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Good practice for dynamic light scattering (DLS) measurements

Bonnes pratiques pour l'analyse de la dispersion lumineuse dynamique (DLD)

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Dynamic light scattering (DLS) is a widely used technique for the characterization of particles with equivalent hydrodynamic diameters below a few micrometres. Modern instruments allow users with minimal training or background to use this technique. The downside is that not all users are familiar with the potential pitfalls, limitations and proper interpretation of results for DLS.

Therefore, this document has been developed as a guidance for good practice in DLS and complements ISO 22412:2017.

Good practice for dynamic light scattering (DLS) measurements

1 Scope

This document provides practical guidance for performing and interpreting measurements using dynamic light scattering (DLS) that goes beyond the treatment of measurement artefacts in ISO 22412:2017.

This document is intended to help users with experiments planning, in particular with respect to obtaining the necessary information on the sample and deciding whether DLS is the most appropriate method. It provides information on how to prepare samples in an appropriate way, verify the proper functioning of the instrument and interpret the data correctly, including ways to assess data quality.

This document focuses on the practical steps required to obtain DLS results of good quality, rather than on theoretical considerations, and covers not only the measurement of solid particles, but also emulsions and bubbles.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Instrument types

4.1 General

A discussion on what constitutes good practices requires knowledge of the instrument type being considered. Different optical configurations require different adjustment to control the optical layout; different signal processing techniques require different techniques to allow for background conditions; different analysis techniques require different conditioning parameters of the processed signal. Two commonly applied variants are homodyne detection with correlation function processing (see ISO 22412:2017, 9.2) and heterodyne detection with frequency spectrum processing (see ISO 22412:2017, 9.3.

Additionally, good practice, as it relates to instrument type, also depends on the scattering angle used for the measurement. For instance large spurious particles generally scatter more power into forward angles than higher angles, so that samples measured in forward-scatter typically require significantly more care regarding the cleanliness of the cuvette used, prior to filling with sample, the filtering of the sample between the particle size distribution (PSD) of interest and unwanted large size fractions and the dispensing to waste of the first few drops of sample from a syringe filter to remove filter spoil. Additionally, the single-scattering relaxation time is known to be well approximated by higher order scattering from concentrated samples as the scattering angle approaches 180 °, thereby allowing the characterization in backscatter of concentrated samples, so long as bulk scattering losses through

concentrated media can be avoided. Losses are mitigated in many commercial instruments by moving the optical detection point closer to the cuvette wall using opto-, mechanical or opto-mechanical means.

4.2 Information prior to analysis

4.2.1 Sample information

The customer or submitter of a sample for DLS analysis provides, as available, all information relevant to the measurement of the sample. Absence of information does not preclude analysis, but availability of information aids the analyst with respect to sample preparation, measurement design and interpretation of results. In general, the more information is available about a sample, the more likely the analysis will be successful and the results meaningful for the customer. Availability of this information can also reduce the uncertainty for the overall measurement result.

The following questions are answered where possible.

a) Questions related to the analysis step in DLS.

   1) What is the primary composition of the sample?

      The composition will determine the scattering properties and the complex refractive index, as well as colloidal stability.

   2) What is the crystallographic phase (if known)?

   3) What is the density of the sample? Has a Stokes' law calculation been carried out to show the settling rate for particles of different sizes?

   4) Is the sample coated (e.g. is there a polymeric coating, ligand or surfactant that modifies the surface functionality and stability)?

   5) What is the colloidal stability (sedimentation/agglomeration/dissolution…)?

      If the colloid sediments or agglomerates, measurement results will change with time. This does not necessarily invalidate the results, but it is important to know whether such changes are expected and what information the user wants to obtain from the analysis (see 4.6.5.1, 4.6.5.2 and 4.7.3).

   6) What is the complex refractive index of the particles?

      The complex refractive index consists of a real and imaginary part. The former defines the light scattering behaviour, the latter defines the light absorption behaviour. The real and the imaginary part of the refractive index of the particle are necessary for converting intensity-weighted to volume-weighted results.

b) Questions related to sample preparation.

   1) What is the dispersing medium?

   2) If the sample requires dilution, a diluent of similar chemical composition, ionic strength, pH and that contains the same other additives is chosen.

   3) What is the refractive index of the medium?

      The refractive index of the medium is required for analysis. If unknown, it can be looked up in tables or measured.

   4) Does the medium contain surfactants necessary to maintain stability? If so, the surfactant and its concentration are identified.

5) What is the viscosity of the dispersion medium?

The viscosity of the dispersion medium is required for analysis. The viscosity of common fluids is available in tabulated forms. If this is not available, it can be measured.

6) Can the sample be filtered to remove large scatterers such as dust or residual aggregates?

This depends on the size of the principal component and desired information from analysis of sample.

7) If the sample is in suspension, is it clear? Does it contain sediment?

8) Are specific ingredients or procedures required for preparation of the sample?

9) Does the medium show non-Newtonian behaviour?

c) Questions related to the choice of the appropriate particle concentration.

1) What is the mass concentration of the sample (e.g. 0,01 mg/ml)?

d) Questions related to the selection of the appropriate evaluation algorithm.

Is the sample polydisperse (e.g. does it contain multiple size populations or a very broad size range, is it agglomerated) and what is the anticipated size distribution?

Providing any available information about degree and nature of polydispersity can help to set up the analysis.

The size distribution is in most cases the purpose of the measurement, but an expectation can help not only to set up the experiment, but also allows checking the plausibility of the result.

Many modern instruments are capable of characterising multi-modal samples with distribution-based analyses such as non-negative least squares. This is often the first step, prior to use of cumulants to provide a z-average size and polydispersity if and only if the sample is monodisperse. Laser diffraction may be considered for large aggregates; however, the user needs to be aware that laser diffraction reports the hard sphere size in comparison to dynamic light scattering which reports the hydrodynamic diameter.

e) Questions related to the identification of potential artefacts.

1) Are there other (non-principal) scattering components in the sample (e.g. proteins, a second solid phase, micelles)?

Providing the known sizes of secondary components helps in the interpretation of results.

2) Are the principal particles highly asymmetric (e.g. rod-like)?

f) Questions related to sample storage before analysis.

1) Are special conditions necessary for sample storage before analysis (e.g. refrigerated, in dark, exclusion of CO_2 uptake etc.)?

2) Is the sample material subject to dissolution?

4.2.2 Desired outcome of analysis

In addition to providing the analyst with basic information about the sample, it is equally important for the customer or submitter to stipulate the purpose and desired outcome of the analysis. This will

determine the level of effort expended and will aid the analyst in the experimental design. The following questions are most relevant.

a) In which context will the results be used?

   1) Applications in quality control (QC) typically require less stringent analysis than applications in research and development or product characterization. Often the goal is detection of change rather than accurate determination of size/size distribution. In these cases, factors that lead to a constant bias (non-Newtonian media, inaccurate knowledge of the refraction indices etc.) will not affect the conclusion drawn from e.g. a time series.

   2) Applications in research and development or product characterization may require higher levels of scrutiny depending on the application need.

b) Is DLS able to deliver results at the required uncertainty level?

   On a material with a narrow size distribution and a median diameter of 100 nm, relative expanded uncertainties of the z-average of ≤3 % are achievable (see ISO 22412:2017, 10.1). For more polydisperse materials, higher uncertainties are expected. More data on uncertainties are given in e.g. References [4],[5], and [6]. Depending on how the result is used, DLS may not be able to deliver results with the required accuracy.

c) Should a mean size and polydispersity index value be reported?

   1) This typically involves cumulants analysis, which delivers robust results for a monomodal Gaussian size distribution. It is not applicable to highly polydisperse systems or samples with a more complex distribution.

      NOTE The scattering intensity into all scattering angles from particles of diameter < 1/10th of the wavelength of the illuminating light beam is well-approximated, to within a few per cent, as proportional the 6th power of the particle radius.

   2) The polydispersity index can be indicative of sample quality and hence for the suitability of DLS to measure the sample in question.

   3) Cumulants may be useful for QC applications in particular, but see caveat in 1) above.

   4) The use of software that generates Gaussian distributions from a mean value and a polydispersity index is deprecated, as the generated distributions may not correlate to the actual particle size distribution of the sample.

d) Is a size distribution rather than just a mean/modal size required?

   1) The basic distribution analysis yields a scattered intensity-weighted hydrodynamic size distribution.

   2) To convert the intensity-weighted distribution to volume or number basis, the complex refractive index for the sample material is required. However, it is deprecated to convert from intensity to volume and especially to number basis due to the inherent errors involved in this process, except for comparative purposes.

   3) Due to low resolution of DLS, decentiles (e.g. x_{10}, x_{20}) can carry high uncertainties, especially those away from the median diameter. Therefore, use of decentiles is not recommended.

NOTE The mean size of the distribution can differ from that obtained by cumulants analysis.

4.3 Appropriateness of samples for DLS analysis

a) If sedimentation is clearly observed over a time period relevant to the measurement, then the sample is not appropriate for DLS analysis.

Sedimentation will manifest itself as a trend towards smaller particle sizes over time, so a simple check for excessive sedimentation is to re-measure the same suspension after some period of time has elapsed.

Potential solutions are filtration to remove the sedimenting fraction or the choice of other techniques, for example laser diffraction.

b) If the sample contains a substantial amount of very coarse particles, then it may not be appropriate for DLS.

Very coarse particles can be removed by filtration unless these very coarse particles are of interest in the analysis.

c) If the medium is shear thinning or generally non-Newtonian, and the zero-shear viscosity is not known or cannot be measured, then the sample is probably not appropriate for DLS analysis.

If the purpose of the analysis is a comparison of different samples of the same composition (for example comparison of different batches or observation of a sample over time) rather than the determination of an absolute size, these effects are not relevant, as they affect all samples in the same way.

d) If the sample is too highly concentrated, multiple scattering, particle-particle interactions and restricted diffusion can influence the result (see ISO 22412:2017, B.2). Measurement of such samples may require dilution or specialized equipment. For example, certain instrument configurations can be used to minimize multiple scattering. Measurement at different dilutions is the method of choice to detect these effects.

NOTE ISO/TR 19997 describes methods for diluting a sample with the dispersing liquid.

e) In some instances, the particles can be excited by the incident light causing fluorescence that interferes with DLS measurements. As is the case for absorption [see f) and 4.4.5], also fluorescence is wavelength dependent.

When fluorescence is a problem, two approaches can be used to avoid or minimize its influence. One is using a different wavelength (a longer wavelength than the original) that does not generate fluorescence. The other is the installation of a narrow bandpass filter that blocks the fluorescent interference from reaching the detector.

f) If the sample is darkly coloured, this may interfere with DLS analysis due to absorption of laser light (see 4.4.5).

Potential solutions include dilution of the sample, measurement at a wavelength in which the medium does not absorb light or measurement in backscattering mode.

4.4 Sample preparation

4.4.1 General

Sample preparation is conducted with due consideration of the purpose of the measurement. This process also considers the light scattering properties of the sample and the way the signal is detected and processed. For instance, in a typical DLS measurement, scattered light is detected from a very small well-defined volume within the suspension. The intensity is, as stated previously, highly dependent on the particle size, and the rate of intensity fluctuations results from interference phenomena due to light scattered by many particles simultaneously. The following are important considerations for sample preparation.

4.4.2 Dispersion

The sample particles are uniformly dispersed in a suspension medium during the measurement in order to perform the measurement with high accuracy and reproducibility. As the measurement volume, which is called “scattering volume”, is very small in a DLS measurement, the uniformity of a sample suspension strongly influences the determination of particle size.

It can be difficult to disperse particles, especially inorganic particles, down to constituent particles without additives or the application of mechanical or ultrasonic energy. To improve the dispersibility of particles, one or more dispersants can be added to the sample suspension, and/or ultrasonic treatment can be used if it does not alter the sample properties. The dispersants, which may be inorganic species, surfactants or polymers, adsorb to the particle surface to increase surface charge and/or provide steric repulsion (this adsorption also increases the apparent particle size by a few nanometres). The principal dissociative groups of dispersants are phosphoric acid, carboxylic acid, sulfonic acid, and amine. The concentration of dispersant is higher than the amount needed to stabilize the suspension but below the critical micelle formation concentration. Typical dispersants for aqueous suspensions are shown in Table 1. The appropriate dispersant depends on the condition of sample suspensions such as particle size, concentration and shape. For further details, see Reference [2].

In addition, pH adjustment can increase the surface charge of particles. The pH of a sample suspension can be adjusted by adding an acid or alkali to make it lower or higher than the isoelectric point of sample particles. For a general discussion on this issue, see Reference [3]. The acid or alkali added to a sample suspension have ions in common to a sample and do not interact with particles.

One needs to filter solvents and other dilution media so as to reach a particle size that is equal to or lower than the expected particle size of the sample. For example, if 20 nm to 70 nm particles are to be measured, then filtration of the dispersion medium through a 0,02 µm filter is recommended. Measurement of the dilution medium (filtered or not) by DLS can ensure that it does not contain particles that interfere with the measurement of the sample.

NOTE Some filter materials can shed particles and contaminate the sample or can interact with the dispersion medium.

Table 1 — Typical dispersants for aqueous suspension

Category	Anion/Cation	Dispersant
Inorganic compound	Anion	Polyphosphoric acid
Surfactant	Anion	Alkylsulfonic acid
	Cation	Quaternary amine
Polymer	Anion	Polycarboxylic acid Polyacrylic acid Naphthalenesulfonic acid
	Non-ionic	Polyethylene glycol

4.4.3 Filtering of sedimenting particle systems

When the average particle size tends to decrease with repeated measurements, sedimentation within the sample is suspected and the accuracy of the measurement might be compromised. If the sedimenting fraction does not contain the target particles, it can be removed by filtration with an appropriate pore size and filter material or by centrifugation.

NOTE Filtration significantly changes the particles available for analysis by removing an unknown fraction of oversize particles/aggregates/agglomerates and potentially also particles of interest. Depending on the use of the results, filtration can be inappropriate.

A filter is chosen that is not changed (in the worst case: dissolved) by the medium, which means that often different filter types are used for aqueous and organic media. When a protein solution of low concentration is filtered, the use of a hydrophilic polyvinylidene fluoride (PVDF) or polyethersulfone membrane filter is recommended because of its low adsorption affinity. Elution from the membrane

or housing unit of the filter sometimes occurs and this depends on the combination of the membrane material and sample suspension. The elution can be reduced by pre-filtering with the solvent before sample filtering.

If filtering is not appropriate, the sedimentation rate can be reduced by dilution with a high viscosity solvent such as glycerol or ethylene glycol solution on condition that the diluent does not alter the sample property and the viscosity of the solvent is known.

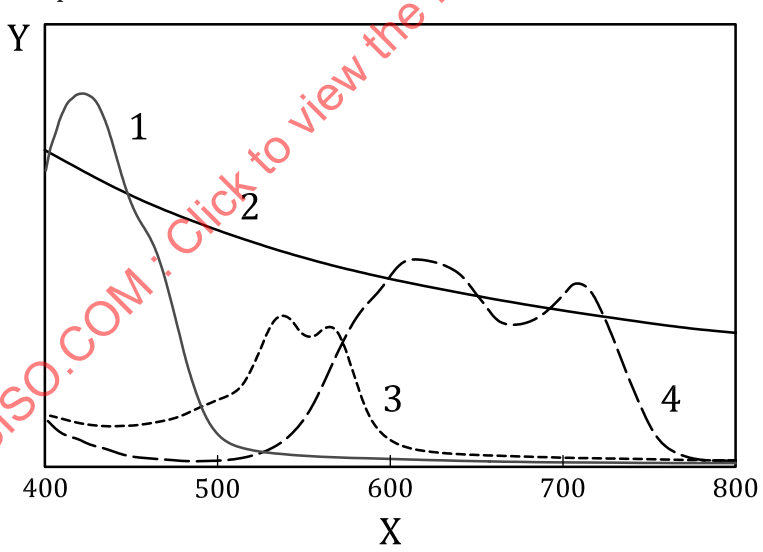
4.4.4 Dissolution and expansion

The size of particles dispersed in a liquid can change due to dissolution or swelling. For example, organic particles such as microcapsules can expand when heated or after the addition of a small amount of organic solvent. Metal nanoparticles may become smaller by dissolution, either after initial dispersion or after changing the temperature, pH adjustments or addition of detergents to existing dispersions. Gelatine particles, which have a network structure by weak non-covalent bonds between particles, may separate into constituent particles by dilution or ultrasonic treatment.

The extent of these size changes can be determined by measuring the suspension several times and checking if there is a trend towards larger or smaller particle sizes.

4.4.5 Colour of samples

Due to the absorption of laser light, especially red light sources such as He-Ne laser, some samples with a dark colour such as black, dark blue and dark brown can be difficult to measure. Absorption depends on the wavelength and Figure 1 shows typical absorption spectra of inks for inkjet printers in the visible region, as an example.



Key

X wavelength (nm)
Y absorbance
1 yellow
2 black
3 magenta
4 cyan

Figure 1 — Examples of absorption spectra of inks for inkjet printer

Two effects are possible.

a) Absorption of the incident and scattered light can mean that the intensity of the scattered light at the detector is insufficient for a reliable measurement. This effect is mainly observed in correlation analysis with a 90° viewing angle and is less pronounced in frequency analysis and backscattering setup.

   The scattered intensity at the detector is checked to determine if it is sufficiently high for analysis (follow recommendations of the instrument manufacturer).

b) Absorption of laser light may create temperature inhomogeneities in the sample, which interfere with particle size measurement.

Measurements at different concentrations, at different laser powers, using laser wavelengths different from the absorption maximum of the sample or using optical filters can be performed to check whether the effect is significant. If the results are unchanged, the effect is likely to be small. Alternatively, when measuring a sample that absorbs laser light, it is recommended to measure the sample suspension at the lowest possible concentration.

4.4.6 Dilution

As the optimum particle concentration depends on the particle size and refractive index difference between particles and medium, it is hard to give general guidance. Measurement in highly concentrated dispersions can show effects of multiple scattering, particle-particle interactions and restricted diffusion (see ISO 22412:2017, B.2). On the other hand, a sample suspension of extremely low concentration increases the measurement uncertainty because of the low intensity of the scattered light. In addition, if the number of particles in the detection volume is too small, the statistical error increases. At least 1 000 particles in the scattering volume are required to minimize the number fluctuation in DLS measurement[8]. An intensity fluctuation of 3 % or less shows that a sufficient number of particles are present in the detection volume, so that the entering or exiting of one or two particles into the measurement volume during measurement does not cause a significant change of scattering intensity. In cases of low intensity or low particle numbers, standard deviation will increase and longer run times are necessary for a given precision (see 4.7.2 for an example of the effect of longer run times).

At high concentrations, the apparent particle diameter is influenced by multiple scattering and particle-particle interactions. The effects of these two mechanisms are shown schematically in Figure 2. While the effects of multiple scattering can be reduced using backscattering or cross-correlation, particle-particle interaction and restricted diffusion effects can only be eliminated by dilution.

a) Multiple scattering **b) Particle-particle interactions**

Key

X volume fraction

Y diameter

NOTE The onset of both effects is usually not at the same concentration. At high concentrations both effects are present and the net effect is not predictable.

Figure 2 — Effects of multiple scattering and particle-particle interactions

Therefore, to check whether the measurement is influenced by high concentration effects, a dilution-ladder is performed for each measurement. For this, the suspension is diluted several times and measured. A change in the measured mean size suggests that multiple scattering is present. The results of any concentration in the flat region of the concentration/diameter curve are used.

NOTE 1 It is important that the chemical composition of the diluent matches the chemistry of the original dispersion liquid as closely as possible to avoid destabilization of dispersions and emulsions.

NOTE 2 In rare cases, the effects of multiple scattering and particle-particle interactions compensate and cancel each other. This is, however, only true for selected samples and is not a general rule.

4.4.7 Generation of air bubbles

When measuring a cooled sample at room temperature, care is taken of air bubble generation. As the temperature rises, the dissolved gas can be released as air bubbles, which behave like large particles. Allowing sufficiently long equilibration and lightly tapping the cell to remove bubbles will generally mitigate this effect. The sample can also be degassed prior to analysis (e.g. under vacuum).

4.4.8 Measurement cell (only relevant when using cuvettes in a homodyne setup)

The measurement cell may be re-usable optical glass or quartz, or disposable plastic. The material of the cell is resistant to the dispersion medium and sample particles. The measurement cell is clean and does not have any scratches on its surface. As the light intensity scattered from particles with diameters < 100 nm is proportional to the sixth (homodyne setup) or third (heterodyne setup) power of the particle size, even a tiny amount of dust such as visible particles and fibres can obstruct accurate measurement when the specimen contains very small particles and is dilute.

If disposable plastic cuvettes are used, the protective packaging material is put back on the unused cuvettes to avoid accumulation of dust. It may be necessary to precondition cells by cleaning them with filtered ethanol and water if they are too dusty.

4.5 Instrument verification

4.5.1 General

DLS is a method based on first principles and does not require calibration by the user with a particle size reference material. However, the metrological traceability of the DLS results depends on the accuracy of several input factors that are verified at regular intervals. In addition, the method performance is checked at regular intervals to ensure reliability.

4.5.2 Operation qualification

The operation qualification is performed at least yearly and after each major change of the instrument (such as repairs, change of location, etc.), or in case of doubt about the validity of measurement results. The following parameters are checked initially by the manufacturer of the instrument and later during routine maintenance either by the user or the service technician.

4.5.2.1 Temperature correctness

The temperature of the measurement cell is verified. To this end, the temperature of the cell is set to a certain temperature and the correctness of this temperature is verified with a (calibrated) thermometer/thermocouple. This procedure is repeated with at least a second temperature.

The temperatures cover the measurement range observed in practice.

The temperature given by the instrument is in compliance if it differs by at most 0,3 °C.

For some instruments, considerable knowledge and practice is necessary to minimize experimental errors. If the sample temperature does not match, the manufacturer should be contacted.

Sufficient time is required to allow equilibration of thermometer with the sample. Also not all instruments are configured to allow this verification.

4.5.2.2 Backscattered light intensity

The following test verifies the proper functioning of the complete optical system (laser, optical fibres, mirrors and photodetector). It is performed at least yearly, but more frequent tests are recommended.

A high purity solvent (e.g. chromatography grade) with sufficient scatter intensity (e.g. toluene) is filtered using a nominal pore size of 20 nm and put into a quartz cuvette and placed in the instrument.

The count rate is measured in the backscattering mode according to the manufacturer's specifications.

The acceptable ranges for the count rate depend on the instrument specifications.

4.5.2.3 Correctness and repeatability of the size of a certified reference material with a narrow size distribution

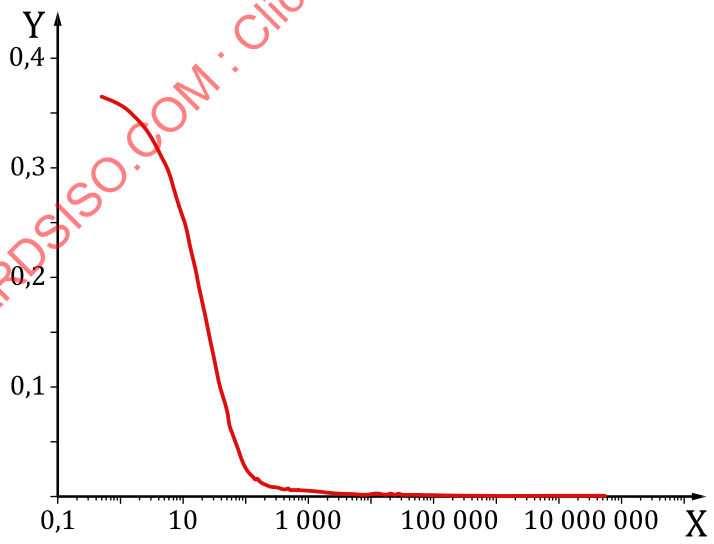
See ISO 22412:2017, Clause 10.

4.6 Data quality and interpretation: Correlation analysis

4.6.1 Interpretation of correlograms

The correlogram is essentially displaying the correlation coefficients in each time delay channel and provides information about the sample. The shape of the curve can indicate certain problems, such as noise/spikes. Noisy data is often due to factors such as the count rate being too low, the sample being unstable or external effects such as vibration or interference.

Figure 3 shows an initial rapid decay indicative of small particles being present, followed by a slower gradient indicative of medium range polydispersity, and the long tail, starting at about 1 000 µs, is indicative of large particles or aggregates as the baseline is not flat.



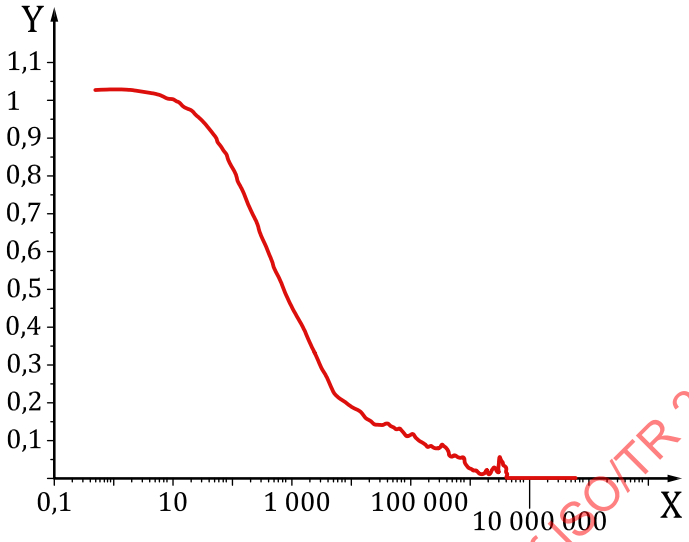
Key

X delay time (µs)

Y correlation coefficient

Figure 3 — Example of a correlogram of a material with some large particles present

The spread of correlogram of Figure 4 indicates high polydispersity. The slope from 1 000 µs onwards is indicative of large particles being present. The lack of a flat baseline is particularly telling. The intercept of the correlation function is > 1 which is indicative of number fluctuations occurring.



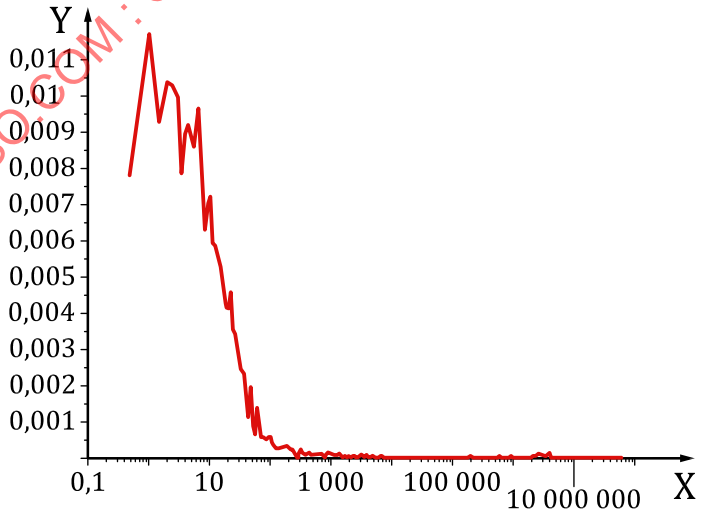
Key

X delay time (µs)

Y correlation coefficient

Figure 4 — Example of a correlogram of a material of high polydispersity

Figure 5 shows a very noisy correlation function with an extremely low intercept. This may be indicative of a sample undergoing fluorescence. Fluorescent light is random and uncorrelated. This adds to the baseline, thereby reducing the intercept in the correlogram, which is tantamount to a reduction in the signal-to-noise in the measurement. The vendor may be able to inform about various filter options, but ultimately a different wavelength laser may be the only option.



Key

X delay time (µs)

Y correlation coefficient

Figure 5 — Example of a correlogram of a material with a low intercept

4.6.2 Interpretation of particle size distribution

The simplest conversion of a correlogram into a particle size distribution is by the cumulants analysis (ISO 22412:2017, 9.2.1) which yields an average particle size distribution and a polydispersity index. This describes an assumed Gaussian distribution, but just in terms of a mean and spread. If a distribution is required, then a distribution calculation algorithm is required (see ISO 22412:2017, 9.2.2).

As pointed out in ISO 22412:2017, 9.1, DLS is not capable of resolving narrowly spaced peaks in the particle size distribution. While it is mathematically possible to calculate percentiles, values away from mass-median-diameter, D_{50}, will have increasingly large uncertainties and are primarily used for qualitative and not quantitative comparisons.

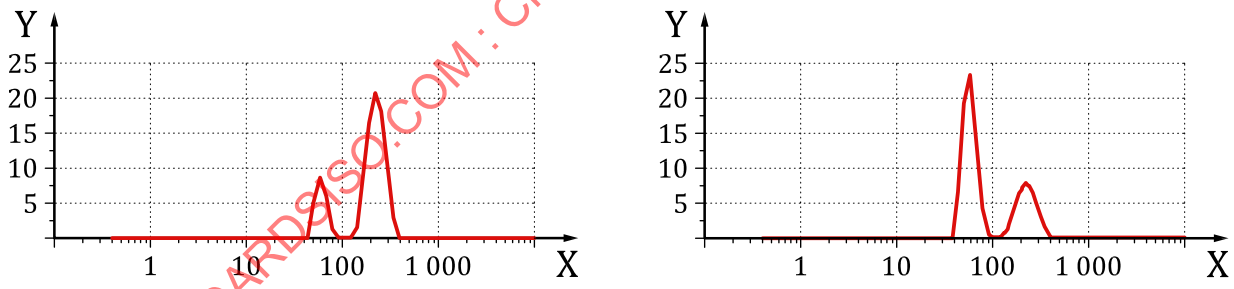
Results from inverse algorithms are far more sensitive to the quality of the measured data than the cumulants procedure. When a bi- or multimodal distribution is detected, measurement at different scattering angles can be used if available to improve the confidence of inverse algorithms.

4.6.3 Conversion from intensity to volume or number-based results

The original signal obtained by DLS is intensity based. Conversion to volume or number results is possible in many instruments. As described in ISO 22412:2017, 9.1 errors will be magnified, so conversion is generally deprecated.

DLS will overestimate the width of peaks in the distribution and further transformation will compound this issue. Volume and intensity distributions are only used for estimation of the relative amounts of material in separate peaks. The widths are less reliable. For multimodal distributions the intensity PSD is used for reporting the size of each component peak, but volume can provide useful insight into relative quantities. In the case of a multicomponent sample with separate peaks with a user requiring the volume contribution, a volume conversion can provide some information (as shown below).

For example, in a 2:1 volume mixture of 60 nm and 220 nm polystyrene latex standards, the intensity distribution shows sizes correctly, but the relative percentages are not correct (see Figure 6). A transformation to volume recovers the relative amounts correctly. The results of the intensity and volume-based distributions are given in Table 2.



a) Intensity-based distribution **b) Volume-based distribution**

Key

X diameter (nm)
Y intensity (%)

X diameter (nm)
Y volume (%)

Figure 6 — Intensity based and volume-based distribution of a 2:1 volume mixture of particles of 60 nm and 220 nm diameter polystyrene latex particles

Table 2 — Results of the intensity-based and volume-based distribution of a 2:1 volume mixture of 60 nm and 220 nm polystyrene latex particles

Peak	Hydrodynamic diameter nm	% intensity	% volume
1	59	21	67
2	220	79	33

4.6.4 Influence of the observed scattering angle

High angle/backscatter measurements are often used to measure samples at high concentrations. Traditional 90° systems primarily use lower concentrations.

While there still may be differences between measurements at different angles, with a modern instrument these would normally be reduced due to improvements in electronics sensitivities. There may be differences in amounts detected: lower angles will show more sensitivity to larger material than high angles and vice versa. Ideally when comparing instruments, the same angle would ideally be used.

4.6.5 How to judge good data quality

The quality of data obtained from the measurement is essential in determining how well the distribution algorithm (if used) will perform. There are several parameters which can be examined which can provide useful insight into the state of the sample

4.6.5.1 Raw signal repeatability

At least three consecutive measurements are performed on the same sample, the raw signal (photon count rate, photocurrent etc.) for stable samples varies by no more than a few percent. If there is a larger variation, the trend is examined.

An increasing raw signal from successive measurements will be indicative of particle aggregation.

A decreasing raw signal from successive measurements will be indicative of particle sedimentation/creaming or particle dissolution.

Large random raw signal variations between measurements can be due to general material instability or poor sampling.

4.6.5.2 Average diameter repeatability

The estimate for the central value (harmonic mean, median, z-average, etc.) obtained from repeat measurements is within 2 % of one another.

Increasing average diameters will normally be indicative of particle aggregation.

Decreasing average diameters will normally be indicative of particle sedimentation/creaming or particle dissolution. In rare cases, a steady change in temperature can, through a steady change of viscosity, also lead a trend in the measured size.

If the sample has a different polydispersity index or a different size distribution from the one expected, then several approaches may be considered. The sample could actually be too polydisperse for the evaluation algorithm chosen or for DLS measurements in general, so an orthogonal technique such as laser diffraction might be more suitable. Filtration of large particles from the sample by a device such as a syringe filter will remove the large particles from the system.

4.6.5.3 Multiple scattering/concentration effects

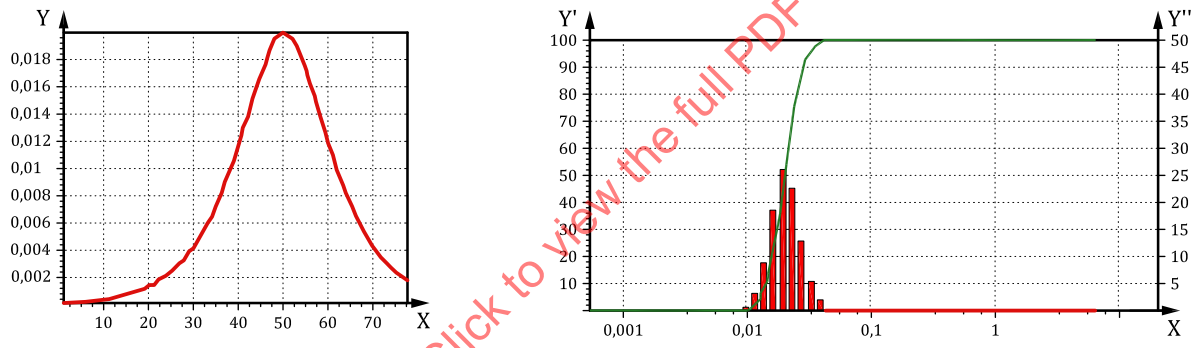
As described in Annex B of ISO22412:2017, it is recommended that, as part of method development, a dilution series is performed on a material to check the consistency of the answer upon dilution. If the sample is similar in size upon dilution, the occurrence of multiple scattering or particle-particle interaction is unlikely. If a size change is observed, further dilutions are performed until a concentration-independent region is reached. Alternatively, extrapolation to zero concentration may be feasible.

4.7 Data quality and interpretation: Frequency power spectrum analysis

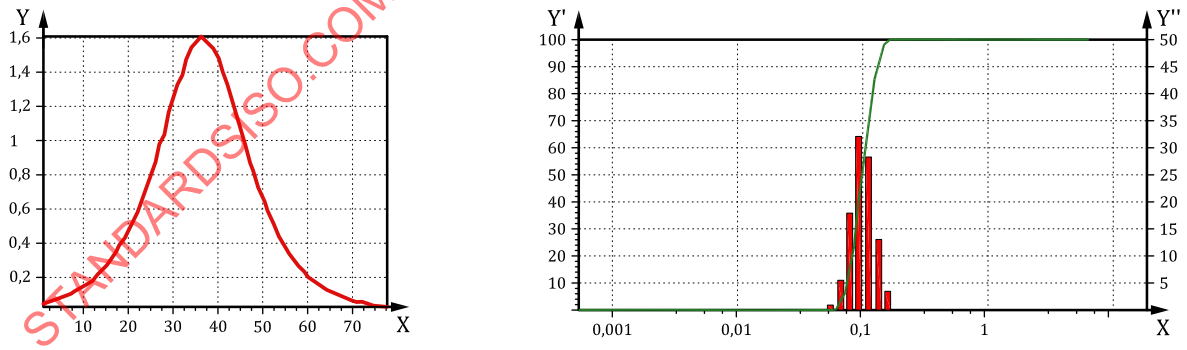
4.7.1 Frequency power spectrum

A second type of instrument uses heterodyne optical detection, frequency power spectrum signal processing and iterative deconvolution particle distribution analysis. For a narrow single mode particle size distribution, the aspect of the power spectrum for different particle sizes is shown in Figure 7. The power spectrum for each size is the same bell curve shape but positioned at a characteristic frequency channel. Large particles at the low frequency uses channels to the left and small particles at the high frequency channels to the right. From the channel location of the power spectrum maximum the particle size can be approximated.

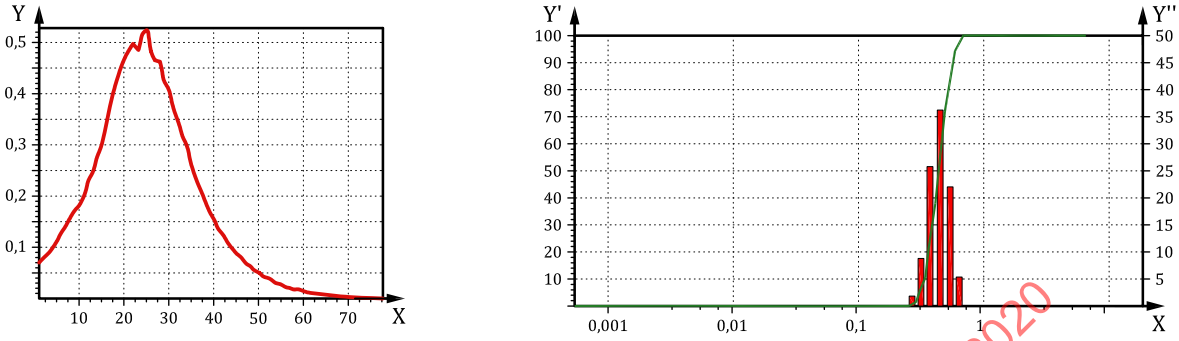
The analysis of the power spectrum results in a PSD which is narrow, without structure and repeatable.



a) Mean particle diameter 20 nm



b) Mean particle diameter 100 nm



c) Mean particle diameter 450 nm

Key

X	channel	X	diameter (µm)
Y	amplitude	Y'	cumulative undersize (%)
		Y"	% channel

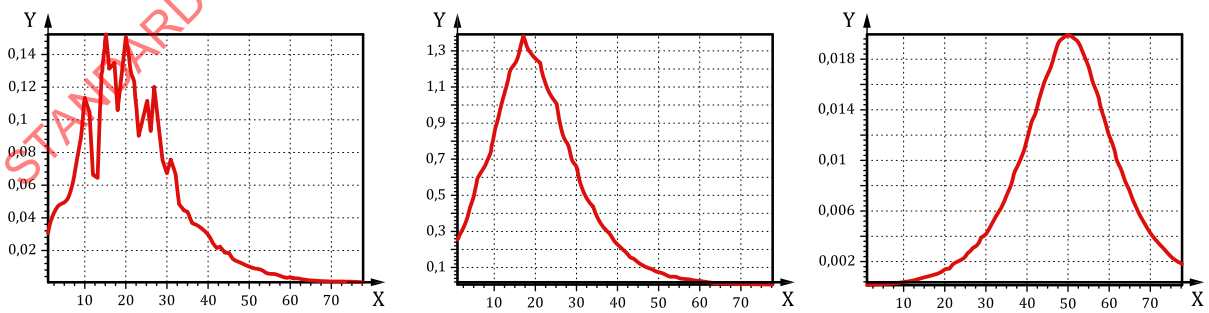
Figure 7 — Typical power spectra and intensity-based particle size distributions of monomodal samples of low polydispersity of mean particle diameter of 20 nm, 100 nm and 450 nm

4.7.2 Precision and run time

The frequency power spectrum is collected by continuously averaging the measured power spectrum over the full selected run time. Statistically, the longer the data averaging the smaller the statistical uncertainty in each frequency channel of the power spectrum. The uncertainty in the calculated PSD will follow the uncertainty in the power spectrum. The variation is reduced by the square of the run time for a power spectrum.

The uncertainty of low frequency channels populated from large particle scattering is larger than the high frequency channels populated from small particles. Therefore, longer run times are required for larger particles to obtain the same uncertainty as a measurement of smaller particles. To determine the appropriate run time, repeated runs at various run times are performed and compared to the standard deviations obtained.

Examples of power spectra for large and small particles are shown in Figure 8.



a) Short run time/large particles **b) Long run time/large particles** **c) Short run time/small particles**

Key

X channel

Y amplitude

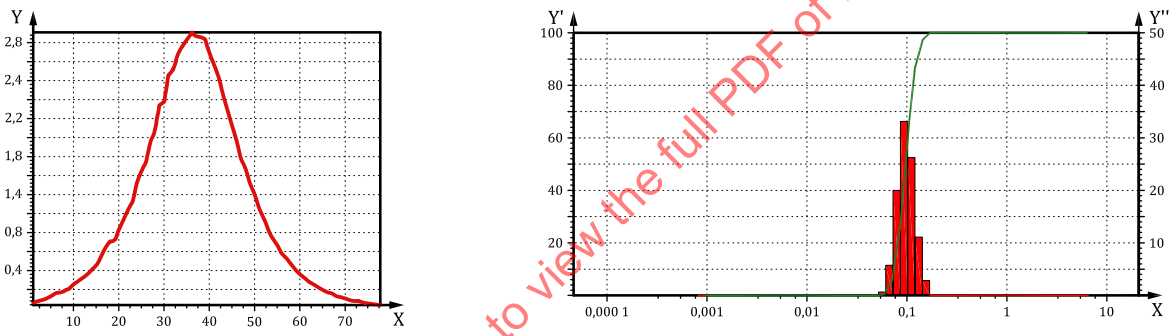
Figure 8 — Power spectrum in relation to the total run time

The required PSD accuracy determines the run time required to produce the needed power spectrum accuracy. Good practice is to perform test measurements of the power spectrum at various run times. At the run time that produces acceptable power spectra and PSD, the user performs multiple runs and determines the coefficient of variation for the parameter of interest. Increasing the run time will improve the results.

4.7.3 Sample quality

4.7.3.1 Contamination — Agglomeration — Settling

A single mode sample run with a long run time will have a power spectrum which is symmetric with negligible structure (see Figure 9).



Key

X channel

Y amplitude

X diameter (µm)

Y' cumulative undersize (%)

Y" channel (%)

Figure 9 — Power spectrum and intensity-based particle size distributions of a monomodal material

A power spectrum showing structure at the low frequency side is indicative of large size particle content (see Figure 10). A mode is detected in the particle size distribution. Good practice is to repeat the measurement and determine the stability of the suspension. If this mode content remains constant, it is indicative of a contaminant.