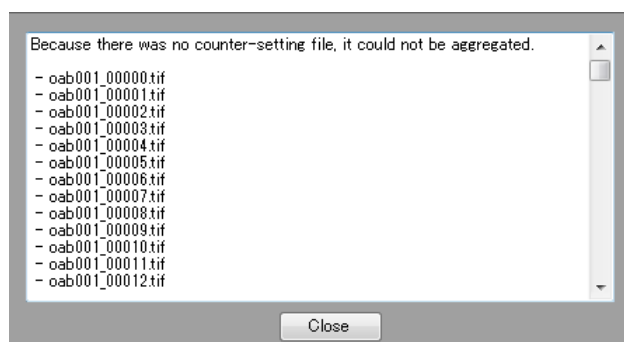


- x. When you have completed the process, the graph will be displayed in double logarithmic plot. The graph can be enlarged or reduced by scrolling the mouse or by dragging the range

to be enlarged by a left-click. You can select the graphs to be displayed by checking or unchecking the boxes below and clicking [Redraw]. Use [Clear] to delete all. You can also display graphs by dragging & dropping dat files onto this figure.



- xi. If there is an error in the counter file selected to conduct the normalization of scattering intensity, the circular averaging processing may finish immediately. More concretely, this may occur if the count value of the image data to be processed does not exist in that file. In this case, select the correct file again.



- xii. There are three data rows output in the output data. The selected horizontal axis ( $Q(\text{\AA}^{-1})$  for example) has been output in the first row, the scattering intensity  $I$  in the second row and the standard error of the scattering intensity  $\sigma I$  in the third row. In case the normalization of the scattering intensity has been indicated, the values in the second and third rows are the ones after normalization. The condition of calibration used for circular averaging, the explanation for each data row and the header for the image file are output in the file header.

```

# Date: 2015/05/29 19:14:08
# Type: ImgAverage
# BeamCenter X: 731.71402/ Y: 799.99149
# Wavelength: 1.00000
# Camera distance: 2027.09893
# Tilts H: 0.00000/ V: 0.00000
# Image: D:\OA_std\oa001_00000.tif

# 1st. Column: Q [A-1]
# 2nd. Column: I(export) = I(ORG) / ([C2] x 0.012131288)
# 3rd. Column: Sigma I(export) = Sigma I(ORG) / ([C2] x 0.012131288)

# NewSubfileType: 0
# ImageWidth: 1475
# ImageLength: 1679
# BitsPerSample: 32
# Compression: 1
# PhotometricInterpretation: 1
# ImageDescription:
## Pixel_size 172e-6 m x 172e-6 m
## Silicon sensor, thickness 0.000320 m
## Exposure_time 5.0000000 s      :

```

**(2) In case you monitor selected folders and automatically conduct circular averaging on updated images**

- i. The preparation for processing should be done as above. Just like the (1) from i to vi above, select the paths for input and output and the conditions for normalization.
- ii. [Individual] processing should always be selected, as the automatic processing is done by monitoring the directory selected in [Input]. Check [individual] for [All] in the [Data processing].
- iii. Execute [Autorun] after having completed all necessary input. Individually process image files inside the "Input" folder. When all this processing is complete, the system enters the waiting mode. In the default setting, the folder is checked again in 15 seconds, and processing is executed if there is a newly created image. It is possible to modify the interval of processing time with [Option - ConfigSetting].
- iv. In order to stop Autorun, click the [Autorun] button again. It will restart operation if you click

[Autorun] again, but all the files in the directory will have to be processed from scratch again.

- v. When circular averaging is complete, move on to the “Subtraction” tab.

※How to obtain the standard error

- I. Conduct circular averaging on the data of N pixels located in the distance r from the beam center.
- II. The average value  $x_m$  of N data sets  $x_1, x_2, \dots, x_N$  will be as follows.

$$x_m = \frac{1}{N} \sum_{i=1}^N x_i$$

- III. The variance V for the average value  $x_m$  will be as follows.

$$V = \frac{1}{N} \sum_{i=1}^N (x_i - x_m)^2$$

- IV. As the square root of the deviance V is the standard deviation (SD),

$$SD = \sqrt{V}$$



- V. The standard error (SE) can be obtained with the following relational expression according to the law of propagation of errors. This value is output.

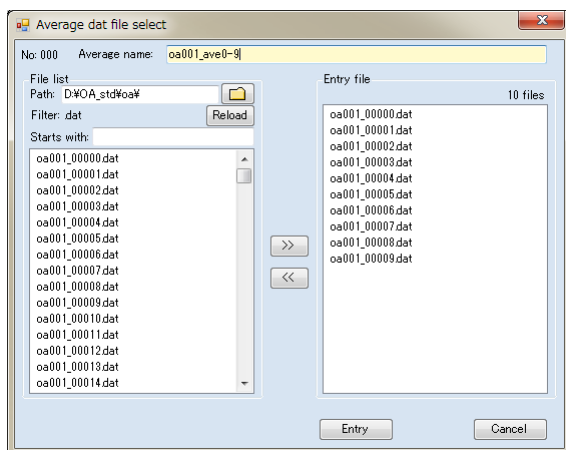
$$SE = \frac{SD}{\sqrt{N}}$$

※ Please be reminded that when circular averaging is performed by averaging several images, all the images are integrated before they are averaged.

⑥ **Average of dat files after circular averaging**

In the [Subtraction] tab, there is a tool to average dat files after circular averaging in the upper row and a tool to subtract the background in the lower row. First, we explain about the averaging of dat files.

- i. In the [Input:], the [Output] directory selected for averaging has been automatically input. If you have started the procedure from here, click  and select the directory where the dat files have been saved. This field can also be directly edited.
- ii. In the [Output:], select the directory where the averaged file should be output. Either click  or directly edit it (if it is the same as for [Input], copy and paste it).
- iii. Click [Add] and select the dat files to be averaged as for the circular averaging executed earlier. After inputting the name of the averaged file to be output, click [Entry] and close the window.



- iv. If you want to average additional data sets, add them by clicking the [Add] button.
- v. After having completed the input, click [Average]. The Averaged file will be output and graphs will be displayed. In the header of the output file, it will be clarified which file was used for averaging, and the values of the horizontal axis including Q, the scattering intensity I and the standard error of the scattering intensity  $\sigma_I$  will be output. The standard error has been recalculated according to the law of propagation of errors.

✂ Error after the averaging of dat files


The average value  $x_m \pm \sigma_m$  of N data sets  $x_1 \pm \sigma_1, x_2 \pm \sigma_2 \dots, x_N \pm \sigma_N$ , having the error of  $\sigma$ , can be obtained with the following formula:

$$x_m \pm \sigma_m = \frac{\sum_{i=1}^N x_N}{N} \pm \frac{\sqrt{\sum_{i=1}^N \sigma_N^2}}{N}$$


※ When averaging images with the [Averaging] tab, all the images are first added before being processed. Therefore, the output result will be different from when averaging individual images after circular averaging.

## ⑦ Subtracting the background

This processing is meant to subtract background data from the sample data.

- i. In the [Output:] field, select the directory where the calculation result should be output. Click  or edit directly.
- ii. In the [Background file] field, select the file to be treated as “background”. You can either click [Browse] to select the file or drag & drop it here. Input one single background file.
- iii. In the [Sample file] field, select files from which you want the background to be subtracted. You can select as many files as you want at once (no limits in principle). Click [Browse] to select files, but in case you select multiple files, use the ctrl or the shift key. You can also drag & drop files here. Select multiple files at once and drop them.
- iv. Both on the [Background] and [Sample] fields, you can multiply the scattering intensity by a value if you enter it in the [Multi.] section. This value is normally set to 1.000. Moreover, for the [Sample], you can also enter [Conc. (sample concentration)]. The value of concentration will be recorded in the header of the output file and will be automatically loaded during the analysis later.
- v. If you want to perform calculation taking into account the partial specific volume, check the [Use PSV] box. Although the default value of partial specific volume is 0.734 with BSA (bovine serum albumin), it is also possible to modify this value. In case you subtract the background taking account of the partial specific volume, you need the exposure data of an empty cell (sample cell without anything inside). Prepare and select a file measured and normalized under the same condition as the sample. You can select a file by clicking [Browse] or drag & drop a file onto this field (absorption correction should also be done in order to subtract the empty cell correctly).

Subtraction

Output: D:\OA\_std\oa\bksub 

Use PSV

Cell file	Conc.	Multi.

PSV: 0.734

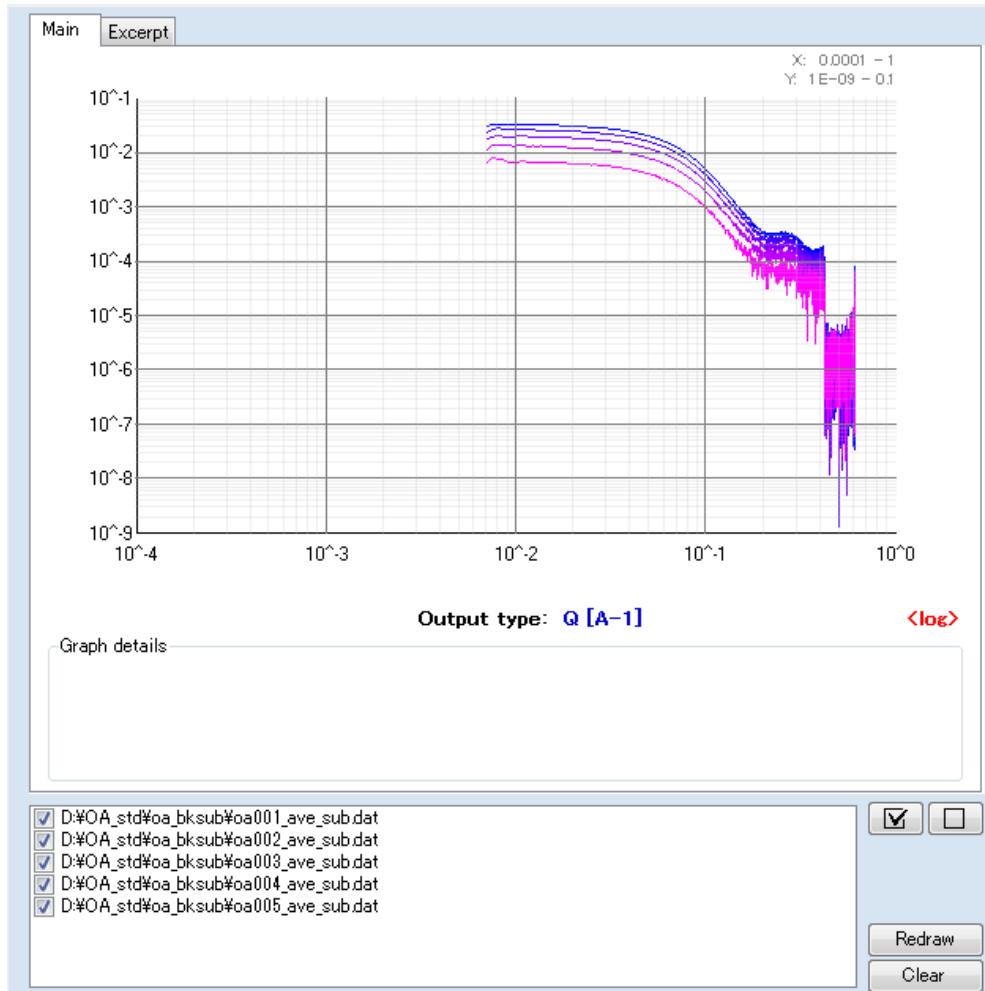
Background file

Background file	Conc.	Multi.
D:\OA_std\oa\oab001_ave.dat	---	1.000

Sample file

Sample file	Conc.	Multi.
D:\OA_std\oa\oa001_ave.dat	5.000	1.000
D:\OA_std\oa\oa002_ave.dat	4.000	1.000
D:\OA_std\oa\oa003_ave.dat	3.000	1.000
D:\OA_std\oa\oa004_ave.dat	2.000	1.000
D:\OA_std\oa\oa005_ave.dat	1.000	1.000

- vi. When preparation is complete, click [Subtract]. The files will be output and the graph will be displayed in double logarithmic plot. The graph can be enlarged or reduced by scrolling the mouse or by dragging the range to be enlarged by a left-click. You can select the graph to be displayed by checking or unchecking the box below and clicking [Redraw]. Use [Clear] to delete all. You can also display the graph by dragging & dropping the dat file onto this field.

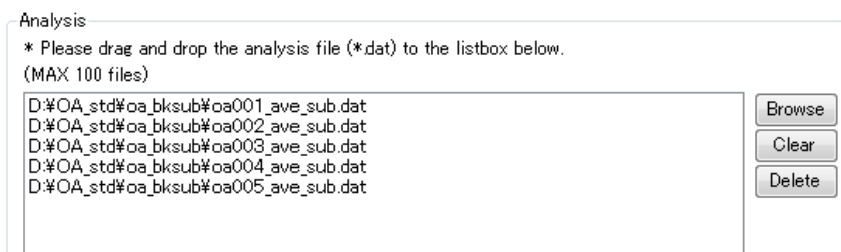




## ⑧ Analysis

The Analysis tab is used to conduct analysis by creating a graph from obtained data.

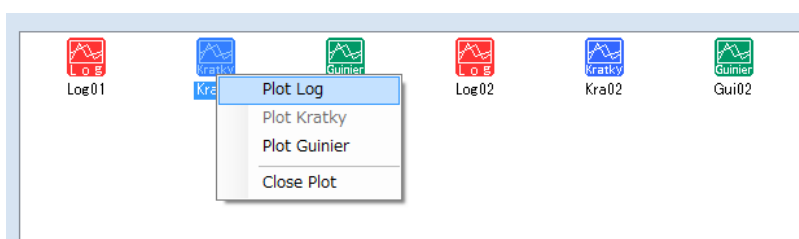
- i. First, load the data. If you select multiple data sets and load them using the [Browse] button, make selection using the ctrl key or the shift key. You can also drag & drop files onto this field. Select multiple files at once and drop them (maximum 300 files).



- ii. Currently, with SAngler, it is possible to create three types of plots;
  - 1) "Log plot (LogY)", which displays in the form of a common logarithm the scattering intensity  $I$  on the Y axis,
  - 2) "Guinier plot", indicating as natural logarithm the scattering intensity  $I$  on the Y axis with the X axis showing the squared value,
  - 3) and "Kratky plot", made by multiplying the scattering intensity  $I$  on the Y axis by the square of the X-axis value.

By clicking each button, the graph will be shown on a separate window. As the selected file list remains until you push the [Clear] button, you can display three graphs in turn. If you want to modify the list, clear it by pushing [Clear] and load a new file.

- iii. When the graph is displayed, it will also be shown in the form of an icon in the right of the main window. If you right-click this icon, you can close the graph or create a new graph with a different format. In this way, you can create multiple graphs. Next, the functions available at each plot window will be explained in turn.

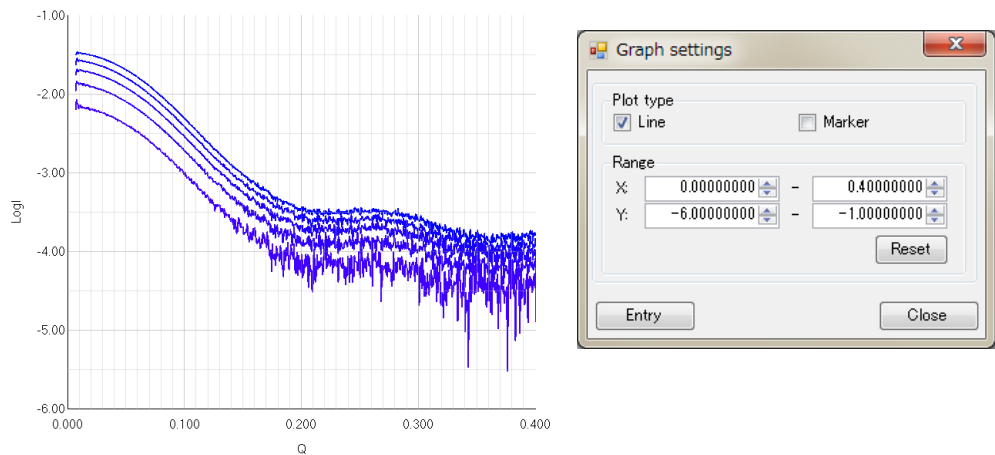


### (1) "LogY plot"

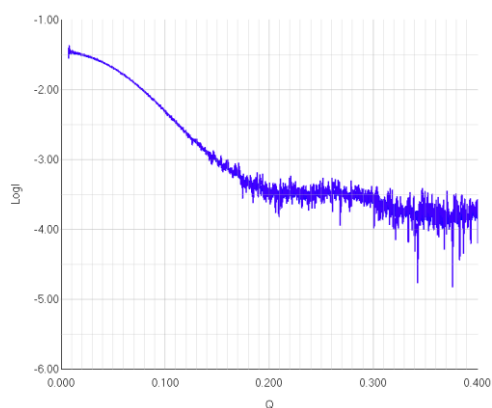
- A) A graph is displayed in the left of the newly opened graph window, while the file list is shown in the right. The list is composed of [Factor] and [Conc.]. In the [Conc.], the value of concentration inputted at the subtraction is loaded. If you enter a value in [Factor], you can multiply it by the Y-axis value. In addition, when normalization is carried out as explained later, the normalization factor will be automatically displayed. Enlarge or reduce by scrolling the mouse pointer on the graph. In

addition, if you select the range of display by dragging with a left-click, you can enlarge it. You return to the original state by right-clicking. If you want to add data to the graph, you can do it either by loading data from the [File] - [Input] buttons in the file menu or dragging & dropping the data onto the file list field in the right of the window.

- B) With the [View] - [Graph settings] button located in the file menu, you can set the plot style or the display range of the X and Y axes of the graph. Click [Entry] after having selected the range.



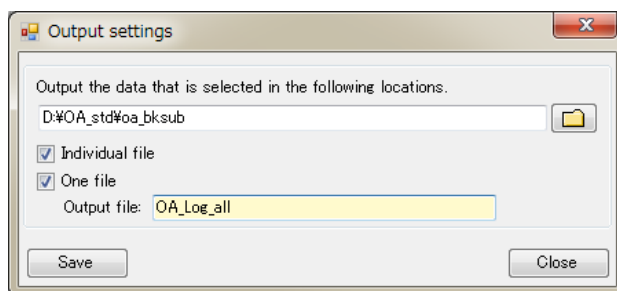
- C) With the [Tools] - [Normalization] button, values on the Y axis can be normalized. With [Scale], the data on the Y axis in the selected range of the X axis will be used for normalization. If [Concentration] is selected, the value in the [Conc.] will be used for normalization. **The normalization will be performed using as reference the data marked with ● in the [STD] section in the file list.**



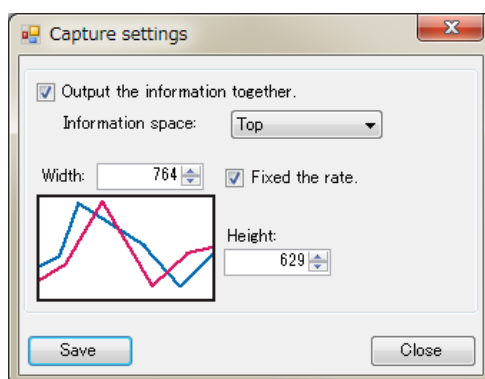
- D) A dialog will be displayed if you select [Scale]. If you select [Normalization by the peak value in the displayed range], normalization will be performed using the peak value in the displayed range of the graph. If you select [Normalization by the values in the specified range], the range of the X axis to be normalized will be

selected and normalization will be performed covering this selected range. If you want to conduct normalization on a specific point of the X axis, enter the same value on the two blanks. The command will be executed if you click [Entry]. This is the same with [Concentration]. You get back to the original state by executing [Reset].

- E) It is possible to output the data in the displayed graph. Execute the command with the [File] - [Output] - [Output data file] buttons. Set the output directory, and check either or both of the [Individual] option, used to output each data separately, or the [One file] option to record on one single file in order. When selecting [One file], enter the file name. The data will be output by pushing [Save]. In the file, the X-axis value, the scattering intensity  $I$ , the standard error of the scattering intensity  $\sigma I$ , the X-axis value in the graph, the common logarithm of the scattering intensity  $\text{Log } I$ , and the standard error of  $\text{Log } I$ ,  $\sigma \text{Log } I$ , are recorded in this order. Moreover, in case normalization has been performed, the values after normalization are also output as  $\text{Log } I_{\text{norm}}$  and  $\sigma \text{Log } I_{\text{norm}}$ .

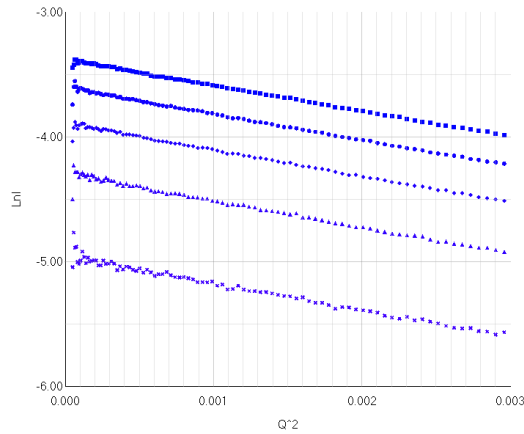


- F) It is also possible to output the displayed graph as an image (jpg). Execute [File] - [Output] - [Capture image]. In the dialog, if you want to save the file list together as part of the image, check [Output the information together]. In order to save the aspect ratio of the image, the “Fixed the rate” box is checked in the default setting. In order to enlarge the image size and save it, modify the width or the height (number of pixels). When setting is complete, click [Save]. Next, after you select where to save the image file, it will be output.

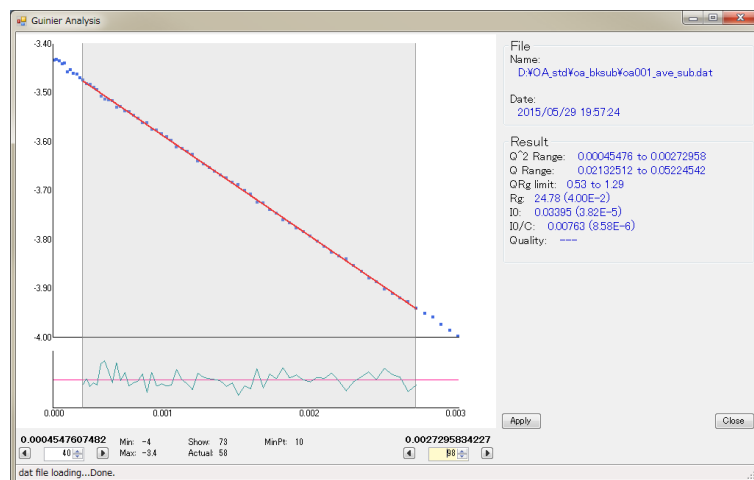


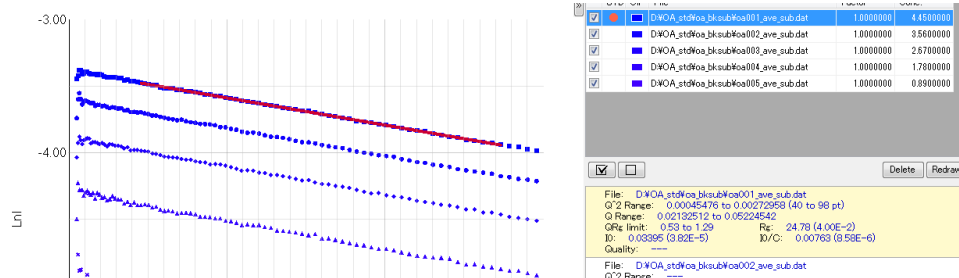
(2) "Guinier plot"

A) Although its basic function is the same as that of Log plot, Guinier is equipped with functions that can be used for Guinier analysis (linear approximation). First, set the display range and enlarge the small-angle region. In addition, it may be better to adopt [Marker] for the display style, as linear approximation will be performed.

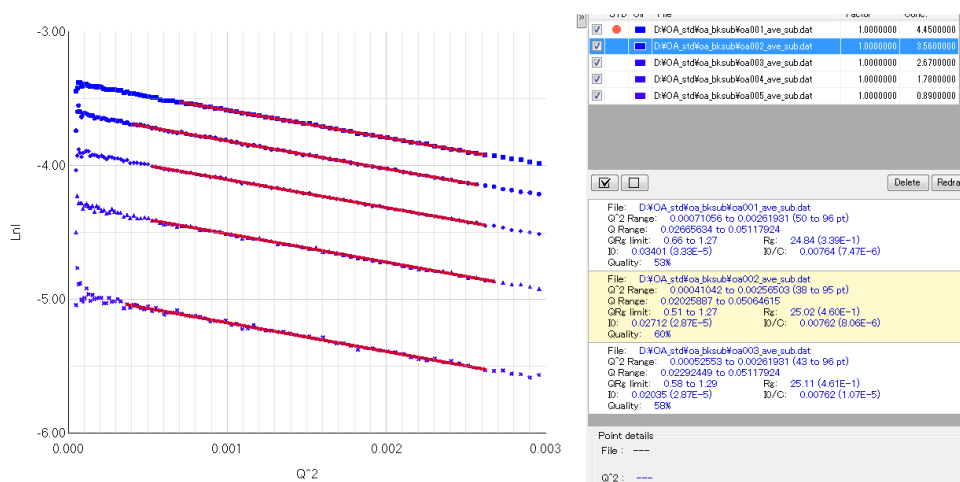


B) Execute [Tools] - [Guinier Analysis]. There are in total three menus, namely [Manual], [Auto(autorg)] and [All auto(autorg)]. In the file list field, select and right-click the data, and the same menu will be displayed. If you select [Manual], a separate window for manual linear approximation will be displayed. Adjust by moving the cursor to determine the starting point and the ending point for linear approximation. The value of  $Q \times R_g$  ( $QR_g$  limit) is also displayed in the right of the window, and the differential values between the straight line and each data point are plotted in the lower side of the graph. Please use these values for reference. When you finish this operation, click [Apply] to close the window. A straight line will appear on the original graph window and the resulting values will also be displayed.





- C) Under the environment where ATSAS has been installed, it is possible to perform analysis using autorg of ATSAS. When you execute [Auto (autorg)], ATSAS autorg will be executed and the result will be displayed in a separate window as in the case of the manual analysis mentioned above. If there is no problem, confirm the result by clicking [Apply]. If you want to modify the result, take the same procedure as in [Manual]. Please be reminded that after the execution of autorg, the “autorg\_csv” folder is created in the same directory as the data, where the output result of autorg (csv file) is automatically saved. In the csv file, information such as the used file,  $R_g$ , the error of  $R_g$ ,  $I(0)$ , the error of  $I(0)$ , the data points of the starting and ending points of linear approximation, quality, and possibly aggregated results have been output. With SAngler, based on this output result,  $I(0)/c$  will be calculated from  $I(0)$  and the concentration, and the range of  $Q^2$ ,  $Q$ , and  $Q \times R_g$  will be calculated from the starting and ending points of linear approximation, and these values will be output on SAngler.
- D) Autorg can be applied to all data. If you execute [All auto(autorg)], autorg will be executed on all the data in the file list and the result will be displayed.



- E) When analysis is complete, you can output images of the data or the graph as with Log plot. If linear approximation has been carried out, the X and Y coordinates of the starting and ending points of the approximated straight line has been output,

together with such information as the X-axis value on the graph, the scattering intensity  $I$ , the standard error of the scattering intensity  $\sigma I$ , the square of the X-axis value, the natural logarithm of the scattering intensity  $\ln I$ , and the standard error of  $\ln I$ ,  $\sigma \ln I$ . Therefore, if you create a Guinier plot using the square of X and  $\ln I$  on graphic software like Excel, and connect two points with a straight line adding the data on the starting and ending points of this line, it is possible to superimpose the approximated straight line on the experiment data just as in SAngler. Moreover, in case of Guinier, it is possible to output the result of Guinier analysis in the form of a table. With [File] - [Output] - [Output result data] button,  $R_g$ ,  $I(0)$  and  $I(0)/c$  values, as well as information on the range of linear approximation (ranges of Q,  $Q^2$  and  $QR_g$ ) will be output. Autorg also outputs information identifying aggregation such as “Possibly aggregated”. On the table, the display will be either “0= None” or “1=Possibly aggregated”.

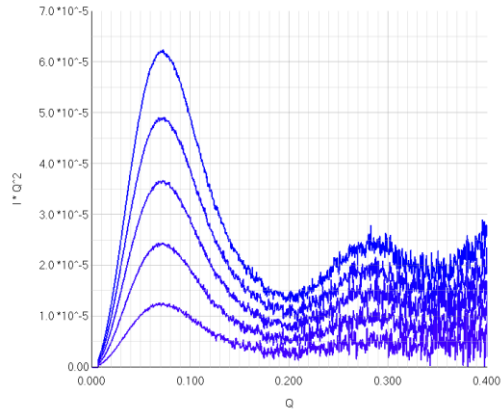
# No.	Q^2Start	Q^2Last	QStart	QLast	QRgLimitM	QRgLimitMConc	Rg	dRg	I0	dI0	I0/Conc	dI0/Conc	Quality	Aggregated	
1	0.000711	0.002619	0.026656	0.051179	0.96	1.27	4.45	24.84	3.39E-01	0.03401	3.33E-05	0.00764	7.47E-06	0.53	0
2	0.00041	0.002565	0.020259	0.050646	0.51	1.27	3.56	25.02	4.60E-01	0.02712	2.87E-05	0.00762	8.06E-06	0.6	0
3	0.000526	0.002619	0.022924	0.051179	0.58	1.29	2.67	25.11	4.61E-01	0.02035	2.87E-05	0.00762	1.07E-05	0.58	0
4	0.000526	0.002674	0.022924	0.051712	0.56	1.3	1.76	25.23	6.74E-01	0.0136	3.00E-05	0.00764	1.66E-05	0.52	0
5	0.000368	0.002619	0.019193	0.051179	0.49	1.3	0.89	25.39	9.72E-01	0.00702	2.31E-05	0.00766	2.60E-05	0.55	0

※1) On the output data, the coordinates of the starting and ending points of the approximated straight line are expressed in the form of data rows as LineX and LineY. In each of these data rows, values are included in the first two points, representing the starting point and the ending point. Meanwhile, values from the third points are “zero” for both data rows. This is a measure taken to smoothly load data simultaneously with other data rows in such software as Igor pro, and these zeros mean nothing. Please delete them after loading.

※2) Depending on the conditions of the data, the “autorg” analysis may fail and output no data. Sometimes you may obtain ambiguous results which make you wonder why you have succeeded here and failed there. In such cases, perform analysis in the “manual” mode.

### (3) “Kratky plot”

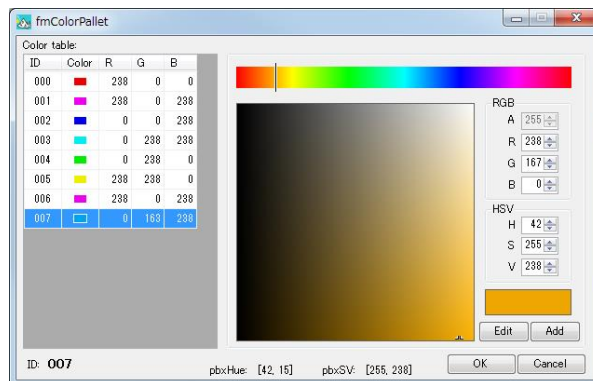
A) The functions for the Kratky plot is basically the same as those for LogY plot. Refer to the explanation on the LogY plot for operation.



### ✘ How to modify the graph colors

Although the colors of the graph are set to be automatically attributed by the software, they can be modified manually following the procedure below.

- (1) On the file list in the right side of each graph window, click the [Clr] (color) button of the data that you want to modify, and a separate window [fmColorPallet] will appear.



- (2) In the left side, the color table used for the current graph is displayed. If you want to replace the graph color with the one proposed in the table, click the color (ID) and then [OK].
- (3) If you want to add another color, adjust the color on the palette in the right and click [Add]. Then, the newly created color is added to the table in the left. Select it and click [OK] as in (2).
- (4) If you click [OK], you will be asked "Regist Color table data?" Click "Yes".
- (5) The color has now been modified.

iv. Information on linear approximation and errors

Below is supplementary information on the calculation of linear approximation and errors of various values in Guinier analysis. All these values have been obtained according to the law of propagation of errors. In addition, it has been confirmed that the output results are completely the same as with PRIMUS.

(1) Regarding linear approximation

Perform regression analysis using the primary linear regression line  $y=A+Bx$  in case  $y_i$  has an error of  $\sigma_i$  at the data point  $(x_i, y_i)$ . Consider the weight of each data point  $\omega_i$  depending on the errors.

$$\omega_i = \frac{1}{\sigma_i^2}$$

$$\Delta = \sum \omega_i \sum \omega_i x_i^2 - \left( \sum \omega_i x_i \right)^2$$

If we make definitions as above, the values A and B can be obtained with the following formulas.

$$A = \frac{1}{\Delta} \left( \sum \omega_i x_i^2 \sum \omega_i y_i - \sum \omega_i x_i \sum \omega_i x_i y_i \right)$$

$$B = \frac{1}{\Delta} \left( \sum \omega_i \sum \omega_i x_i y_i - \sum \omega_i x_i \sum \omega_i y_i \right)$$

In addition, the respective errors for the values A and B,  $\sigma_A$  and  $\sigma_B$ , can be described as below.

$$\sigma_A = \sqrt{\frac{\sum \omega_i x_i^2}{\Delta}}$$

$$\sigma_B = \sqrt{\frac{\sum \omega_i}{\Delta}}$$

In Guinier analysis, from the following formula of Guinier approximation,

$$\ln I(q) = \ln I(0) - \frac{R_g^2}{3} q^2$$

The  $I(0)$  value is obtained from the Y intercept  $A = \ln I(0)$ , and the  $R_g$  value is calculated from the slope  $B = -R_g^2/3$ .

$$I(0) = e^A$$

$$R_g = \sqrt{(3 \times |B|)}$$

The error for each value can be expressed as follows.

$$\sigma I(0) = e^A \times \sigma_A$$



$$\sigma R_g = \frac{1}{2} (3 \times |B|)^{-\frac{1}{2}} (3 \times \sigma_B)$$

- (2) The errors of the common logarithm and natural logarithm of the scattering intensity  $I$ , respectively  $\text{Log}I$  and  $\text{Ln}I$ , can be described as follows according to the law of propagation of errors.

$$\sigma \ln I = \frac{\sigma I}{I}$$

$$\sigma \log I = \frac{\sigma I}{(I \times \ln 10)}$$

- (3) Source books and websites

We have used following textbooks and sites for reference.

• *Introduction on Error Analysis in Measurement*, John R. Taylor, translated by Shigeo Hayashi and Ryo baba, Tokyo Kagaku Dojin Publisher

• *(Illustration) Exercises on calculus*, edited by Yoshiya Suzuki et al, Kyoritsu Shuppan Co., Ltd.

• Web page on the “Uncertainty of chemical standard materials”, managed by Toshihide Ihara, chief researcher at the Measurement Standards System Division, Metrology Institute of Japan, National Institute of Advanced Industrial Science and Technology (AIST), and Shigemitsu Shin, technical staff member at the Surface and Thin Film Standards Section, Materials Characterization Division, Metrology Institute of Japan.

<https://staff.aist.go.jp/t.ihara/>

※ These references have been very useful. We are deeply grateful.

#### 4. Reference for publishing

- ① We are now preparing a paper on SAngler for submission. We will update the information immediately if it is accepted. On the other hand, if you present a paper using SAngler in the meantime, indicate the following page as reference.

<http://pfweis.kek.jp/~saxs/software/SAngler.html>

- ② Do not forget to refer to ATSAS if you have used ATSAS autorg in Guinier analysis. Describe that you have used SAngler to make the data one-dimensional and subtract the background and ATSAS for Guinier analysis.

Petoukhov, M.V., Franke, D., Shkumatov, A.V., Tria, G., Kikhney, A.G., Gajda, M., Gorba, C., Mertens, H.D.T., Konarev, P.V. and Svergun, D.I. (2012)

New developments in the ATSAS program package for small-angle scattering data analysis. *J. Appl. Cryst.* **45**, 342-350.



※The above message will be displayed if SAngler is linked to ATSAS.

## 5. Supplementary information

- ① For SAngler, Nobutaka SHIMIZU has been in charge of the specification and concept of the program, as well as the determination of calculation and processing methods in each stage, while Ms. Keiko YATABE has made the program. We have confirmed whether calculation has been performed as specified by correlating the processing results in each stage with the results processed without SAngler using Igor pro or Excel. Moreover, using as reference PETRAIII, ATSAS (mainly PRIMUS and autorg) developed by Dr. D. I. Svergun's group at EMLB (European Molecular Biology Laboratory), as well as the Igor Pro macro Nika developed by Dr. Jan Ilavsky at APS (Advanced Photon Source), we have confirmed that the results obtained with SAngler are consistent with the output results obtained with these other software.

[ATSAS software]

<http://www.embl-hamburg.de/biosaxs/software.html>

["Nika" package of 2D -> 1D SAS data reduction macros for Igor Pro]

<http://usaxs.xray.aps.anl.gov/staff/ilavsky/nika.html>

- ② Although the program is far from perfect, especially in terms of the graph display functions, we will continue improving it on the basis of the current version. Please contact us by email if you have questions about the program or if you find bugs or other problems.
- ③ Please contact us if you want some plots to be newly added to the [Analysis] tab.
- ④ The program is displayed in English. However, there are some dialogs where users are asked to select between yes and no. As these messages depend on the OS language, you will see [Hai (yes)] and [Iie (no)] on the Japanese OS, while the English OS displays [Yes/No].

- ⑤ Currently, there are only quadrangular masks. We are now working to create circular, polygonal and other masks. Please wait for a moment.
- ⑥ PILATUS is scheduled to be compatible with the cbf format from fall 2015 or later.

## 6. Change log

- ① The software version updated to 1.1.6 on October 15<sup>th</sup>, 2015.
  - Since the description way of a system file was changed, it became impossible to start software by the system file of version 1.0.0. To update a program, it is necessary to replace the whole program folder.
  - The function of making the mask region of a circle and a polygon is available.
  - In the Analysis tab, the color pallet is installed in order to be able to change the display color of a graph arbitrarily.
- ② The software version updated to 1.1.12 on November 12<sup>th</sup>, 2015
  - The function of regulation of the brightness of the image in a mask drawer.
  - It supports CBF format of PILATUS.
  - A correction of the position of icons of Kratky and Guinier on the Analysis tab.
  - The modification of management of a mask data.
  - The modification of display of a file name in ListView of Averaging.
  - The addition of Detector tab.