Characterization of the volume-based or number-based size distribution for silica nanoparticles using a unique combination of online dynamic light scattering having a unit-tau multi-bit correlator and high resolution centrifugal field-flow fractionation separator.

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Abstract

This paper presents a study of colloidal nanoparticles’ size distributions using an online dynamic light scattering (DLS) unit with a uni-tau multi-bit correlator (UMC) combined with a centrifugal field-flow fractionation (CF3) separator.

Conventionally, FFF-UV-MALS system utilizing field-flow fractionation (FFF) combined with UV detector and multi angle light scattering instrument (MALS) could be used to obtain the particle size distribution of colloidal nanoparticles.

Lately, DLS as a technique to measure size distributions of colloid materials has become prevalent. However, DLS instrument will practically measure only the large particles in a multi-modal particle mixture.

Therefore, the CF3-DLS w/UMC system were developed consisted of a CF3 unit connected to an online DLS instrument with UMC. The system could measure the volume- or number-based size distribution with excellent quantitatively and accurate histograms for multi-modal sample. The size distributions were validated with size distributions obtained by images of the atomic force microscope (AFM). Two types of colloidal silica nanoparticles with different distribution widths were used in this study.
1. Introduction:

Nanotechnology is utilized for nanoparticle research, manufacturing of nanostructures, or to evaluate those features and characteristics. Furthermore, nanotechnology has attracted great interest on a worldwide scale, and many kinds of nanomaterials are synthesized, especially particles below 100 nm (the so-called nanoparticles) which show different properties from bulk materials.

Recently, nanomaterial users had faced the lack of or inconsistent definition of the nanomaterials until the European Commission adopted a definition of a nanomaterial in 2011 - Recommendation on the definition of a nanomaterial, 2011/696/EU. According to the recommendation, a nanomaterial means; A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm. However, it is difficult for a nanoparticle manufacturer to decide if a material is a nanomaterial, because particles in suspension include not only primary particles but also aggregated particles. In the field of powder technology, synthesized nanoparticles’ size and size distribution are normally evaluated by dynamic light scattering (DLS) or multi angles light scattering (MALs) that is considered to be one of the most effective methods for measuring colloidal nanoparticles; however, this method has difficulties in distinguishing small nanoparticles from large particles because the scattered light from small nanoparticles is weak and the scattered light from large particles is strong. Therefore, these instruments can measure only large particles in a multi-modal particle mixture.

In order to rectify this problem, classification of the particles before measurement can be done. There have been recent studies on the nanoparticles classification by size exclusion chromatography (SEC), Asymmetric flow field-flow fractionation (AF4) or
Centrifugal field-flow fractionation (CF3)\(^5\). Yamaguchi et al.\(^6\) combined a SEC with an online DLS (SEC-DLS w/UMC) to measure silica nanoparticles in the liquid, and compared by images of scattering electron microscope (SEM). The SEC classified injected silica nanoparticles into several fractions of mono-dispersed nanoparticles. The classified nanoparticles were introduced from the SEC to the flow cell in the DLS w/UMC sequentially. The size distribution obtained from the integration was the size distribution of the total injected nanoparticles. However, SEC was not effective as SEC had several columns that were filled with porous silica packing material that had different pore sizes in order to expand the classification size range. Unfortunately, it was also possible for nanoparticles to be trapped in a column’s packing material.

On the other hand, field FFF was effective as AF4 and CF3 had wider classification size range than SEC, the AF4 could produce better collection rate by using surfactant in the sample. Kato et al.\(^7\) had reported that CF3 that used a flow cell with a spacer could allow almost a 100% collection rate by using a suitable surfactant concentration and it was possible to have a wide measurement range by controlling the centrifugal speed. Jores \(et\ al.\)^\(^8\) investigated on the structure of solid lipid nanoparticles and oil-loaded solid lipid nanoparticles. They characterized the sizes by AF4-MALS that was compared with DLS, laser diffraction (LD) and transmission electron microscope (TEM). The three types of apparatus showed much different diameters, because they have each different principle of measurement, and the shapes of solid lipid were not spherical. Kato \(et\ al.\)^\(^9\) determined the accurate Z-average diameter of spherical polystyrene latex (PSL) particles by DLS and AF4-MALS. The Z-average diameter determined by DLS and AF4-MALS agreed well within the estimated uncertainties, although the size distribution of PSL determined by DLS was less reliable in comparison with that determined by AF4-MALS. Aureli \(et\ al.\)^\(^10\) developed a new method, AF4-MALS-ICP-MS/MS to allow dimensional and mass
determination of silica particles over the size range of approximately 20-200 nm. Caputo 
et al.\textsuperscript{11} measured the particle size distribution of lipid-based nanoparticles of moderate 
poly-disperse by using AF4-MALS-DLS over the size range of approximately less than 
100 nm. The AF4 had good performance for smaller nanoparticles’ size classification, but 
the larger, sub-micrometer particles could not be classified effectively and moved on the 
surface of membrane filter with the flow of elution liquid. The AF4-MALS could measure 
particles with geometric diameters of less than 100 nm with high resolution while AF4- 
DLS could measure only mean diameters for particles larger than 40 nm without any 
distribution. There was a need to combine AF4 and MALS and DLS to measure wider 
particle size range. The DLS used had an exponential correlator and this could affect the 
flow rate of the sample from AF4 to the online DLS. Caldwell \textit{et al.}\textsuperscript{12} used CF3-UV and 
a batch style DLS to measure particle sizes of polystyrene latex beads. Polystyrene latex 
beads were classified by CF3 coupled with an UV detector to generate a fractogram. Some 
size fractions at several elution timings were corrected, after which, the batch style DLS 
would measure the particle sizes for each fraction. It was combination of three techniques. 
It was not an online system between CF3 and DLS and it was done with size fraction 
collection at several elution timings with respect to the sample volume. A shortfall of the 
setup was although poly-disperse sample was classified into mono-disperse fractions after 
the CF3, the mono-disperse fractions would finally combine back into a single poly-
disperse sample in the batch cell of DLS. Galyean \textit{et al.}\textsuperscript{13} introduced a new method for 
optimizing the separation of poly-disperse samples using AF4, and demonstrated the 
applicability using MALS-DLS with polystyrene bead mixtures. Scattered light intensity 
by MALS was used to determine the concentration while hydrodynamic radius was 
measured by DLS at each elution time slice. The DLS used had an exponential correlator 
that would affect the flow of the sample. A combined system of CF3-MALS-DLS was
used but sample distribution broadening and dead volume effect in the cell were not discussed. Chu et al.\textsuperscript{14} characterized particle size and size distribution of multi-sized polymer lattices by centrifugation with DLS. Confirmation was done by result comparison with TEM, FFF, and capillary hydrodynamic fractionation (CHDF). A fractionated supernatant was measured with several rotation speeds of centrifugation. Some limitation of the method include the fact that it remains as an off-line technique, and it is difficult to apply to samples with complex particle size distribution.

The authors provided a summary in the previous paragraph on the many studies that used combined AF4/CF3 with UV detectors or MALS to measure particle concentration, and combined DLS/MALS to measure particle size and size distribution by both off-line and on-line methods. In those studies, an exponential correlator was used in DLS, and AF4 and CF3 were combined with an UV detector to produce a fractogram between elution time and absorption. There are shortcomings in those papers to measure poly-dispersed nanoparticles in the liquid. Firstly, it is difficult to know the particle concentration using an UV detector, because the UV detector detects both light absorbed and light scattered that depended on the refractive indexes of the material and the solvent. It is especially, problematic for smaller particles because larger particles scattered light much more strongly. Secondly, batch DLS has been mixing mono-disperse samples during collection time. Combined systems such as FFF-UV-MALS-DLS has a long tubing length and multiple dead volume in the connection parts, causing peak broadening after FFF. Thirdly, all DLS systems used in the studies had exponential correlators for the calculation of particle size. The sampling times of each channel of exponential correlator to produce the auto-correlation function is longer than that of a linear correlator and can significantly affect the flow rate used in the cell of the online DLS.

The authors had developed a new system that could rectify the above shortcomings that
consisted of only a CF3 and an online DLS w/UMC (CF3-DLS w/UMC) that could measure particle concentration from scattered light intensity and particle size from the diffusion coefficient of the particles’ Brownian motion. The classified fractions of mono-dispersed nanoparticles were introduced from the CF3 unit to the online DLS unit sequentially. The DLS unit would measure Z-average diameter calculated from the auto correlation function with linear sampling gate timings (not exponential) and particle count per second (CPS) using scattered light intensity as particle concentration. The UMC is more effective than exponential correlators, and when low-channel-number channels were used to produce the auto-correlation function for the calculation of the Z-average diameter, it would be less likely to be affected by the flow movement in the cell. A particle size distribution based on the scattered light intensity would be derived using the Z-average diameter and the CPS. Number-based and volume-based size histograms were calculated using the Mie theory and had relationship of scattering light intensity per unit volume and per unit number respectively. The histograms showed the size distributions of the total injected nanoparticles. Four kinds of colloidal silica nanoparticles, SI-100, SI-200, SI-A and SI-B with different distribution widths were used in this study. Both SI-100 and SI-200 had a mono-dispersed size distribution. Quantitative accuracy by volume was confirmed by calculating the volume-based size distribution by mixed ratio of those colloids. Both SI-A and SI-B had a poly-dispersed size distribution. The number-based size distributions were compared and validated with size distributions obtained by images of the atomic force microscope (AFM).

2. Experiments:

2.1 Samples:
A standard PSL was used to verify the DLS system. The PSL was provided by Thermo Scientific K.K. Ltd, Catalogue No. 3100A, nominal diameter of 102 nm, uncertainty of 3 nm, and particle concentration of 1% by mass. The PSL used for the CF3-DLS w/UMC was diluted with ultra-pure water before measurement. The concentration of the particles was 1 mg/L. We also analyzed four colloidal silica nanoparticles dispersed in water. Surfactant-free aqueous suspensions of silica nanoparticles (SI-100, SI-200, SI-A, and SI-B, National Institute of Advanced Industrial Science and Technology certified reference material, Japan) were used. The respective concentrations are listed in Table 1. The concentrations were calculated from the mass of the particles. The nanoparticle sizes were obtained using SEM (Quanta 250 FEG, FEI Co., USA) equipped with a field emission electron gun. The electron diffraction of a selected area was investigated at 2–20 kV with secondary electrons in the high-vacuum mode. The samples were prepared on a hydrophilic-treated flat silicon wafer. Fig. 1(a) and Fig. 1(b) show SEM images of SI-100 and SI-200. Fig. 1(a) shows that the mean diameter was 100 nm and the standard deviation was 2.0 nm. Fig. 1(b) shows the mean diameter was 200 nm and the standard deviation was 2.8 nm. Fig. 1(c) and Fig. 1(d) show AFM images of SI-A and SI-B obtained using AFM (Hitachi AFM4000).

2.2 CF3-DLS w/UMC:

Fig. 2 shows the block diagram of the CF3-DLS w/UMC. The CF3-DLS w/UMC consisted of a CF3 unit and a DLS unit with a flow cell. The correlator used in the DLS unit was a uni-tau multi-bit correlator.

2.3 CF3 unit:
CF3 unit uses centrifugal force as the separation mechanism. A circulated flow cell with a sample holding space is usually used, and the rotation or the centrifugal force pushes nanoparticles to the outer wall of the cell. The centrifugal force is proportional to the particle diameter to the third power. This means that large particles are more strongly affected, while a weaker force is exerted on small particles. The separation of the particles by size is made possible with the altering of the centrifugal force or the adjustment of the rotation speed. In essence, the rotation of the flow cell is increased initially to force all particles towards the outer wall of the cell, virtually keeping them stationary at the outer wall. As the rotating speed starts to decrease, the centrifugal force for the smallest particles will be weaken and the smallest particles will be moving again and can be transported by carrier liquid out of the flow cell. Further drop in rotation speed will allow the next fraction of slightly bigger particles to escape the centrifugal pull and be transported out. This is the principle of particle classification of CF3 unit. In this study, a CF3 unit (FFF-C8030 prototype, Shimadzu Corporation, Japan) was used and it had the followings: channel shape was rectangular, radius of rotation was 99 mm, tip-to-tip length was 568.5 mm, width was 20 mm, nominal spacer thickness was 0.25 mm, wall material was made of stainless steel, stop flow time was 2.5 min and void time was 3.6 min. The complete CF3 unit setup consisted of a liquid chromatography (Prominence HPLC, Shimadzu Corporation, Japan) unit with an auto-sampler (SIL-20AC), a degasser (DGU-20A3R), a pump (LC-20AD), and an UV detector (SPD-M20A, 215nm). We used an applied field that was programmed to decay with time during the run to reduce the run time of a single measurement. The field strength was reduced using a power decay function according to equation (1):
\[ RPS(t) = RPS_0 \left( \frac{t}{t_a} \right)^4 \]

where \( RPS(t) \) is the rotational angular speed expressed in revolutions per second at time \( t \), \( RPS_0 \) is the initial rotational speed during application, and \( t_1 \) is the period of the constant field preceding the field decay. In this study, \( t_1 \) and \( t_a \) were fixed at 10 min and -80 min, respectively. The \( RPS_0 \) was 2,500 rpm for the mixture of SI-100 and SI-200, and SI-A, \( RPS_0 \) was 10,000 rpm for SI-B. The mobile phase flow rate was 1.0 mL/min, using FL70 surfactant (Postnova analytics, GmbH). It was 0.1% by mass, so its viscosity was almost the same as pure water. The injection volume was 20 µL. A PEEK tube having a diameter of 0.13 mm was used to connect a DLS unit to the CF3 unit setup.

2.4 DLS unit:

The DLS unit used was a customized dynamic light scattering nanoparticle size analyzer (SZ-100-AIST, HORIBA, Ltd, Japan). It consisted of a diode-pumped laser (532 nm, 100 mW), a digital correlator, two angled photomultipliers, and a flow cell. The flow cell was having an inner dimensions of a width of 1 mm, a depth of 1 mm, and a height of 25 mm. In this study, the DLS unit would be using a photomultiplier perpendicular (90 degree) to the direction of the laser light path, and a uni-tau multi-bit correlator to generate the auto-correlation function. The scattering volume between the laser optics and the detector optics was a 50 µm sphere. A PEEK tube having a diameter of 0.25 mm and total length 0.5 m was used in the DLS unit. Total liquid volume was 50 µL in the DLS unit.

2.5 Methods for CF3-DLS w/UMC:

Fig. 3 shows the result determination process for the CF3-DLS w/UMC to obtain the volume-based and number-based distributions. The measurement process started with the
collection of scattered light intensity $N(t_0)$ of the eluent without the particles flowing into the DLS unit. The blank eluent count rate was equal to 10,000 counts per seconds. After the injection of nanoparticles, they were classified into several fractions of mono-dispersed nanoparticles by the CF3 unit. The classified particles would flow into the DLS unit sequentially from smaller to larger ones. The DLS unit measured each fraction of mono-dispersed nanoparticles in order and obtained scattered light intensity from the particles, $N(t_i)$, forming an autocorrelation function from $t_0$ to $t_{\text{end}}$ repeatedly at 20 s intervals. The Z-average $D(t_i)$ was calculated from the autocorrelation function and particle count per second (CPS) of scattering light, $N(t_i)$, that was measured for each measurement period. The Z-average $D(t_i)$ was calculated by using the viscosity of water as the viscosity of eluent. The gray dots were real data, and the black dots were approximation value by least square. The scattered light intensity is a function of the concentration of the nanoparticles and their diameters. Total elution time taken for one sample was 150 minutes maximum from $t_0$ to $t_{\text{end}}$. The scatter plot based on the scattering light intensity $F_i(D)$ was created by the Z-average diameter $D(t_i)$ and the CPS, $N(t_i) - N(t_0)$. After which, a number-based histogram $F_n(D)$ or volume-based histogram $F_v(D)$ was calculated by using the Mie theory that had relationship of scattering light intensity per unit volume or per unit number accordingly. The histogram displayed the size distribution of total injected nanoparticles.

2.6 AFM:

AFM images were obtained from an atomic force microscope (AFM4000, Hitachi High-Technologies Corporation, Japan) coupled with silicon probes (OCML-AC160-TS, Olympus Corporation, Japan) at room temperature and pressure using the dynamic force mode. Sample preparation was conducted using the sandwich freeze-drying method\textsuperscript{15}. 

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This technique deposits nanoparticles on a silicon substrate using -80 degree C freezer and freeze dryer. This sandwich method involves freeze-drying of a thin film formed from a suspension.

3. Results and Discussions:

3.1 Online-DLS unit:

Conventional DLS system measures the translational velocity of particles in Brownian motion in a fluid (practically, a liquid) where there is no forced circulation due to mechanical movement, heat or any external applied force (other than natural gravity). However, an online-DLS measures the Brownian motion with added fluid flow movement with forced circulation due to a pumping device. We ascertained the precision of the online-DLS unit as shown in Fig. 4 at flow rates of 1.0 mL/min by using a PSL suspensions (nominal diameter: 100 nm, concentration: 1 mg/L).

The data precision of an online-DLS unit is proportional to the integration time. Long integration time for data sampling produces higher signal to noise ratio than short them, but particles with several sizes size fractions will be mixed with a long integration time. Therefore, the data sampling time was set to less than 20 s to measure the classified mono-dispersed particles in the cell in this study. A UMC was selected because a UMC has more data channels than an exponential correlator using fast delay time. Fig. 5 shows a comparison of measurement stability for 30 data points between the use of all the 512 channels and just only with 1-50 channels from the measurement of 100 nm PSL at 5 mg/mL. The first cumulant method described in ISO 22412 was used to determine the average decay rate and subsequently the Z-average diameter. The Z-average diameter calculated by the first 50 channels of the autocorrelation function exhibited better repeatability due to the fact that usually the autocorrelation function would not be affected.
by the flow of particle at fast delay times (represented by physical channels of low channel numbers, for example, 1<sup>st</sup> to 50<sup>th</sup> channels).

### 3.2 CF3-DLS w/UMC:

A mixture of mono-dispersed SI-100 and mono-dispersed SI-200 was measured by the CF3-DLS w/UMC. The sample was mixed with same volume at 1 mg/mL concentration each. Injection volume was 20 µL. Fig. 6(a) shows the UV fractogram with two peaks, left side peak is for smaller particles and right side peak is for larger particles. CF3 unit could separate the mixed sample into two groups with different sizes. The area ratio of two intensity’s peaks was 1:2.3, but theoretically the area ratio should be 1:2 when it was the same mixing volume for them. The difference was almost 15%. This result illustrated the situation where UV detection was not a reliable method to measure the sample concentration because the detected intensity was having both the transmitted light and scattered light, especially for the larger particle. It is usual that a larger particle has a stronger light scattering than a small particle, therefore, the UV fractogram included both the transmitted and scattered light intensity for the larger particles resulting in 15% higher ratio. On the other hand, Fig. 6(b) shows the results of the CF3-DLS w/UMC as shown in Fig. 2. The result shows only the scattered light intensity without transmitted light intensity and the Z-average diameters that were measured when the scattered light intensity were stronger than the scattered light intensity of the dispersant without particles. The graph shows that the average of the Z-average diameters of the left side peak is about 100 nm and the average of the Z-average of the right side peak is about 200 nm. The gray dots were real data, and the black dots were approximation value by least square. The area ratio of two scattered light intensity’s peaks was 1:5.7 when theoretically the area ratio should be 1:5.8 when it was the same mixing volume for them. The difference was
about 2%. Therefore, this result illustrated the situation where it reasonable to use scattered light intensity to measure the sample concentration. Fig. 6(c) shows the results of the DLS w/UMC without the CF3 unit as shown in Fig. 4. The injected sample used was the same as the previous experiment. The graph shows only a single scattered light intensity peak with an average of the Z-average diameters of about 170 nm because the injected sample went through the cell without any separation. The gray dots were real data, and the black dots were approximation value by least square. Auto-correlation function for the mixed mono-dispersed SI-100 and SI-200 obtained was analyzed by the first cumulant method described in ISO 13321 to determine the average decay rate. The apparent diffusion coefficient $D_i$ obtained from DLS w/UMC was used in the Stokes–Einstein relation of the following form to determine the hydrodynamic particle size of the nanoparticles:

$$D = \frac{k_B T}{6\pi\eta D_i}$$

Here, $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, $\eta$ is the viscosity of the solvent, and $D$ is the calculated hydrodynamic diameter of the nanoparticles. Therefore, it is important to have CF3-DLS w/UMC to measure reliable particle size distributions as per the above results.

Fig. 7(a) shows the scattered light intensity-based size distribution of the mixed SI-100 and SI-200 calculated from the scattered light intensity and Z-average diameters of Fig. 6(b). Fig. 7(b) shows the volume-based size distribution of the mixed SI-100 and SI-200 calculated from the Mie theory using the relationship between scattered light intensity and diameter. The result shows a bimodal size distribution. The mean diameter of the smaller peak’s size distribution corresponding to the SI-100 is 110.0 nm, and the mean diameter of the larger peak’s particle size distribution corresponding to the SI-200 is
216.1. Fig. 7(b) shows the area ratio of two peaks is 48:52 in the volume-based size distribution. This result demonstrated the high resolution and effective quantitative capability of CF3-DLS w/UMC.

The SI-A with wide distribution was measured by CF3-DLS w/UMC. The sample was at 5 mg/mL. Injection volume was 20 µL. Fig. 8(a) shows the UV fractogram of a peak with wide distribution. Fig. 8(b) shows the result of the CF3-DLS w/UMC. The result shows the scattered light intensity and the Z-average diameters that were measured when the scattered light intensity of the particles were stronger than the scattered light intensity of the liquid dispersant without particles. The Z-average diameters measured were within 90 and 160 nm. The gray dots were real data, and the black dots were approximation value by least square.

Fig. 9(a) shows the number-based size distribution of the SI-A. The result shows that the mean diameter is 108.7 nm and the standard deviation is 4.8 nm. Fig. 9(b) shows the number-based size distribution by AFM. The sample for AFM was the same SI-A without classification by CF3. Particle count for analysis was found to be 3370, the mean diameter determined was 106.8 nm and the standard deviation was 8.8 nm. The number-based size distribution of CF3-DLS w/UMC obtained closely matches the number-based AFM’s size distribution from these results. Those mode sizes agree well, but the width of size distributions has a difference of two times different from the standard deviation. The reasons for the difference is due to band broadening effect that occurred in CF3 separation. Kato et al. [9] illustrated the phenomenon with certified PSL standard.

The SI-B with wide distribution was measured by CF3-DLS w/UMC. The sample was 5 mg/mL. Injection volume was 20 µL. Fig. 10(a) shows the UV fractogram of a peak with wider distribution. Fig. 10(b) shows the result of the CF3-DLS w/UMC. The result shows the scattered light intensity and the Z-average diameters were measured when the
scattered light intensity of the particles were stronger than the scattered light intensity of liquid dispersant without particles. The Z-average diameter measured were within 80 and 200 nm. The gray dots were real data, and the black dots were approximation value by least square.

The sample was 5 mg/mL, and injection volume was 20 µL. Fig. 11(a) shows the number-based size distribution by CF3-DLS w/UMC. The mean diameter was found to be 126.2 nm with a standard deviation of 13.3 nm. Fig. 11(b) shows the number-based size distribution by AFM. The sample for AFM used was the same SI-B without classification by CF3. Particle count for the analysis was found to be 864, the mean diameter was 124.9 nm and the standard deviation was 18.2 nm. The number-based size distribution of CF3-DLS w/UMC is quite similar to the size distribution obtained by AFM. Those mode sizes agree well, but the width of size distributions has a difference of about 27% from the standard deviation. The standard deviations of CF3-DLS w/UMC is slightly narrower. The reason for the difference is due to band broadening effect occurred in CF3 separation.

The results by using CF3-DLS w/UMC for SI-A and SI-B had poly-dispersed size distribution indicated to measure accurate size distributions. The mean diameters from the size distributions and the width from the standard deviation were validated with AFM imaging size distributions. The results including broadening effect were slightly different from the width of size distributions by using AFM image technique, but the CF3-DLS w/UMC represent a major advanced in accurate measurement of poly-dispersed colloid.

4. Conclusions:

In this study, we developed a unique system to evaluate the size distribution of silica nanoparticles in a liquid by using CF3-DLS w/UMC. We established that this system
could measure correctly volume-based or number-based size distribution histograms calculated by using the Mie theory related with scattering light intensity per unit volume or per unit number respectively. Furthermore, we had proved that this system was not affected by the flow rate in the cell for size measurement, by the material’s reflective index for concentration measurement, by the broadening of the size distribution in the connected parts, or by hiding small particles by the large particles in a multi-modal particle mixture. We validated that the volume-based size distribution with bi-modal silica colloid mixed could be measured with excellent quantitativeness. We validated that the number-based size distributions determined by CF3-DLS w/UMC and AFM agreed well. This novel system could be a practical method for researchers to achieve some degree of concordance in nanoparticle size distribution determination in industrial field.

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7. **Nomenclatures:**

\[ D: \text{diameter} \quad [\text{nm}] \]

\[ k_B: \text{Boltzmann coefficient} \quad [\text{J/K}] \]

\[ T: \text{Absolute temperature} \quad [\text{k}] \]

\[ \eta: \text{Viscosity of the solvent} \quad [\text{mPas}] \]
$D$: Diffusion constant \quad [\text{m}^2/\text{s}]

ti: sampling time \quad [\text{sec}]

$N (t)$: counts numbers of scattering light intensity \quad [\text{-}]

$F_l (D)$: scattering light intensity-based size distribution \quad [\text{-}]

$F_v (D)$: volume-based size distribution \quad [\text{-}]

$F_n (D)$: number-based size distribution \quad [\text{-}]

$RPS (t)$: Rotation speed at time $t$ \quad [\text{rpm}]

RPSo: Initial rotation speed \quad [\text{rpm}]

ti: The period at the initial rotation speed \quad [\text{rpm}]

ta: Decay parameter \quad [\text{rpm}]
**Figure Captions**

Fig. 1 SEM images; (a) SI-100, (b) SI-200, AFM images; (c) SI-A, (d): SI-B.

Fig. 2 Block diagram of CF3-DLS w/UMC.

Fig. 3 Result determination process of CF3-DLS w/UMC.

Fig. 4 Block diagram of online DLS w/UMC.

Fig. 5 A comparison of measurement stability for the online-DLS with different correlator channel selections by using PSL suspension.

Fig. 6 Results for mixed SI-100 nm and SI-200 nm. (a) Fractogram and rotation speed by CF3 unit, (b) The scattered light intensity and Z-average by CF3-DLS w/UMC, (c) The scattered light intensity and Z-average by online DLS w/UMC.

Fig. 7 Results for mixed SI-100 and SI-200 suspension. (a) Scattered light intensity-based size distribution by CF3-DLS w/UMC, (b) Volume-based size distribution by CF3-DLS w/UMC.

Fig. 8 Results for SI-A. (a) Fractogram by CF3 unit, (b) Count rate and Z-average by CF3-DLS w/UMC.

Fig. 9 CF3-DLS w/UMC results for SI-A. (a) Number-based size distribution by CF3-DLS w/UMC, (b) Number-based size distribution by AFM without CF3.

Fig. 10 Results for SI-B. (a) Fractogram by CF3 unit, (b) Count rate and Z-average by CF3-DLS w/UMC.

Fig. 11 CF3-DLS w/UMC results for SI-B. (a) Number-based size distribution by CF3-DLS w/UMC, (b) Number-based size distribution by AFM without CF3 unit.
Fig. 1
Fig. 2
$F_i(D)$: Scattering light intensity-based size distribution

$F_v(D)$: Volume-based size distribution

$F_n(D)$: Number-based size distribution
Fig. 4
Fig. 5

(a) Absorption - Rotation speed

(b) Count rate

(c) Count rate
Fig. 6
Fig. 7
Fig. 8