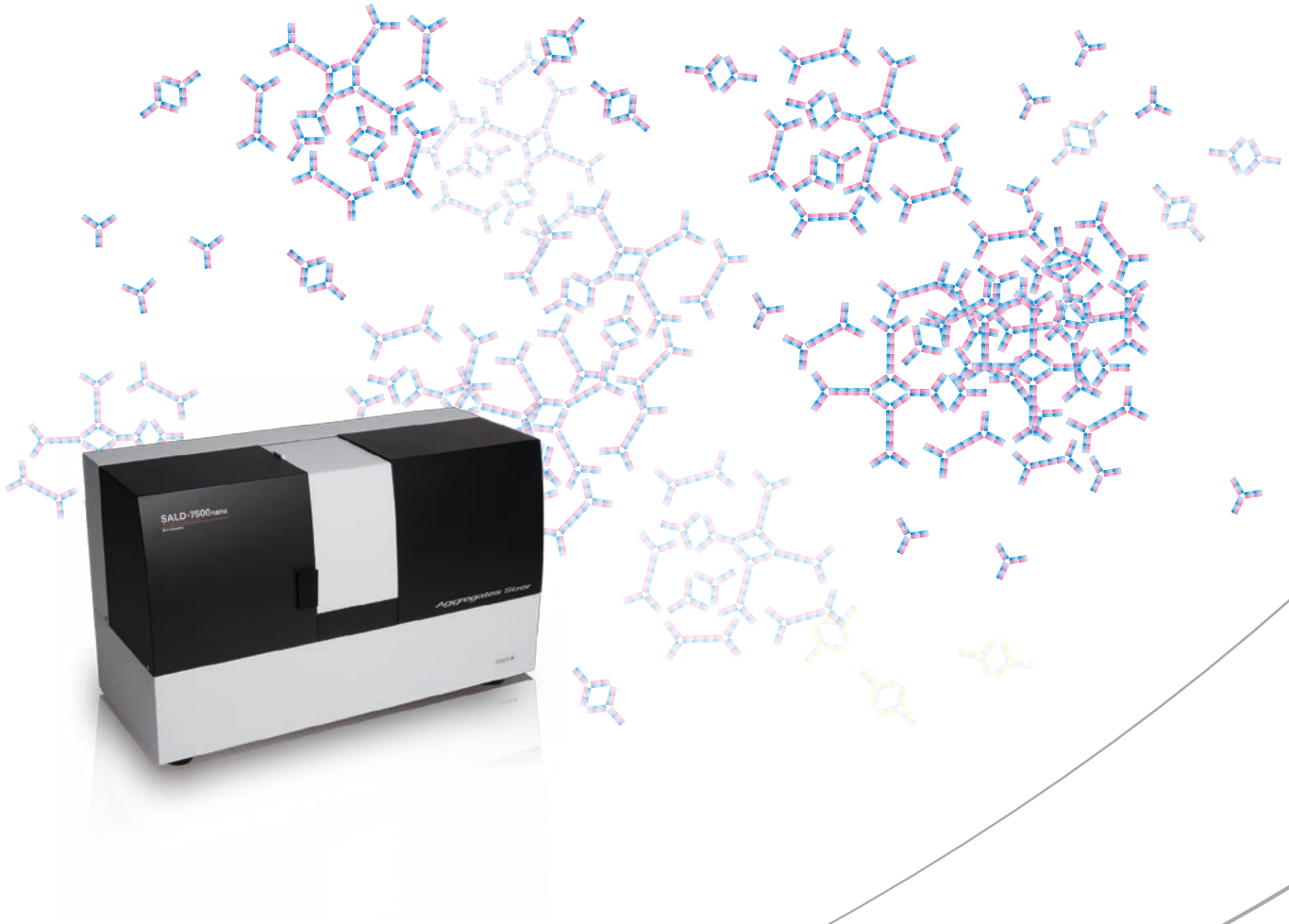
C060-E010A

SHIMADZU
Excellence in Science

Aggregation Analysis System for Biopharmaceuticals

Aggregates Sizer

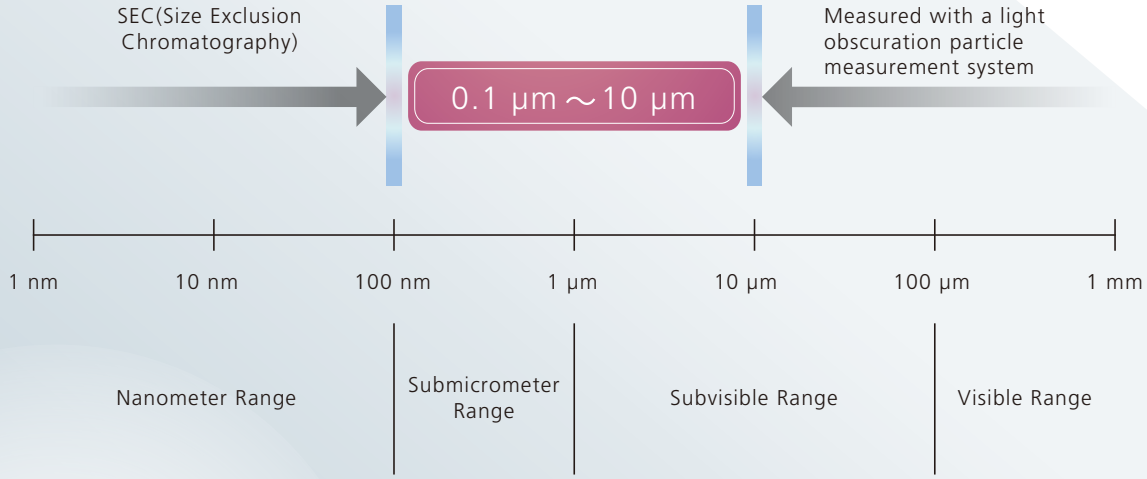


Measures the concentration of 100 nm to 10 µm aggregates of biopharmaceuticals

The Aggregates Sizer aggregation analysis system enables the quantitative evaluation of particle amounts in the 0.1 µm to 10 µm range as a concentration (units: µg/mL or particles/mL)*1.
Aggregations of biopharmaceuticals can be categorized*2 into 4 ranges: nanometer (<100 nm), submicrometer (100 nm to 1 µm), subvisible (1 to 100 µm), and visible (>100 µm), according to their particle size.
Until now, particles in the submicrometer to subvisible portion of this range (100 nm to 10 µm) were generally measured by combining multiple methods.

*1 As per the categories in the USP (1787)
*2 In this system, the laser diffraction method is adopted as the principle behind the measurement of the particle size distribution. The concentration (units: µg/mL or particles/mL) is determined after calibration using measurement results of polystyrene latex (PSL) standard particles.

Aggregates Sizer by laser diffraction method



1 Quantitative evaluation of aggregate concentrations across a wide particle size range

This system enables the quantitative evaluation of the concentration of aggregates (units: µg/mL or particles/mL) from 100 nm to 10 µm in size.
A wide range of particle sizes from the submicrometer to the subvisible regions can be evaluated with a single system.

2 Measures aggregates with small sample amounts

Measurements can be performed accurately with small sample amounts, using micro cells for 0.125 mL samples.

3 Quantitative evaluation of the aggregation process with the real-time measurement function

Changes in aggregates (particle size or concentration) can be measured and recorded in real time (with a 30 second minimum interval). Further, utilizing a batch cell (sample amount: 5 mL), the aggregation process can be measured while stress is applied by stirring. In addition, stirring rods made of different materials are provided, so the tendency to aggregate due to differences in wetted materials can be evaluated.

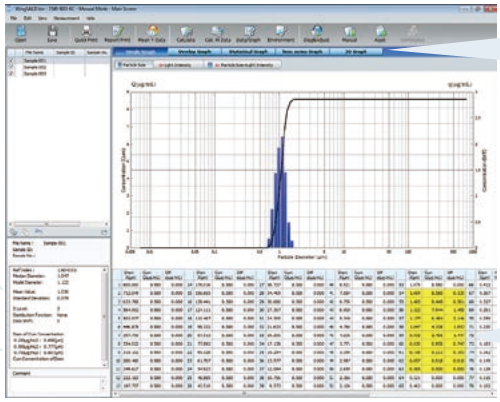
4 Temperature control function (when equipped with the Aggregates Sizer TC temperature control function)

Aggregate changes, which tend to depend on temperature, can be measured while a constant temperature (20 °C to 42 °C) is maintained.

5 Software compliant with FDA 21 CFR Part 11 (optional)

Particle sizes from 100 nm to 10 µm can be measured with a single system

Particle concentrations (µg/mL or particles/mL) can be evaluated quantitatively using WingSALD bio, special software for the Aggregates Sizer.



List of data read on memory Change in data types and Display/Not display can be operated easily.

Changes in the graph, such as overlay and statistical processing, can be easily performed with one click.

Unit of vertical axis is concentration; Q means cumulative amount and q means differential amount.

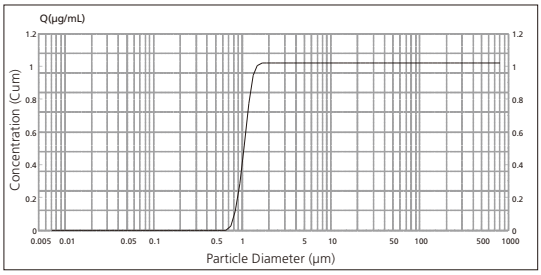
Summarized data such as mean value and standard deviation.

Aggregate concentrations included in user-defined particle size intervals can be displayed.

The position where the particle amount is not 0 can be indicated with yellow color for easy understanding.

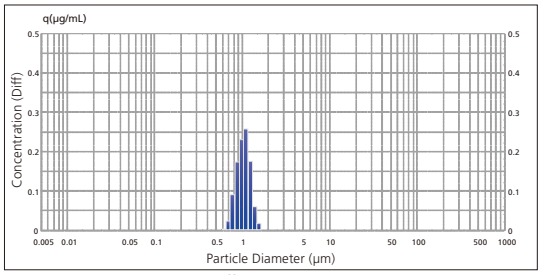
After measurement, the latest data can be added to this list. Confirmation, analysis and comparison can be easily performed.

Cumulative graphs are displayed with polylines. These indicate the total cumulated amount of particles equal to or smaller than the particle size shown on the horizontal axis, representing this as a numerical value Q (µg/mL or particles/mL) on the vertical axis.



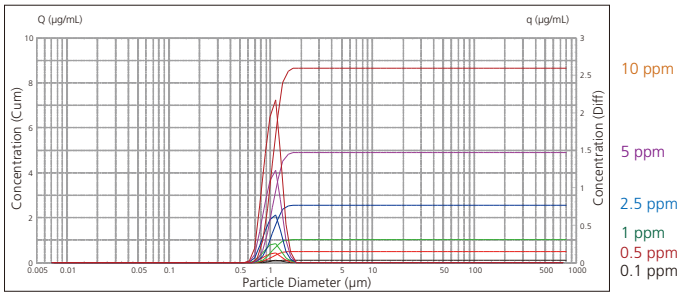
Cumulative graph

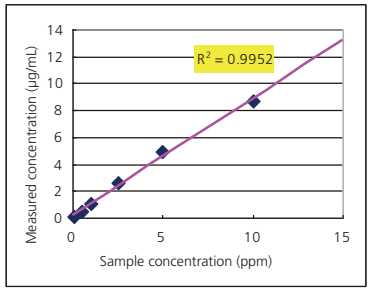
Differential graphs are displayed with histograms. These indicate the amount of particles contained in the particle size range shown on the horizontal axis as a numerical value q (µg/mL or particles/mL) on the vertical axis.



Differential graph

High linearity of concentration





Sample : PSL standard particles of 1 µm
Conditions of concentration : 0.1 ppm, 0.5 ppm, 1 ppm, 2.5 ppm and 5 ppm

Vertical axis indicates the measured concentrations by Aggregates Size and horizontal axis indicates concentration as measurement conditions of 0.1 ppm, 0.5 ppm, 1 ppm, 2.5 ppm and 5 ppm. This graph shows good linearity of concentration measurement by Aggregates Sizer.

High repeatability

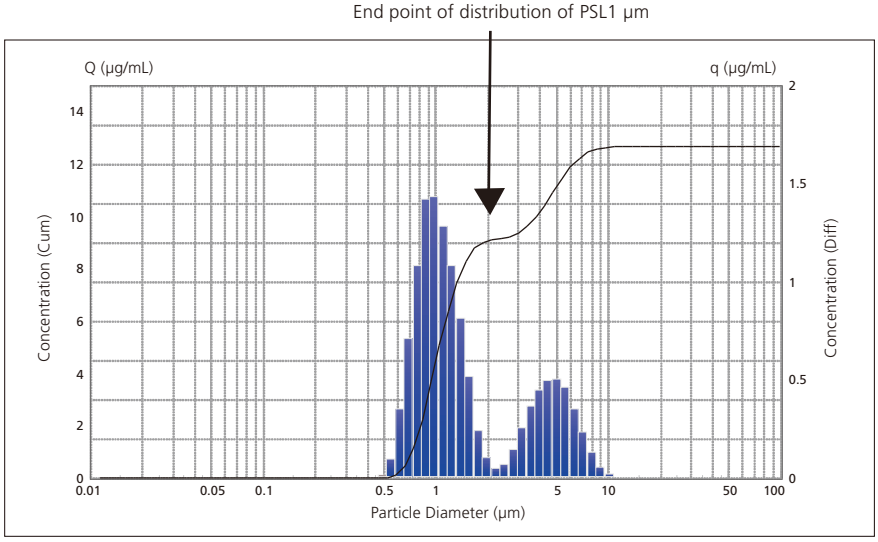
The stable optical system used with the laser diffraction method enables accurate detection of scattered light.

The following table shows measurement results using a PSL standard particle of 1 µm, changing the concentration conditions (0.5 ppm, 1 ppm, 2.5 ppm and 5 ppm) to confirm the repeatability. CV values for all conditions are less than 3%.

Concentration (ppm)	Measured Concentration(µg/mL)					Average	StDev	CV (%)
	1st	2nd	3rd	4th	5th			
0.5	0.540	0.513	0.513	0.511	0.513	0.518	0.012	2.380
1.0	1.024	1.036	1.025	1.025	1.033	1.029	0.006	0.535
2.5	2.566	2.600	2.586	2.586	2.589	2.585	0.012	0.475
5.0	4.900	5.001	5.018	5.035	5.058	5.002	0.061	1.219
						Average CV		1.152

High resolution – Detect multiple peaks accurately

The intensity of scattered light from large particles is high and changes frequently within the forward small angle. In contract, the intensity of scattered light from small particles is very low and changes slowly within the large angle.
Aggregates Sizer uses Wing sensor II, which consists of 78 concentric sensor elements, and the area of the respective element can increase logarithmically from center to outer. Therefore, Wing sensor II can effectively detect the scattered light intensity pattern of a wide particle size range, enabling high resolution.



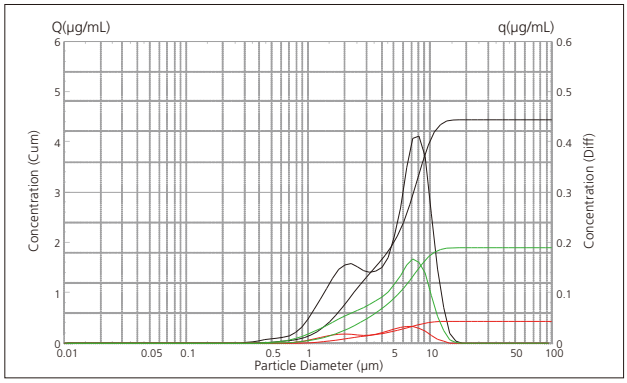
Particle Diam. X(µm)	0.463	0.521	0.585	…	1.875	2.106	2.366	…	9.573	10.756	12.084
Cumulative Q(µg/mL)	0.000	0.012	0.104	…	9.015	9.114	9.157	…	12.645	12.664	12.667

Mixture of PSL 1 µm and 5 µm

From this graph and table, we can determine that the concentration of PSL 1 µm is 9.114 µg/mL and that of PSL 5 µm is 3.553 µg/mL, which can be calculated by subtracting 9.114 from total value of 12.667 µg/mL.

The particle amount can be quantitatively evaluated in the 100 nm to 10 μm range

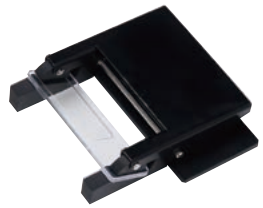
Changes in particle size distribution of γ globulin by changing pH



This graph shows the measurement results of γ globulin, which is dispersed by pure water, phthalate buffer solution (pH4) and phosphate buffer solution (pH7.4).
Concentration conditions are 1 mg/mL. The size of aggregation is mainly distributed from 1 μm to 10 μm. In the case of pure water, the concentration is about 4.4 μg/mL, the largest value. In the case of phosphate buffer solution, the concentration is about 0.4 μg/mL, the least value. This ratio is more than 10 times.

Measurement with small sample amounts

Micro cell
Measurement of 0.125 mL samples is possible.



Time series changes of aggregations can be confirmed quantitatively

Continuous measurements with a minimum interval of 30 seconds

With the high-speed measurement function, a feature of the Aggregates Sizer, changes in aggregates (size or amount) can be quantitatively confirmed at a minimum interval of 30 seconds. As a result, not only the status before and after the change, but also the process as it happens can be observed, enabling evaluation of the speed of the change. Conversely, continuous measurements can be used to evaluate the absence of change, confirming that a sample is stable.

POINT

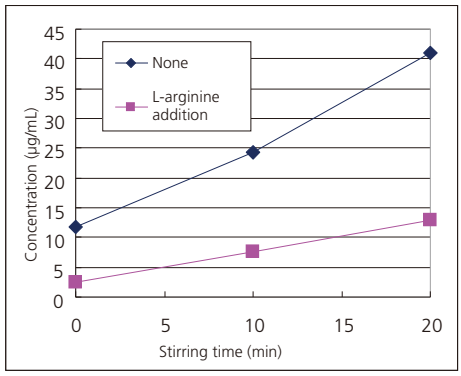
1. A single laser light source system has been adopted, enabling high-speed measurements with a minimum interval of 30 seconds. Time series changes in protein aggregations can be evaluated.
2. Using a batch cell, biopharmaceuticals can be measured while they are stirred. The aggregation of biopharmaceuticals has been reported to increase due to stirring. Accordingly, the system can also be used for properties evaluation screening with respect to the aggregation of biopharmaceuticals.

Accelerated test of aggregations by mechanical stimulus

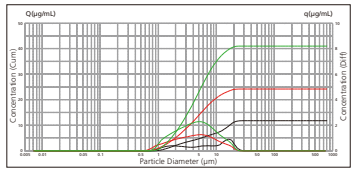
Several days aggregation analysis can be reduced easily and dramatically

Changes in samples caused by mechanical stimulus using the batch cell's stirring function can be observed. This system enables accelerated test without additional equipment and software, and this process can be used for the screening of proteins to confirm the properties of aggregations.

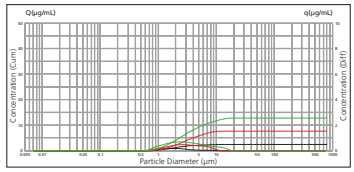
Inhibition effect to prevent aggregations by the L-arginine addition



Relationship between the concentration of aggregations and stirring time



Without L-arginine addition

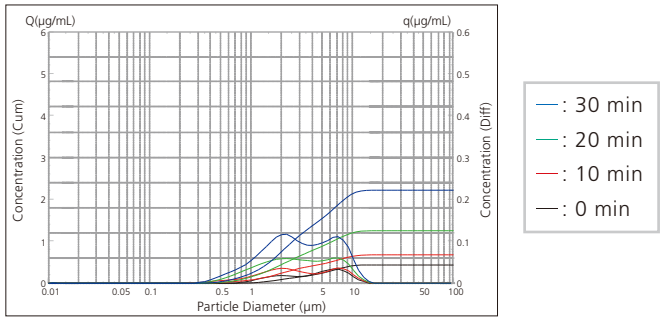


L-arginine addition 100 mM

The batch cell's stirring function can accelerate the aggregation and reduce the total observation time of this kind of experiment.

This graph shows the relationship between the concentration of aggregations and stirring time.

Accelerated testing of aggregations by mechanical stimulus



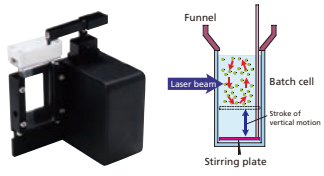
This graph shows the time series changes in particle size distribution and amount when gamma globulin is dispersed by a phosphorus acid buffer solution (PH 7.4), and is stirred in an Aggregates Sizer batch cell using the stirrer mechanism provided as standard.

The concentration of aggregate particles was approximately 0.4 μg/mL when unstirred (0 min). However, stirring for 30 minutes resulted in more than a five-fold increase to approximately 2.2 μg/mL.

Evaluation of the tendency to aggregate using the stirring function

Batch cell

Mechanical stimulation can be applied to samples by the vertical motion of the stirring plate. (Required liquid volume: 5 mL)

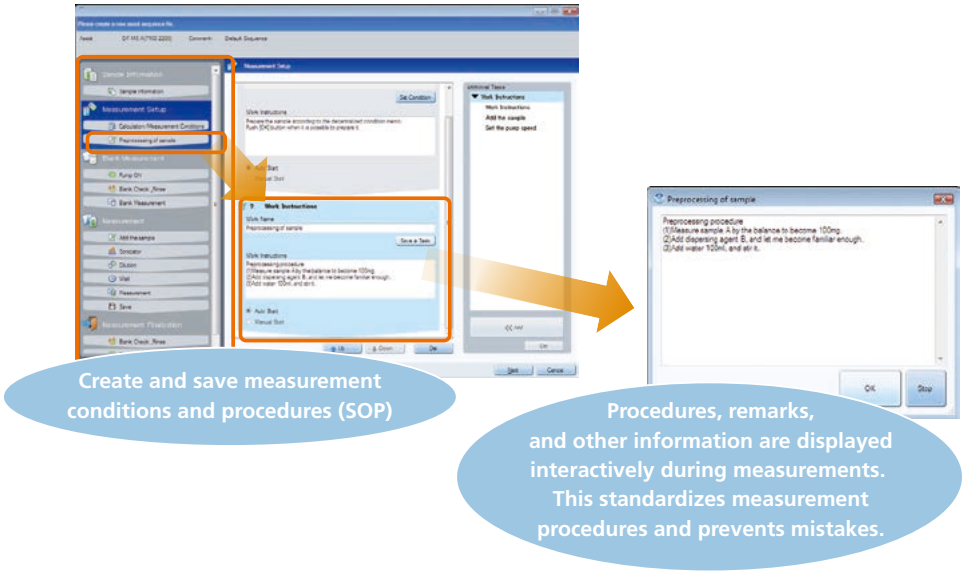


Features

Measurement Assistant Function Allows Preparing an SOP to Ensure Measurements Are Always Performed Using the Same Conditions and Procedures

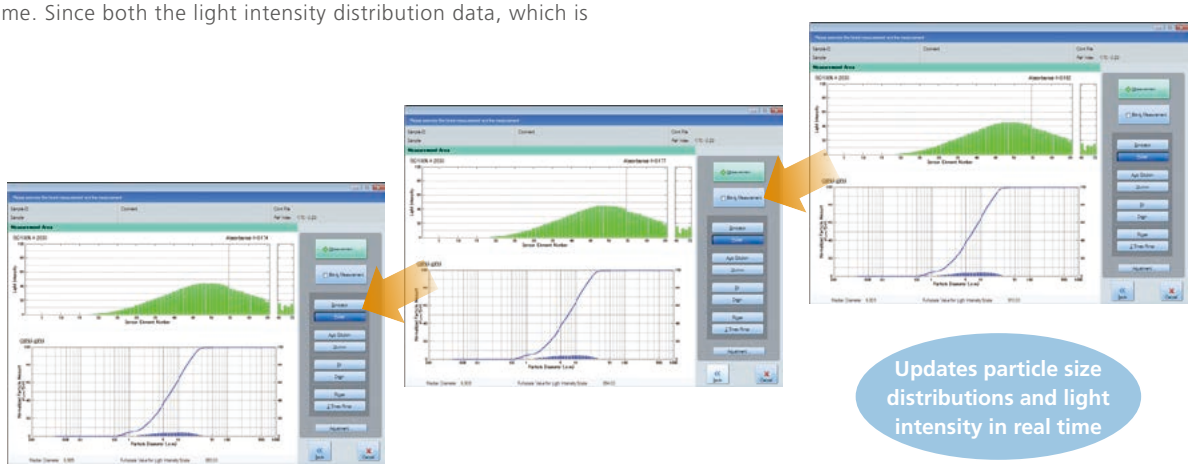
Creating, saving, and sharing measurement conditions and procedures, including pretreatment methods and conditions, ensures measurements are performed using the same conditions and procedures, even if performed by a different operator or at a different location or plant, and allows safely comparing data. Furthermore, when the measurement assistant function is used, instructions for the operator are displayed on the screen. This enables even inexperienced operators to perform measurements correctly.
In addition, administrators and operators can be assigned different operating privileges to ensure security.

Note: SOP is an acronym for Standard Operating Procedure.



Monitors Changes in Sample Status in Real Time
– Evaluation of the Aggregate Production Process from Two Aspects –

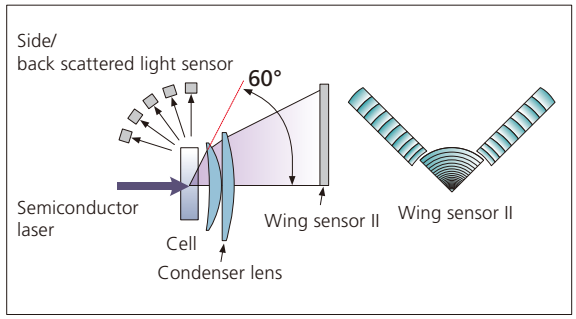
Particle size distribution data and light intensity distribution data can be displayed in real time. This means that sample changes over time or shifts in the dispersion status can be monitored in real time. Since both the light intensity distribution data, which is the raw data, and particle size data can be monitored simultaneously, they can be compared to keep track of any changes in the status of samples.



Seamless measurement over the entire range using a single measurement principle, single optical system, and single light source. The SLIT optical system continuously captures forward-scattered light at up to 60° on a single detection plane

The target particle size range is covered using a single measurement principle, single optical system, and single light source to achieve a perfectly seamless single wide range. Accurate particle size distribution measurements are possible across the entire measurement range using a single standard, as the instrument does not incorporate multiple optical systems that create discontinuities in the data.

The application of the SLIT optical system, based on sophisticated scattered light intensity tracing technology, smashes conventional wisdom to continuously capture forward-scattered light at up to a wide 60° angle on a single-detector face. This achieves high resolution in the fine particle region.



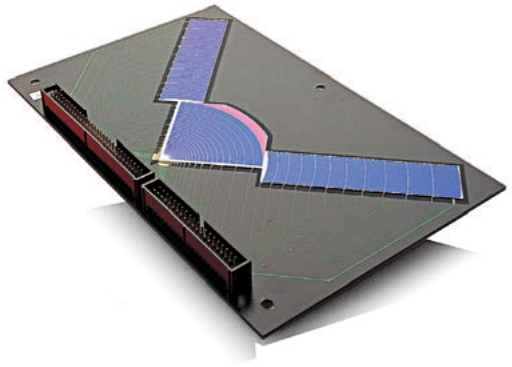
SLIT (Scattered Light Intensity Trace)

More Stable Optical System

The Omnidirectional Shock Absorption Frame (OSAF) is employed to fully isolate all elements of the optical system from the disturbances of shocks and vibrations.

High-Resolution/High-Sensitivity Wing Sensor II

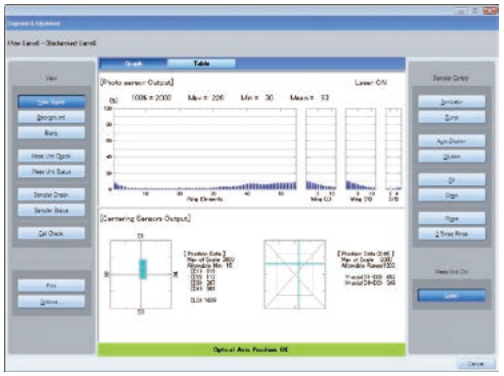
Forward diffracted/scattered light is detected by a "Wing sensor II", a 78-element sensor that was developed using semiconductor manufacturing technology of the highest level. This sensor can detect greatly fluctuating small-angle forward scattering with a high level of resolution and wide-angle scattering of low optical intensity with a high level of sensitivity. Also, side scattered light is detected by 1 sensor element and back scattered light is detected by 5 sensor elements. Accurately capturing light intensity distribution patterns with a total of 84 sensor elements enables the high-resolution, high-precision measurement of particle size distributions over a wide particle diameter range.



Wing sensor II

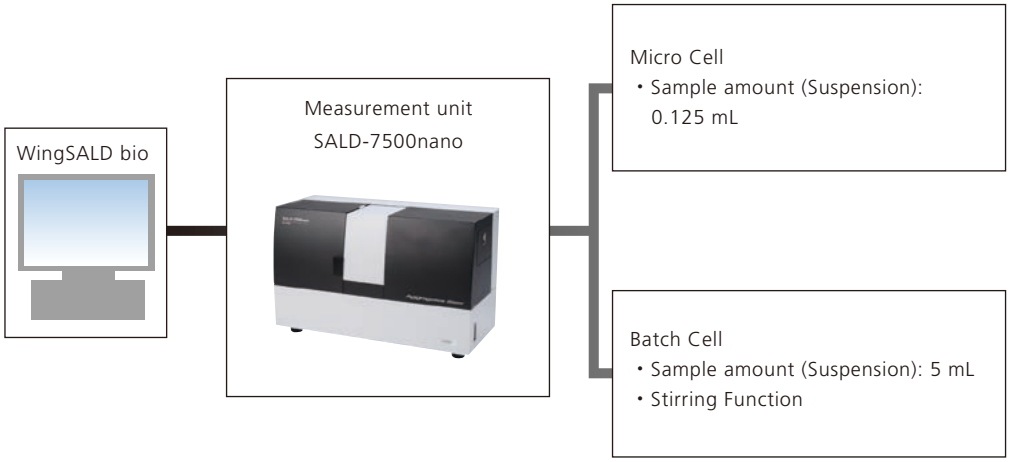
Self-Diagnostic Functions Ensure Easy Maintenance

These analyzers incorporate powerful self-diagnostic functions. The output signals sent by the sensors and detecting elements, and the instrument operating status, can be checked, facilitating easier maintenance. Using the Operation Log function, detailed information about, for example, the instrument usage status and contamination of the cells is included with all the measurement data, making it possible to investigate the validity of measurement data obtained in the past.

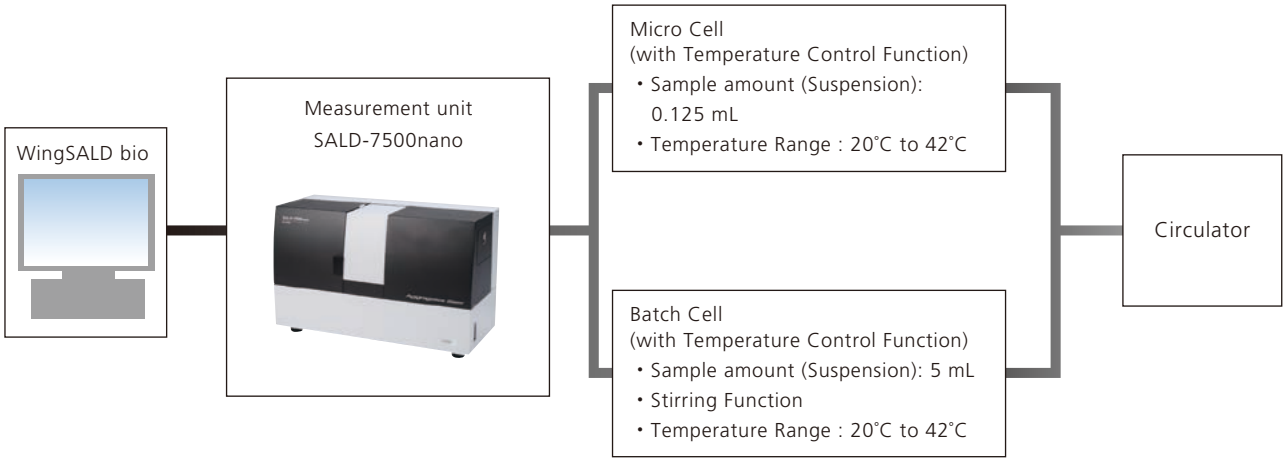


System Configuration

Aggregates Sizer



Aggregates Sizer TC (with Temperature Control Function)

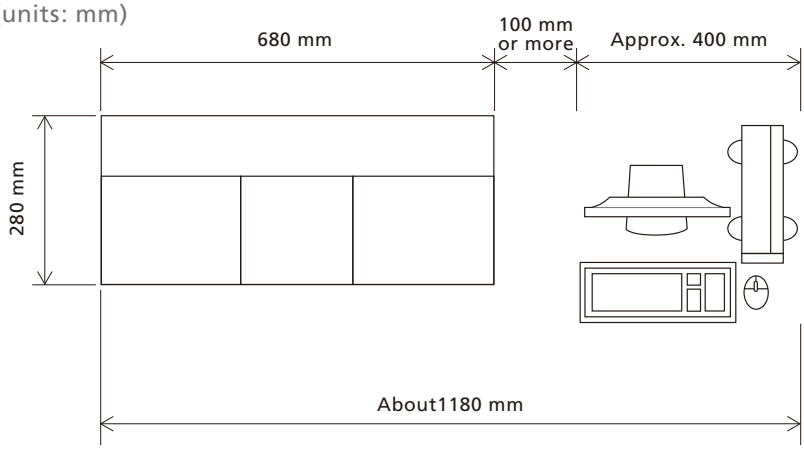


Nano Particle Size Analyzer

SALD-7500nano

- Violet semiconductor laser (wavelength: 405 nm) is used for the semi-permanent light source. Maintenance, such as gas replacement, is unnecessary.
- The detector incorporates 78 elements at the front, 1 element at the side, and 5 elements at the back, for a total of 84 elements. Additionally, high-sensitivity light receptors that support Violet semiconductor laser wavelengths are adopted with all detectors.
- The fixed parts of the cell and cell holder can be pulled out at the front of the unit using a slide mechanism, as shown in the photo on the left. This makes it easy to mount and replace cells, and to perform maintenance.
- WingSALD bio standard software is supplied as standard. It offers versatile data processing and simple, high-speed operation to suit every purpose and processing requirement.

External Dimensions (units: mm)



Measurement of 0.125 mL samples is possible

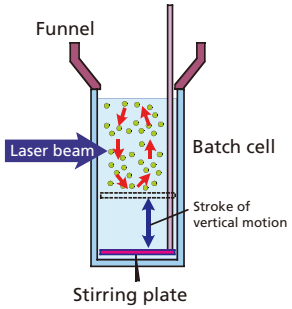
Micro cell

Micro cell

- Measurement is possible with small amounts of sample.
- The micro cells, made of quartz glass, can be cleaned with chemical agents as well as ultrasonic cleaning.

Optimal for evaluating the tendency to aggregate due to stirring stress

Batch Cell



- The tendency to aggregate can be evaluated by accelerating aggregation through application of mechanical stimulation using the vertical motion of the stirring plate.
- Three stirring plate materials are provided, so that the tendency to aggregate due to differences in wetted materials can be evaluated. (Stainless steel, glass, and polyether ether ketone (PEEK))
- With the funnel, sample handling is easy, with minimal concerns about sample spillage.

Specifications

Hardware

General Specifications

Measurement Principle	Laser Diffraction Method
Measurement Range	Particle Size Range : 100 nm to 10 μm (Size Distribution Displayed : 40 nm to 20 μm)
Concentration Measurement Accuracy	± 30 or less
Concentration Range	Particle size 100 nm : 2 μg/mL to 12 μg/mL Particle size 1 μm : 0.5 μg/mL to 10 μg/mL Particle size 10 μm : 10 μg/mL to 180 μg/mL

Note 1: The measurement range varies according to the shape, etc. of the particle.
Note 2: When concentration measurement accuracy measures the reference sample of our specification in a regular procedure.
Note 3: The concentration range varies according to the shape, etc. of the particle.

Measurement Unit: SALD-7500nano

Light Source	Semiconductor laser (Wavelength 405 nm)
Light Detector	Detector elements for violet semiconductor laser Total 84 elements (78 forward, 1 side, 5 back)
System Compliance	Class 1 Laser Product, CE compliant
Required Power Supply	115 or 230 VAC as ordered 100 VA
Dimensions & Weight	Approx. W680 mm × D280 mm × H430 mm, Approx. 31 kg
Operating Environment	Temperature: 10 to 30°C, Humidity: 20 to 80% (no condensation)

Note 4: Reference sample and USB cable (2 m) supplied as standard

Batch Cell

Cell Material	Quartz glass, PTFE
Required Liquid Volume	Approx. 5 mL
Stirrer Mechanism	Up-and-down movement of blade
Stirring Plate Material	Stainless Steel, Glass, PEEK
Temperature Range (with Temperature Control Function)	20°C to 42°C±1°C, set from PC (constant temperature)
Operating Environment	Temperature: 10 to 30°C, Humidity: 20 to 80% (no condensation)

Micro Cell

Cell Material	Quartz glass, PTFE (Cell Cap, with Temperature Control Function)
Required Liquid Volume	Approx. 0.125 mL
Temperature Range (with Temperature Control Function)	20°C to 42°C±1°C, set from PC (constant temperature)

Circulator (When Equipped with the Aggregates Sizer TC Temperature Control Function)

Required Power Supply	AC100V、13A、50/60 Hz
Dimensions & Weight	Approx. W230 mm × D420 mm × H610 mm, Approx. 31 kg

Software

WingSALD bio

Measurement and Data Display Functions	
Measurement of Particle Size Distribution	Allows measurements using measurement assistant function (interactive process based on SOP)
Real-Time Display	Particle size distribution/light intensity distribution simultaneous display
Display of Particle Size Distribution Data	Displays overlay of max. 200 distributions
Display of Light Intensity Distribution	Displays overlay of max. 200 distributions
Diagnostics/Adjustments	Self-diagnostic function and cell check function
Statistical Data Processing	Max. 200 sets of data (also allows overlaying max. 200 data sets)
Time-Series Processing	Max. 200 sets of data
3-Dimensional Graphing	Max. 200 sets of data
Data Transfer via Clipboard	[Image Output]: Outputs entire data sheet or graph only. [Text Output]: Outputs summary data, particle size distribution data, or light intensity distribution data.
Data Sorting	Sorts by file name, sample ID, sample number, or refractive index
Output Conditions	
Particle Size (μm) Divisions	Fixed 51 divisions, User-settable 51 divisions
Concentration (μg/mL) Divisions	Fixed 49 divisions, User-settable 51 divisions
Distribution Basis	Count or Volume
Expression of Cumulative Distribution	Oversized or undersized
Expression of Frequency Distribution	q
Report Function	Single data sets (8 templates), overlaid data (9 templates), statistical data, time-series data, or 3D data can be selected and output using batch processing
Data Analysis Functions	
Continuous Measurement Function	Continuously measures changes in particle size distributions and particle diameters over time, at intervals as short as 30 seconds, and saves the results.

PC Requirements

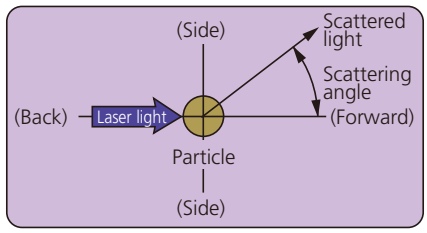
The software is included standard on a CD-R with the Aggregates Sizer.
Install the software on a PC that meets the following specifications.

OS	Windows 10 (32/64-bit version)
CPU	Core i5 or i7
MEMORY	4 GB min
HDD	Min. 1 GB of free space required.
CD-ROM Drive	Required for software installation
USB Port	1 port
Display	SXGA (1280 × 1024 pixels) min.
Printer	Must be compatible with operating system.

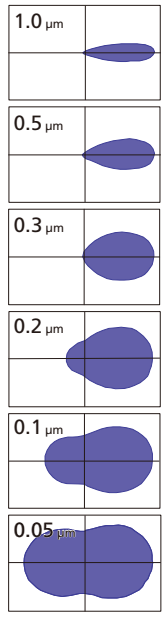
Principle of the Laser Diffraction Method by Violet Laser

There is a one-to-one correspondence between the particle diameter and the light intensity distribution pattern

When a particle is irradiated with a laser beam, light is emitted from the particle in every direction. This is "scattered light". The intensity of the scattered light varies with the scattering angle and describes a spatial intensity distribution pattern, known as a "light intensity distribution pattern". If the particle diameter is large, the scattered light emitted from the particle is concentrated in the forward direction (i.e., the direction of the laser beam), and fluctuates intensely in an angular range too small to be represented in a diagram. Compared to the light emitted in the forward direction, the intensity of all other light is extremely low.



Diffraction/Scattering by Particle

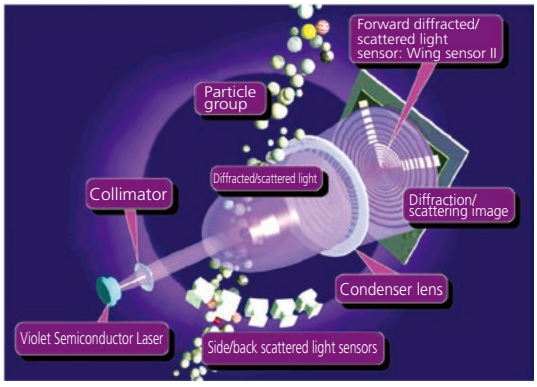


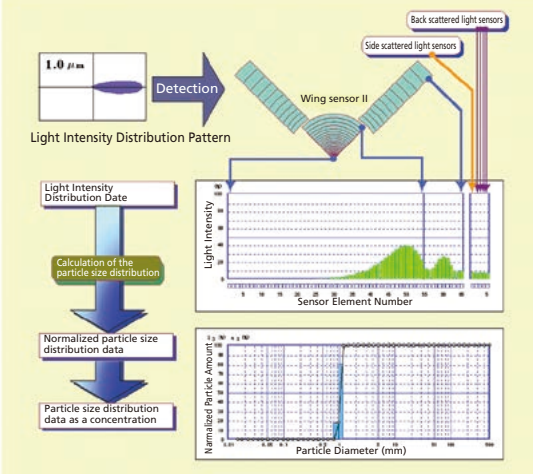
Relationship between Particle Diameter and Light Intensity Distribution Pattern

As the particle diameter becomes smaller, the pattern of the scattered light spreads outwards. As the particle becomes even smaller, the intensity of the light emitted to the side and backwards increases. The light intensity distribution pattern becomes gourd-shaped and spreads out in every direction. Therefore, there exists a one-to-one correspondence between the particle diameter and the light intensity distribution pattern. This means that the particle diameter can be ascertained by detecting the light intensity distribution pattern.

Violet laser allows accurate measurements of ultra-small particles

The light intensity distribution pattern varies little relative to the particle size distribution when the particle size drops to several tens of nanometers. This is the reason for the minimum limit of detection of the laser diffraction method. A violet laser creates clearer differences in the light intensity distribution pattern at ultra-small particle sizes than a red laser. Consequently, a violet laser is used to enhance the measurement performance for ultrafine particles of the order of several tens of nanometers.





Measurement is performed on particle groups

Particle size distribution measurement is not performed on individual particles, but on particle groups made up of a large number of particles. Particle groups contain particles of different sizes, and the light intensity distribution pattern emitted by a group is composed of all the scattered light emitted from all the individual particles. The particle size distribution, in other words, what particle sizes are present in what proportions, can be obtained by detecting and analyzing this light intensity distribution pattern. This is the basic principle behind the laser diffraction method.

Optical System in Aggregates Sizer

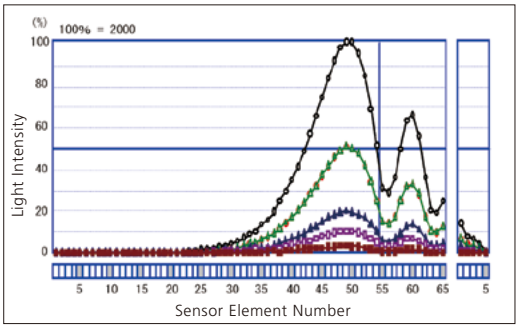
The laser beam emitted from the light source (semiconductor laser) is converted into a thick beam with a collimator, which is directed at the particle group. The scattered light emitted from the group in a forward direction at up to a 60° angle is concentrated with a lens, and concentric scattering images are formed at a detecting plane positioned at a distance equal to the focal length. This is detected with the Wing sensor II in which light-receiving elements are arranged concentrically. The scattered light emitted to the side and backwards is detected with side and back-scattered light sensors. The light intensity distribution data can be obtained by detecting scattered light data.

Overall Flow of Light Intensity Detection and Data Processing

With the Aggregates Sizer, particle size distributions are calculated using the light intensity distribution data. The overall flow of detection and data processing is shown in the diagram to the left. In measurement, the whole range of operations from the detection of scattered light intensity distribution patterns to the calculation of the particle size distribution is executed as one process, and the particle size distribution data is output.

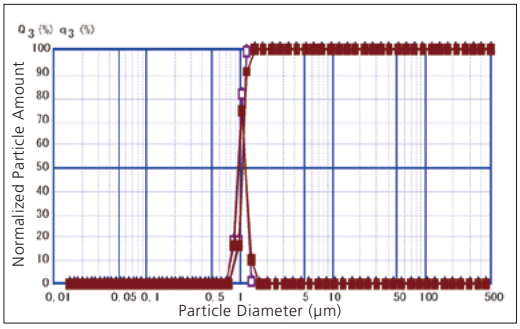
Previously, particle size analysis by laser diffraction method could only obtain a normalized particle amount whose summation is 100%. Particle size analysis by Aggregates Sizer can obtain a concentration (Unit:μg/mL) by referring scattered light intensity according to calibration using PSL standard particles.

Why can particle size distribution as a concentration be measured using the laser diffraction method?

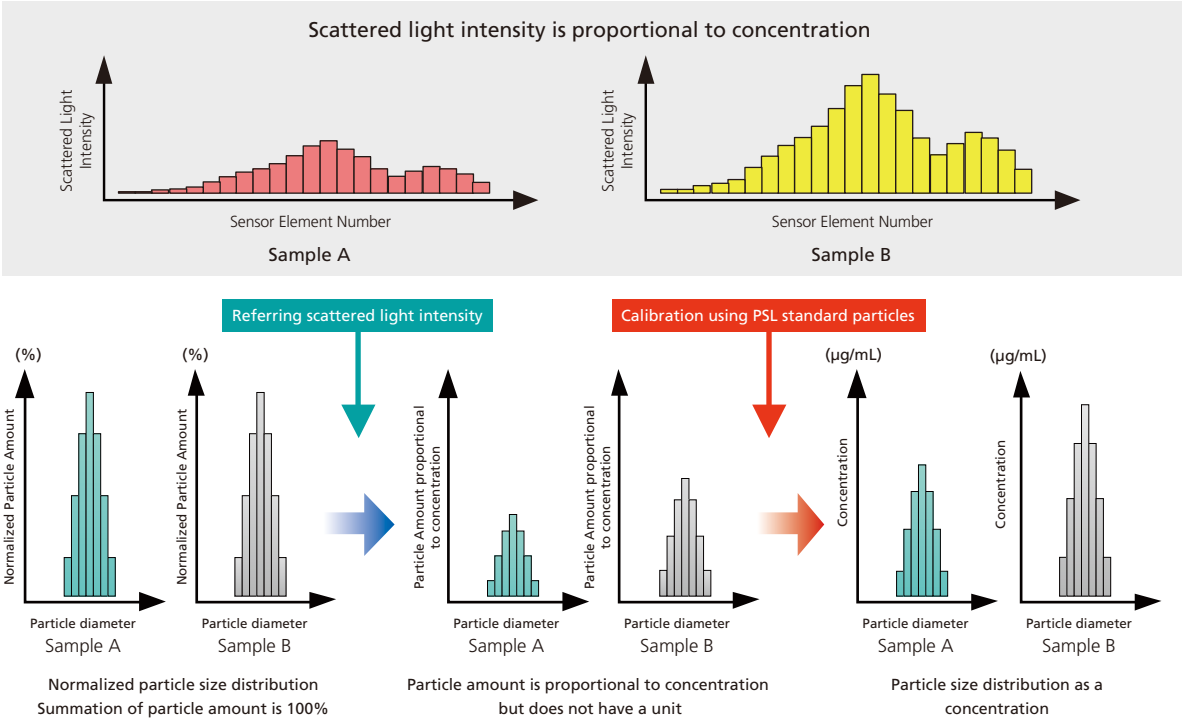


This figure shows scattered light intensity data when the same samples have been measured under the different concentration conditions.
Relationship patterns between the sensor element number and light intensity are similar, and the respective light intensity detected by the sensor element of Wing sensor II is proportional to the concentration.

Calculate particle size distribution by analyzing light intensity distribution data



Previously, normalized particle size distribution could not be obtained as a concentration.



The difference between sample A and sample B cannot be evaluated by particle size analysis using a normalized particle amount whose summation is 100%.
By referring to scattered light intensity, the particle amount becomes proportional to concentration but does not have a unit.
Via calibration using a PSL (polystyrene latex) standard particle, we can obtain particle size distribution as a concentration.

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