



BSI Standards Publication

Particle size analysis — Dynamic light scattering (DLS)

National foreword

This British Standard is the UK implementation of ISO 22412:2025. It supersedes BS ISO 22412:2017, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee LBI/37, Particle characterization including sieving.

A list of organizations represented on this committee can be obtained on request to its committee manager.

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Published by BSI Standards Limited 2025

ISBN 978 0 539 23661 3

ICS 19.120

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 September 2025.

Amendments/corrigenda issued since publication

Date	Text affected



**International
Standard**

ISO 22412

**Particle size analysis — Dynamic
light scattering (DLS)**

Analyse granulométrique — Dispersion lumineuse dynamique (DLD)

**Third edition
2025-09**

Reference number
ISO 22412:2025(en)

Full document details: BS ISO 22412:2025

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CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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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 document 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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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

This third edition cancels and replaces the second edition (ISO 22412:2017), which has been technically revised.

The main changes are as follows:

- inclusion of multi-angle dynamic light scattering (MADLS);
- inclusion of imaging dynamic light scattering (DLS);
- addition of [Clause A.5](#) on polarisation-separated backscatter photon cross-correlation spectroscopy;
- extension of [Annex B](#) on online measurements;
- incorporation of the content from ISO TR 22814 into [Annexes C](#) and [D](#).

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

Particle size analysis in the sub micrometre size range is performed on a routine basis using the dynamic light scattering (DLS) technique, which probes the hydrodynamic mobility of the particles. The technique is successful because it provides estimates of the average particle size and size distribution within a few minutes, and because user-friendly commercial instruments are available. Nevertheless, proper use of the instrument and interpretation of the result involve certain precautions.

The principle of DLS for a concentrated suspension is the same as for a dilute suspension. However, specific requirements for the instrument setup and specification of test sample preparation are specified for concentrated suspensions. At high concentrations, particle-particle interactions and multiple light scattering can become dominant and can result in apparent particle sizes that differ between concentrated and dilute suspensions.

DLS is also referred to as “quasi-elastic light scattering (QELS)” and “photon correlation spectroscopy (PCS),” although PCS is actually one of the measurement methods.

Several methods have been developed for DLS. These methods can be classified in several ways:

- a) by the difference in raw data acquisition (autocorrelation, cross-correlation and frequency analysis, spatial correlation);
- b) by the difference in optical setup (homodyne mode versus heterodyne mode);
- c) by the angle of observation.

In addition, instruments show differences with respect to the type of laser source and often allow application of different data analysis algorithms, e.g. cumulants, non-negative least squares (NNLS), CONTIN, etc.

Particle size analysis — Dynamic light scattering (DLS)

1 Scope

This document specifies the application of dynamic light scattering (DLS) to the following:

- measurement of average hydrodynamic particle size;
- measurement of the size distribution of mainly sub micrometre-sized particles, emulsions or fine bubbles dispersed in liquids.

This document is applicable to the measurement of a broad range of dilute and concentrated suspensions.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1 particle

minute piece of matter with defined physical boundaries

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: A particle can move as a unit.

[SOURCE: ISO 26824:2022, 1.1, modified — Note 3 to entry has been deleted.]

3.2 average hydrodynamic diameter

$\bar{x}_{\text{DLS}}$

hydrodynamic diameter that reflects the central value of the underlying *particle* (3.1) size distribution

Note 1 to entry: The average particle diameter is either directly determined without calculation of the particle size distribution, or calculated from the computed intensity-, volume- or number-weighted particle size distribution or from its fitted (transformed) density function. The exact nature of the average particle diameter depends on the evaluation algorithm.

Note 2 to entry: The cumulants method yields a scattered light intensity-weighted harmonic mean particle diameter, which is sometimes also referred to as the “z-average diameter.”

Note 3 to entry: Arithmetic, geometric and harmonic mean values can be calculated from the particle size distribution according to ISO 9276-2.

Note 4 to entry: Mean values calculated from density functions (linear abscissa) and transformed density functions (logarithmic abscissa) can differ significantly (see ISO 9276-1).

Note 5 to entry: $\bar{x}_{DLS}$ also depends on the particle shape, polydispersity and the scattering vector (and thus on the angle of observation, laser wavelength and refractive index of the suspension medium).

Note 6 to entry: The hydrodynamic radius may also be specified.

3.3

polydispersity index

PI

dimensionless measure of the broadness of the size distribution

3.4

scattering volume

volume defined by the intersection of the incident laser beam and the scattered light intercepted by the detector

3.5

scattered intensity

intensity of the light scattered by the *particles* (3.1) in the *scattering volume* (3.4)

3.6

count rate

I_s

number of photon pulses per unit time

Note 1 to entry: In frequency analysis, the term photocurrent captures a similar concept. The current at the photodetector is proportional to the scattered intensity (3.5) as measured by a detector.

3.7

validation

proof with *reference material* (3.8) that a measurement procedure is acceptable for all elements of its scope

Note 1 to entry: Evaluation of trueness requires a *certified reference material* (3.9).

3.8

reference material

RM

material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

Note 1 to entry: Reference material is a generic term.

Note 2 to entry: Properties can be quantitative or qualitative, e.g. identity of substances or species.

Note 3 to entry: Uses may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control.

Note 4 to entry: ISO/IEC Guide 99:2007 has an analogous definition, but restricts the term “measurement” to apply to quantitative values. However, Note 3 of the definition in ISO/IEC Guide 99:2007 specifically includes qualitative properties, called “nominal properties”.

[SOURCE: ISO 17034:2016, 3.3]

3.9

certified reference material

CRM

reference material (3.8) characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

Note 1 to entry: The concept of value includes a nominal property or a qualitative attribute such as identity or sequence. Uncertainties for such attributes may be expressed as probabilities or levels of confidence.

Note 2 to entry: Metrologically valid procedures for the production and certification of reference materials are given in, among others, ISO 33405.

Note 3 to entry: ISO 33401 gives guidance on the contents of reference material certificates.

Note 4 to entry: ISO/IEC Guide 99:2007 has an analogous definition.

[SOURCE: ISO 17034:2016, 3.2]

3.10 qualification

proof with *reference material* (3.8) that an instrument is operating in agreement with its specifications

4 Symbols and units

For the purposes of this document, the following symbols and units apply.

$C(\Gamma)$	normalized distribution function of decay rates or characteristic frequencies	dimensionless	
D_T	translational diffusion coefficient	metres squared per second	m^2/s
D_c	collective diffusion coefficient	metres squared per second	m^2/s
D_s	self-diffusion coefficient	metres squared per second	m^2/s
f	frequency, $f = \omega/(2\pi)$	Hertz	Hz
$g^{(1)}(\tau)$	normalized electric field correlation function	dimensionless	
$G^{(2)}(\tau)$	scattered intensity correlation function	arbitrary units	
$G(\Gamma_j)$	normalized distribution function of the individual decay rate Γ_j	arbitrary units	
I_s	scattered intensity, count rate, photocurrent	arbitrary units	
I_0	intensity of the incident light	arbitrary units	
M	number of steps in the histogram	dimensionless	
n	refractive index of the suspension medium	dimensionless	
$P(\omega)$	power spectrum	arbitrary units	
PI	polydispersity index	dimensionless	
$\Delta Q_{\text{int},i}$	scattered light intensity-weighted amount of particles in size fraction i , i.e. $x_{i-1} < x \leq x_i$	dimensionless	
x	hydrodynamic diameter of a particle (within this document)	nanometres	nm
$\bar{x}_{\text{DLS}}$	average hydrodynamic diameter	nanometres	nm

$\bar{f}$	scattered light intensity-weighted average value of the distribution function of the decay rate or characteristic frequency	reciprocal seconds	s ⁻¹
$\Gamma_{\max}$	maximum decay rate (histogram method)	reciprocal seconds	s ⁻¹
$\Gamma_{\min}$	minimum decay rate (histogram method)	reciprocal seconds	s ⁻¹
η	viscosity of the suspension medium	Milli Pascal seconds	mPa·s
θ	scattering angle	degrees	°
λ_0	wavelength of the laser light in vacuum	nanometres	nm
μ_2	second cumulant of the distribution function of decay rates or characteristic frequencies	reciprocal square seconds	s ⁻²
ρ	particle density	grams per cubic centimetre	g/cm ³
τ	correlation time	seconds	s
q	modulus of the scattering wave vector	reciprocal nanometres	nm ⁻¹
φ	particle volume fraction	dimensionless	
ω	angular frequency	radian per seconds	rad/s

5 Principle

Particles suspended in a fluid are in constant Brownian motion as the result of the interaction with the molecules of the suspending fluid. In the Stokes-Einstein theory of Brownian motion,^[1] particle motion of smooth spheres at very low concentration is determined by the suspending fluid viscosity and temperature, as well as the size of the particles. Thus, from a measurement of the particle motion in a fluid of known temperature and viscosity, the particle size can be determined.

The DLS technique^{[2],[3],[4],[5],[6]} probes the particle motion optically. The suspended particles are illuminated with a coherent monochromatic light source. The light scattered from the moving suspended particles has a time-dependent phase imparted to it from the time-dependent position. The time-dependent phase of the scattered light can be considered either as a time-dependent phase shift or as a spectral frequency shift from the central frequency of the light source. Measured over time, random particle motion forms a distribution of optical phase shifts or spectral frequency shifts. These shifts are determined by comparison either with all scattered light (homodyne or self-beating mode) or by using a portion of the incident light as reference (heterodyne mode). Regardless of the setup, the optical signals received from the particles are related to the scattering efficiency of the particles and are thus scattered intensity-weighted.

Sedimentation of particles, dependent on their density, sets an upper limit to the particle size that can be assessed by the technique; typically, the upper limit is much less than 10 µm.

For very small particles, the impact of the molecules of the dispersing liquid on the particles becomes relevant and thus sets a lower size limit to the validity of the Stokes-Einstein equation.^[7] The bias is usually smaller than 1 % for particles with diameters larger than 10 nm, but it will increase for particles with diameters of less than 10 nm.

DLS was developed for static suspensions. If orthogonal flow and observation axes are adopted, flowing samples may, under some circumstances, be measured if the procedure is properly validated (see [Annex B](#)).

Different modes of diffusion, particle-particle interaction, multiple scattering and fluorescence can significantly influence the apparent particle diameter calculated from a DLS experiment. [Annexes D](#) and [E](#) should be consulted.

6 Apparatus

A typical apparatus consists of the following components:

6.1 Laser, emitting polarized coherent monochromatic light. Any kind of lasers may be used, e.g. gas lasers (He-Ne laser, Ar-ion laser), solid-state lasers, diode-pumped solid-state lasers and laser diodes.

6.2 Optics, lenses and equipment used to focus the incident laser light into a scattering volume and to detect scattered light. Optical fibres are often used as a part of the detection system and for light-delivering optics.

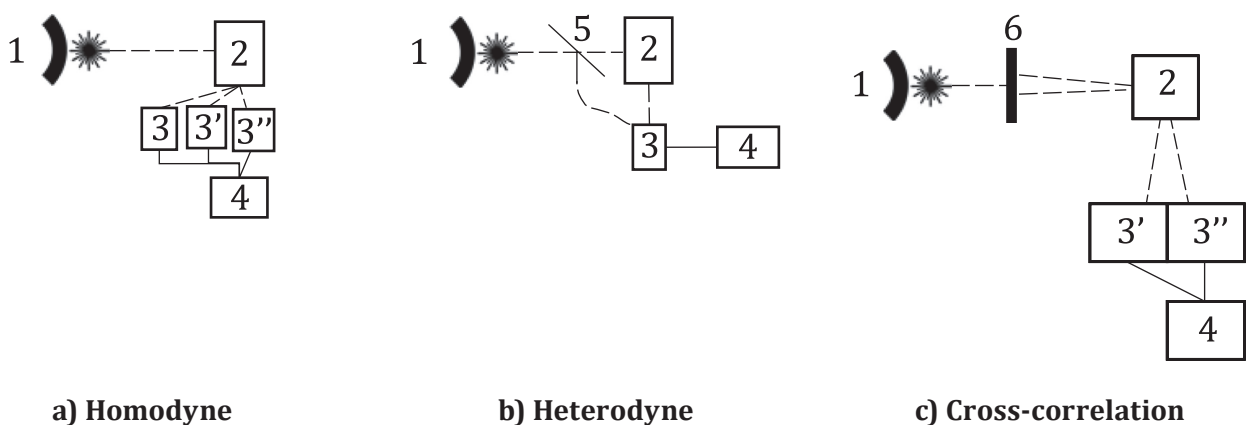
The use of a coherent optical reference allows using interference between the scattered light and the reference to measure the frequency shift of the scattered light. Three methods of referencing are commonly used and are illustrated in [Figures 1 a\)](#) and [b\)](#). A cross-correlation setup using two simultaneous homodyne experiments is shown in [1 c\)](#).

— In homodyne detection (also referred to as “self-beating detection”), the mixing at the optical detector of all of the collected scattered light provides the reference for frequency- or phase-difference measurement. See [Figure 1 a\)](#).

— In heterodyne detection, the scattered light is mixed with a portion of the incident light. The unshifted incident light provides the reference for the frequency- or phase-difference measurement. See [Figure 1 b\)](#).

NOTE In DLS, “heterodyne” is understood as mixing of scattered light with unscattered light from the same source. This convention differs from, for example, the use in optical interferometry.

— In a cross-correlation setup, two homodyne scattering measurements are performed simultaneously in such a way that the two scattering vectors and scattering volumes are the same, but the corresponding wave vectors are not coincidental. These two laser beams produce two correlated fluctuation patterns. The correlation intercept is reduced because both detectors collect light from the other scattering experiment. This cross scattering is suppressed by state-of-the-art instruments (for a description of polarisation-separated backscatter photon cross-correlation spectroscopy (PsB PCCS) see [A.5](#)). The contribution of the multiply scattered light to the detector signals, however, does not contribute to the cross-correlation function, i.e. does not contribute to the PSD, but to an enhanced background. [[Figure 1 c\)](#)]



Key

- | | |
|------------|--|
| 1 | laser |
| 2 | sample |
| 3, 3', 3'' | detector(s): one or several (not limited to three) detectors can be used |
| 4 | correlator, spectrum analyser or computer data processing |
| 5 | reflection area |
| 6 | beam splitter and optics |

Figure 1 — Typical optical arrangements

6.3 Test sample holder, allowing fluctuations of the sample temperature to be controlled to within $\pm 0,3$ °C. While precise knowledge of the sample temperature is required for evaluation, it is not necessary to regulate the temperature to any defined value. However, setting a specific temperature is necessary in some cases to prevent temperature driven effects like convection, demixing, coagulation, etc.

6.4 Photodetector, with an output that is proportionally related to the intensity of the collected scattered light. A photomultiplier tube or an (avalanche) photodiode or a charge-coupled device (CCD)/ complimentary metal-oxide-semiconductor (CMOS) is typically used. In imaging DLS, the detector is a CMOS chip that records spatially distinct signals. Detectors can be placed at any angle, or, like in multi-angle DLS, in more than one angle. Data collection can be performed in a linear or logarithmic manner. In the image DLS and ultrafast image DLS methods, data collection can be performed continuously for hundreds and thousands of images, or only one pair of images.

The position of the detector determines the scattering angle of the measurement, which can have an influence on the measurement result. At particle diameters smaller than about $\lambda/10$, mainly isotropic Rayleigh light scattering is observed, whereas particle diameters $> \lambda/10$ (Mie light scattering) result in a size dependent pattern of minima and maxima.

Detection at backscattering angles (typically about 150° to about 175°) can be used to accommodate different geometries where the 90° (side scatter) can be blocked. Often, backscatter detection means that the path length of light through the sample is shorter and can be less affected by multiple scattering.

The short path length also means that the light intensity from highly-diluted, weak scatterers can be insufficient for a reliable result.

Detection at a scattering angle of about 90° is often recommended for weak scatterers as this setup blocks the flare of laser light at the cuvette wall.

Detection at forward scattering angles (typically about 15°) is especially sensitive to large particles, which scatter more light in the forward direction, and is therefore well suited to monitor few large particles or to monitor agglomeration. However, forward scatter can suffer from the most artefacts due to cleanliness or contaminated systems.

6.5 Signal processing unit, capable of taking the time-dependent scattered light intensity signal and outputting the autocorrelation function or autocorrelation coefficient, cross-correlation function or power spectrum of the input signal. This correlation can be performed by either hardware or software correlators, or both, operating linearly, logarithmically or in a mixed mode.

The resulting output from either mode contains a distribution of characteristic frequencies or time-dependent phases representative of the particle size of the suspended particles. Photon detection has a probability distribution of photon arrival times, which means that a fluctuating signal is obtained even if the intensity of the incident light is constant. The intensity of the photons arriving at varying time intervals is superimposed on this already fluctuating signal. In correlation analysis, the uncorrelated signal is constant, whereas the signal associated with the diffusing particles decays exponentially. In spectrum analysis, the uncorrelated signal is akin to a DC or zero frequency term, which is not recorded. The time-dependent component is analysed to determine the particle-size distribution using the theory of DLS.

6.6 Computation unit, capable of signal processing to obtain either the particle size or particle size distribution, or both. Some computation units also function as the signal-processing unit.

- Evaluation via the autocorrelation function allows determination of a mean diameter without determination of the particle size distribution, but determination of the distribution is also possible.
- Evaluation via the frequency distribution determines the particle size distribution using the power spectrum of the signal.
- Evaluation via photon cross-correlation allows quantification and minimization of the effects of multiple scattering, thus extending the useful concentration range towards higher concentrations (however, the effect of particle-particle interaction cannot be eliminated).

6.7 Instrument location, placed in a clean environment, free from excessive electrical noise and mechanical vibration and out of direct sunlight. If organic liquids are used as the suspension medium, the area shall be well ventilated. The instrument shall be placed on a rigid table or bench to avoid the necessity for frequent realignment of the optical system.

WARNING — DLS instruments are equipped with a low- or medium-power laser whose radiation can cause permanent eye damage. Never look into the direct path of the laser beam or its reflections. Ensure highly reflecting surfaces are not in the path of the laser beam when the laser is on. It is the responsibility of the user of this document to determine the applicability of local regulations for laser radiation safety.

7 Test sample preparation

7.1 General

Test samples should consist of well-dispersed particles in a liquid medium. Dispersion procedures like sonication, filtration, etc. can influence the result and therefore shall be reported. The suspension liquid shall:

- a) be sufficiently transparent (non-absorbing) and non-fluorescent at the laser wavelength to create a clear signal;
- b) be free of particulate contamination;
- c) not dissolve, swell or coagulate the particulate material;
- d) have a known refractive index that is sufficiently different from that of the particulate materials to create a clear signal;
- e) have a known value of viscosity within $\pm 2\%$ over the operational range of temperature to be used;

NOTE As $\bar{x}_{DLS}$ is directly proportional to η , the uncertainty of $\bar{x}_{DLS}$ will always be larger than the uncertainty of η .

- f) meet the guidelines of the instrument for low background scattering.

This can be checked by measuring the count rate for the suspending medium alone and the dark count with no sample or solvent present. The former should be at least one order of magnitude lower than the sample, and the latter should be within the recommended range for the instrument.

Inadequate suppression of the double layer can have a significant influence on the hydrodynamic diameter. A medium with ionic strength high enough to suppress the electric or diffuse double layer can improve agreement between results obtained by DLS and electron microscopy. A conductivity of 1 mS/cm is usually sufficient to achieve this between the hydrodynamic diameter and that obtained by microscopy techniques, especially for small particles.

Water is often used as a suspension medium. The use of freshly deionised and filtered (pore size 0,2 μm) water is recommended. A trace of ionic additive (e.g. NaCl at a concentration of 10 mmol/l = 0,6 g/l) may be added to such samples to reduce the double-layer thickness. However, precaution must be made that such ionic strength adjustment will not make sample unstable or that the additive does not react with the sample (e.g. Cl with Ag ions).

7.2 Concentration limits

The lower concentration limit of the working range of DLS is determined, amongst other factors like particle size, detector sensitivity, etc., by the number of particles that are present in the scattering volume.

The scattered light intensity (e.g. expressed as count rate or I_s) of the sample containing the dispersed particles should ideally be ≥ 10 times the signal obtained by the suspension medium alone. Scattered intensity ratios below 10, either caused by low particle mass fractions or by very broad particle size distributions, will result in a higher variation of results and poorer precision.

The maximum concentration of dispersed particles that can be measured without the concentration influencing the particle size reported is determined by particle-particle interaction and multiple scattering. This concentration limit should be determined empirically by dilution (see [Annex C](#)).

7.3 Checks for concentration suitability

Different instruments adopt differing optical observation angles and optical arrangements. The observations and checks given are for the general case, but the specific instrument operational advice should also be considered.

The following observations and checks are recommended.

- a) Visually inspect the dispersed sample prior to placing it into the instrument. At low concentrations, the sample will look almost transparent. At higher concentrations, a milky or opaque appearance is seen.
- b) Ensure that the sample is placed in the instrument prior to performing the measurement, allowing the sample to equalize its temperature. Check the count rate or signal level. Check the count rate or signal level is appropriate, per the instrument manufacturer's recommendations.
- c) For instruments using correlation analysis in autocorrelation mode, conduct a measurement using appropriate correlator settings and examine the intercept value, which should be above the value specified by the vendor of the instrument. A low value of the intercept value corresponds to the factor B in [Formula A.3](#) and can result from either a poorly aligned optical system, multiple scattering or from very weakly scattering samples requiring the detector aperture be increased, resulting in a multiple coherence area detection. For larger particles, the measurement volume can need to be increased to accommodate an adequate number of particles. This can also reduce the intercept value. A low value of the intercept can also be caused by sample absorbance or fluorescence. All of these factors can reduce the intercept value, requiring further tests to establish the reason.

A low intercept can also result from low sample concentration (at or below the limit of quantitation), inappropriate optical settings (laser power, aperture, attenuation, etc.), or incorrect loading of the sample. Autocorrelation functions that do not decay smoothly to baseline may indicate contamination with large particles, back reflections, or fluorescence.

- d) Measurements performed at different concentrations should give the same results within their measurement uncertainties. A decreasing particle size with increasing concentration indicates a significant amount of multiple scattering, a change of viscosity of the suspension caused by different viscosities of diluent and original suspension and/or collective motion of particles which can result from intermolecular (or particle-particle) interactions. Measurement at different concentrations is also used to extrapolate the particle size to infinite dilution [see [Clause 8 k](#)].

In many applications, a volume fraction (φ) of dispersed particulate material in the range 10^{-5} to 10^{-4} fulfils the requirements for particle sizes below about 500 nm. For cross-correlation or backscatter methods, higher concentrations can be achieved dependent on the sample. For either polydisperse or larger particles, or both, it is potentially not possible to find a concentration that satisfies all requirements without either increasing the coherence aperture of the receiver or increasing the diameter of the incident laser beam in order to increase the measurement volume. If this is the case, then the intercept values obtained will potentially not meet the criterion set out in c). For particle sizes above 1 μm , the requirements c) and d) can only be fulfilled in exceptional cases.

All sample preparation steps (suspension medium, particulate concentration, dispersion procedure) should be recorded. Recommendations for sample preparation are given in [Annex C](#).

8 Measurement procedure

A typical measurement consists of the following steps:

- a) Switch the instrument on and allow it to warm up. Typically, about 15 min to 30 min is required to stabilize the laser intensity and to bring the sample holder to an equilibrium at the desired temperature.

NOTE 1 Monitoring the temperature rather than controlling it is sufficient for many applications, provided a known value of viscosity is available for the reported monitored temperature.

- b) A measurement cell filled only with suspension medium should be checked to ensure a low count rate or signal level, without radical fluctuations, which can indicate particle contamination. A high count rate or signal level can indicate cell flare or dirty cell walls. For instruments that provide background subtraction, measure and store the background signal for the dispersion medium being used.
- c) Visually inspect the sample for the presence of optically visible particles, flocs, fibres and other possible contaminants. If these are present, repeat the sample preparation.
- d) The measurement cell can be disposable (e.g. PMMA, polystyrene) or re-usable (e.g. optical-quality glass or quartz). Compared to disposable plastic cells, glass cells have as main advantage that their use can result in a better signal to noise ratio. The walls of plastic cuvettes are easily scratched and do not provide the optical quality of glass or quartz. Therefore, disposable cuvettes should not be used with weak scattering samples, and should be cleaned only by either rinsing with suspending medium or by using a particle-free air stream to remove loose dust on the cell walls, or both.

The material of the measurement cell must be chemically compatible with the dispersion medium and the particles. Clean and prepare measurement cells according to manufacturer's instructions. Additional recommendations for cell cleaning are given in Annex [C.4](#).

The pore size of the filter used for filtering the water or solvent should be appropriate to the application. Ideally, a membrane with a pore size smaller than the smallest particle to be measured should be used.

Take care not to touch the windows of the measurement cell with bare hands or to wipe the cell surface with any potentially abrasive material (including optical paper or tissue). Transfer a required amount of sample to a suitable and clean measurement cell. Ensure that no air bubbles are entrapped in the test sample for non-air bubble samples. Ensure that no bubbles are attached to the walls of the cell. Place the test sample in the instrument or place the measurement probe into the sample. Allow temperature equilibrium to be established. The temperature value should be known to better than $\pm 0,5$ °C with minimal fluctuation of the value during the measurement.

Uncertainties in particle size determined in aqueous suspensions will be approximately 2 % per degree Celsius at ambient temperature if the test sample has not reached thermal equilibrium.

NOTE 2 For a temperature change of 3 °C, it can take about 10 min for the liquid in the measurement volume of a measuring cell holding a 1 ml of sample to thermally equilibrate.

- e) Record test sample identification, date and time of the measurement and measurement duration, number of individual measurements, measurement temperature, temperature fluctuations during measurement, refractive index and viscosity of the suspension medium, particle concentration or dilution, wavelength of laser and scattering angle, as well as the particle concentration, if known.
- f) Check the average scattered intensity of the sample.

For homodyne optical arrangements, it is preferred that the average scattering intensity be controlled by adjusting the light output power, using neutral density filters, or by minimizing the detector aperture, to maintain detection coherence, while adjusting the receiver sensitivity within the limits specified by the manufacturer.

For homodyne optical arrangements, it is preferred that the average scattering intensity be controlled within the limits specified by the manufacturer. The scattering signal from the test sample should be ≥ 10 times the signal from the dispersing medium alone.

For heterodyne optical arrangements, the reference signal should be substantially greater than the test sample scattering signal (a ratio reference signal:scattering signal of 10:1 should be aimed for). It is preferred that the reference signal can be blocked so that the scattering signal can be assessed as being greater than the suspension signal alone.

- g) Measurements should not be continued if the light signal intensity contains isolated bursts of high count rates, which can indicate contamination of the test sample.

- h) Measurements should not be continued if the correlation function does not decline monotonically or if the power spectrum is not of Lorentz type.
- i) Record the average particle diameter, $\bar{x}_{\text{DLS}}$, and polydispersity index (PI), for each of the measurements performed using the cumulants method.
- j) If a systematic concentration dependence of the average particle size is observed, the results of an extrapolation to infinite dilution (or the results obtained at the lowest acceptable concentration) shall be reported. The dilution shall be performed with particle-free suspension medium, containing the same concentration of salts, surfactants, pH, etc., in order to not alter the particle-solution interactions.

Although the checks described here will minimize biasing effects due to multiple scattering, particle interactions can potentially, in particular for particles below 100 nm (diameter) at volume fractions above 0,01, bias the estimation of the average diameter. Therefore, for unknown dispersed systems, it is recommended that measurements are performed on at least two concentrations varying by a factor of at least two.

- k) Check at the end of the measurement that no significant sedimentation has occurred in the test sample, either by visually checking for sediment or by inspecting the results of multiple, sequential measurements for trends. If sedimentation is found, then it should be decided whether it is small enough so that its effects on the precision of the measurement are acceptable or whether the sample is unsuitable for DLS measurements.

9 Evaluation of results

9.1 General

DLS is a low-resolution technique that is not capable of resolving narrowly spaced peaks in the particle size distribution. The low resolution also means that values away from mass median diameter will have increasingly large uncertainties.

The original signal obtained by DLS is scattered intensity weighted. Many instruments allow calculation of volume (mass) or number-weighted results from the scattered intensity-weighted signal. For most methods, this involves smoothing of the scattered intensity-weighted particle size distribution, Q_{int} , which introduces uncertainties. A second issue affecting all methods is the highly nonlinear dependence of the scattering intensity on the particle size. Therefore, transformation of scattered intensity-weighted distributions to volume or number distributions is not recommended and number distributions from DLS are specifically deprecated, especially for methods involving the smoothing of Q_{int} .

A particle size distribution determined by DLS is based on different physical properties than one determined by, for example, laser diffraction (diffraction of light) or microscopy (transmission of electrons), and therefore they can be different.

For a given size distribution, the distribution $C(\Gamma)$ of decay rates or characteristic frequencies is dependent on laser wavelength and state of polarization and on the scattering angle θ . Hence, x_{DLS} and PI of a given sample depend on those quantities.

Different algorithms exist for the evaluation of results. These algorithms will, for the same sample, often give different results. Reporting of the algorithm and any internal settings or choices, together with the results, is therefore crucial. [Clause 9](#) summarizes those algorithms that are sufficiently standardized today. More information on the theoretical background is given in [Annex A](#) and a very brief overview is found in Reference [8]. Guidance on measurement planning, data interpretation, quality control and potential artefacts is given in Annexes [D](#) and [E](#).

9.2 Correlation analysis

9.2.1 Cumulants method

The cumulants analysis compresses the entire multi-exponential decay distribution into the exponent and then expands the exponent into a polynomial expression. Two parameters describing particle size distributions, i.e. the average particle diameter $\bar{x}_{DLS}$ and the polydispersity index PI, are determined by a variant of the so-called “cumulants method”.^[4]

Obtaining x_{DLS} and PI from the correlation function using the cumulants method can be accomplished by a nonlinear least-squares fitting of the correlation function. Refer to [Annex A](#) for additional considerations with cumulants analysis. The average value and polydispersity obtained from the cumulants method are scattering intensity weighted.

9.2.2 Distribution calculation algorithms

In these algorithms, the distribution of diffusion coefficients for a particle suspension or molecular solution is calculated by applying a multi-exponential fitting algorithm to the correlation function. The output of these algorithms is a particle size distribution from which, if needed, an average particle size can be determined.

9.2.2.1 Regularized non-negative least squares (NNLS)

The NNLS algorithm fits the exponential decay of the correlation function algebraically. There are a variety of parameters that can be altered in an NNLS algorithm, but the two principal ones are the “weighting scheme” and the “alpha parameter” or “regularizer.” Data weighting is used in DLS algorithms to amplify subtle changes in the larger and more significant correlation coefficients over noise in the baseline. In the absence of data weighting, noise in the baseline can lead to the appearance of artefact peaks and erroneous data interpretation.

The regularizer or alpha parameter in NNLS-based dynamic light scattering algorithms controls the acceptable degree of “spikiness” in the resultant distribution. Deconvolution of the DLS-measured correlation function is accomplished using an inverse Laplace transform that is ultimately reduced to a linear combination of eigenfunctions. The caveat to this approach is that when the eigenvalues are small, a very small amount of noise can make the number of possible solutions extremely large, hence the labelling of the DLS method as an ill-posed problem. In order to overcome the problem, a stabilizing term, in the form of the “first derivative” of the distribution solution, is added to the set of eigenfunctions.

The alpha parameter is the multiplier applied to this stabilizing term and defines the emphasis placed upon the derivative of the solution. Large alpha values (0,1) limit the spikiness of the solution, leading to smooth distributions. Small alpha values (0,000 01) decrease the weighting or importance of the derivative, subsequently generating more spiky distributions. Therefore, the alpha parameter can be loosely described as an estimate of the expected level of noise in the measured correlation function. There is no ideal or best alpha parameter. The appropriate value depends on the sample being analysed. For mixtures of narrow mode (low polydispersity) and strongly scattering particles, decreasing the alpha parameter can sometimes enhance the resolution in the scattered intensity particle size distribution.

9.2.2.2 CONTIN

The CONTIN algorithm^{[9],[10],[11]} uses a constrained regularized NNLS method combined with eigenfunction analysis to generate a smoothed solution and to reduce the number of degrees of freedom. The algorithm generates several solutions, of which the least tailed distribution consistent with the data are selected. It can resolve decay rates as long as they differ by at least a factor of 2.

Prior knowledge of the expected distribution is needed, as users must specify a number of constraints, e.g. the expected distribution range and the number of data points. The regularization parameters themselves can be automatically chosen based on an F-test and confidence levels.

9.2.2.3 Histogram method

The histogram method is an iterative method in which an initial distribution of $\Gamma(\Gamma = D_1 q^2)$ is repeatedly processed to give a final answer. In the analysis program, the initial distribution of Γ is a histogram with all the steps being of equal height. The algorithm is repeated to change the histogram to fit the raw data.^[12]

The histogram approach has the advantage that the number of steps M is a variable. Thus, when the data become less precise, the M can reduce correspondingly. Initial values for $G(\Gamma_j)$ and for $\Gamma_{\min}$ and $\Gamma_{\max}$ signalling the start and stop of the range of the histogram are immaterial. The fitting procedure terminates when the limits of error are within the statistical error of measured data. Distributions obtained by this method tend to be broad and have connected peaks.

9.2.2.4 Other algorithms

There are a number of other algorithms and programs which include L-Curve^{[13],[14]} regularized positive exponential sum^[15] and others under various names^{[16],[17]}, which are also based on NNLS analysis. In general, the difference between these algorithms is the optimization of variables (e.g. the “regularizer” or alpha parameter and the weighting scheme implemented) within the NNLS analysis in order to optimize it for a given set of instrument and sample conditions.

9.3 Frequency analysis

In frequency analysis, the signal from the detector is sampled at regular intervals and at a frequency permitting the analysis of the highest frequency required. The sampled values are then subjected to fast Fourier transformation (FFT) to yield a frequency power spectrum.

The basis for the calculation is the iterative fitting of the experimentally obtained power spectrum using a number of predefined size and frequency channels to obtain the complete particle size distribution. For example, a size range of 1 nm to 6 500 nm requires a 16 kHz linear power spectrum. The 16 kHz linear power spectrum is then converted into 80 channels with channel width in a logarithmic progression. Size channels are defined in the same progression. From this distribution, mean diameters, PI and other metrics can be calculated:

- Step 1: Size channels 1-N are defined in a logarithmic progression. Frequency channels 1 to N are defined in the same logarithmic progression and the measured linear power spectrum is converted to logarithmic channels.
- Step 2: A response is calculated for each of the N frequency channels using [Formula \(A.26\)](#) yielding a first approximation of the particle size distribution, PSD.
- Step 3: The calculated response and the measured response are compared and a correction to the approximate PSD is made.

Steps 2 and 3 are repeated until the error between the calculated response and the measured response is minimized, yielding the best fit PSD. Typically, 80 size channels are used between 1 nm and 6 500 nm. The iterative deconvolution results in a full PSD conforming to ISO 9276 (all parts) with all size channels having equal scattered intensity weighting.

9.4 Multi angle dynamic light scattering (MADLS)

9.4.1 Measurement of an angular-independent particle size distribution

Multi-angle dynamic light scattering (MADLS) is an ensemble method capable of measuring an angular-independent particle size distribution.

Different types of MADLS instruments exist, either based on a goniometric set-up of the detector, with a possible range of scattering angles from less than 10° to more than 175°, or a set of fixed detectors, typically capturing backscatter, side-scatter and forward-scatter. In such systems, the time-dependent scattering intensity measured at different angular positions is used to generate different autocorrelation functions for each angle. These correlation functions can be considered independently to provide angular-

dependent scattering information, which can be utilized to gain information about rotational dynamics and concentration, or they may be subsequently concatenated to provide increased size resolution through intrinsic noise reduction.

In the case of concatenated data, a least squares algorithm is used to minimize the magnitude of the residual, that is the difference between the measured and the predicted autocorrelation function, to provide the best fit. It is important to note that if similar weightings are used for each angle, the solution will be disproportionally weighted towards the forward angles of detection. Therefore, each angular contribution to this residual must be weighted separately to minimize the uncertainty expected for each angle of detection but also to account for the expected scattered intensity. For this purpose, the real and imaginary parts of the refractive index of the particles are required for an accurate MADLS fitting.

The particle size distribution can then be calculated. For angle-independent results, the volume weighted distribution is the preferred solution, but intensity and number-weighted can also be determined. Intensity-weighted particle size distributions are always angular-dependent, and therefore are usually displayed as backscatter equivalent.

The main benefits of concatenated MADLS are two-fold:

- a) Noise optimization of the autocorrelation functions, as common components from different angles are emphasized while similarly non-common components, i.e. noise, are suppressed in the analysis.
- b) For non-isotropic scatterers, by utilizing signals from a range of angles, the method reduces the bias of Mie scattering effects. This allows for a better definition of the peak limits in the size distribution and to resolve more closely spaced size populations when compared to single-angle measurements.

The primary result obtained from a DLS measurement is based on the intensity of scattered light. For this intensity-based information, the only optical property required is the refractive index of the dispersant. However, if the results are to be converted to either volume or number, or both, or to a MADLS or particle concentration measurements are to be performed, the optical properties of the particles are required. These are the real refractive index of the material and the imaginary refractive index of the material.

The complex refractive index of the material is required if a volume distribution or number concentration is required and is not needed if the intensity distribution is being used. Three pieces of information are normally required, the refractive index of the dispersant, the real refractive index of the material and the imaginary refractive index of the material. ISO 13320:2020, Annex F contains a section providing sources of information for different measurement methods of the real refractive index. Many of the methods listed will require a large particle to determine the numbers used. An assumption must be made that the refractive index of a sample suitable for dynamic light scattering which is likely smaller than those suitable for laser diffraction is the same.

Some of the methods mentioned in the ISO 13320:2020 Annex F using index matching based on minimising scattering and measuring solution refractive index can be used as described.

The imaginary part of the refractive index is often established empirically or by experience. Traditional methods of measurement use ellipsometry of thin films and values obtained using these techniques are possibly not appropriate at the particle level. An assumption can be made that the imaginary used in dynamic light scattering should be the same or most likely lower than those used in laser diffraction based on the assumption that as particles get smaller they become more transparent. Reference books in various fields can be useful in finding the RI values.^{[50]-[58]}

It is also important to consider the limitations of this method, as constraints present in any of the individual angular measurements are likely to be transposed to the main collective analysis. These constraints will depend on the optical arrangement, including the measurement volume for each angle, and therefore it will be important to ascertain the size and sample concentration limits for this analysis for each instrument and sample type.

9.4.2 General angular and concentration dependence

The repeatability and accuracy of many DLS instruments are not high enough to permit evaluations of the effects of scattering angles and concentrations. It is worthwhile noting the importance of extrapolations based on variations of these two parameters.^{[18],[19]}

By utilizing the multi-angle approach, it is possible to measure the angular and concentration dependences of nanoparticles with sufficient precision to permit extrapolations of these two parameters to zero angle and to zero concentration (infinite dilution). The dependence of the apparent particle size of particle suspensions on the concentration and scattering angle can then be estimated using a dynamic Zimm-type plot based on a bilinear extrapolation of the two dependences. This method provides a means to correct for finite concentration and scattering angle (scattering vector) which will allow for better agreement of any experimental values of particle size obtained by using different instruments under different conditions of scattering angle (and wavelength) and concentrations.

The apparent particle size obtained by the MADLS, D_{app} , often depends on the light scattering angle and the particle concentration of the sample suspension. The dependence can be approximately represented by Formula (1):

$$D_{app}(q, C) = a_{00} + a_{10}C + a_{01}q^2 + a_{11}Cq^2 \quad (1)$$

where C is the particle number concentration of the suspension (number of particles/mL), and q is the magnitude of the scattering vector. The constants a_{00} , a_{10} , a_{01} , and a_{11} can be determined by fitting the equation to experimental data obtained with a MADLS instrument for various particle concentrations and scattering angles.^{[4],[20]} Optically anisotropic particles, electrostatic long-range interaction of particles, or large particle size distribution influence the angular and concentration dependence of MADLS. The "true" particle diameters are theoretically given by $D_{app}(q = 0, C = 0)$ or a_{00} .

9.4.3 Measurement of particles with optical anisotropy

Non-spherical particles such as rodlike, ellipsoidal, and disclike particles, usually exhibit optical anisotropy.

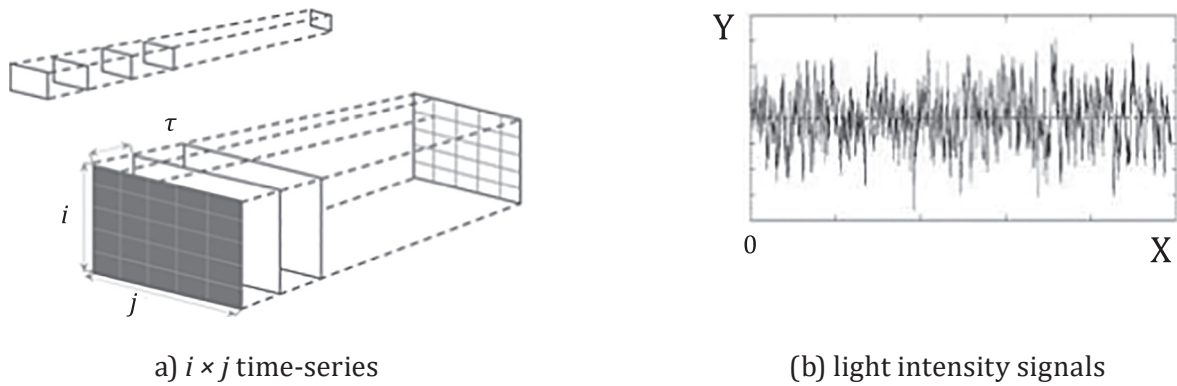
The decay of the correlation function by rotational diffusion is much faster than that by translational diffusion, so that it is often not possible to observe the rotational diffusion, however if the anisotropy is not negligible, the decay rate has two modes expressed by $\Gamma = Dq^2 + 6\Theta$. MADLS instruments fitted with a polarizer in front of the detector can detect the depolarized factor of the scattered light from the anisotropic particles. This enables the estimation of the rotational diffusion coefficient Θ which can serve as input to a numerical model to assess the particle shape.^{[4],[6]}

9.5 Imaging dynamic light scattering (DLS)

9.5.1 Image-based dynamic light scattering (IDLS)

In IDLS analysis, the detector is a CCD/CMOS sensor that captures a pair of images of two-dimensional spatial distribution of dynamic light scattering intensity of nanoparticles in the measurement volume at a very short time interval. The time interval is from milliseconds to microseconds depending on the nanoparticle size, scattering angle and laser wavelength. Due to the Brownian motion of nanoparticles, the images of the spatial distribution of the dynamic light scattering in the images are different.

The image consists of hundreds and thousands of pixels. One pixel or several pixels of the sensor is equivalent to one photomultiplier tube. As shown in [Figure 2 a\)](#), the images are divided into $i \times j$ grids, which are equivalent to the parallel measurement of $i \times j$ photomultiplier tubes. The measurement yields $i \times j$ one dimensional time-series of light intensity signals will be obtained simultaneously as shown in [Figure 2 b\)](#). Therefore, all correlation analysis methods in [9.2](#) may be used to process these time-series of light intensity signals to obtain the size distribution. With this method, the data acquisition time needs only few seconds or less than one second.

**Key**

X time (s)

Y $l(t)$

NOTE Refer to Reference [25] for further information.

Figure 2 — Images division for data processing**9.5.2 Ultrafast image-based dynamic light scattering (UIDLS)**

In fact, the autocorrelation coefficient can be obtained by only a pair of images. The two-dimensional autocorrelation coefficient of a pair of images can be calculated with FFT, then the mean diameter of nanoparticles in the measurement volume at the moment of measurement time can be determined by the Stokes-Einstein equation.

Continuously taking images, a series pair of images in short time will be captured. Then, a series of mean diameters of particles will be determined in a short time. The size distribution of nanoparticles can be obtained by all these results.

10 System qualification and quality control**10.1 System qualification**

Since particle size determination by DLS is an absolute method based upon first principles, calibration of the particle size scale with a particle size calibrant is not necessary. To ensure SI-traceable results, a number of the instrument parameters and test parameters should be measured with traceably calibrated tools (such as test temperature by the user, or laser wavelength and scattering angle by the manufacturer during production of the instrument). In addition, verification of the instrument's performance should be performed with suspensions of particles of certified size after installation of the instrument and at time intervals thereafter or in case of doubt.

Failure of the qualification indicates a problem with this particle suspension, the preparation of the test sample or the instrument.

Certified reference materials with values assigned for DLS using the same evaluation algorithm should be used. Ideally, the chemistry and morphology of the particles should match the test samples as closely as possible.

Alternatively, certified suspensions of polystyrene latex with narrow size distribution such as 5 % CV (standard deviation/average diameter) with average particle diameter as measured by DLS or electron microscopy can be used.

Further information on the proper selection of reference materials is given in ISO/TS 4807.

To assess potential bias, the uncertainty for the mean value of the CRM should be combined with a tolerance for the measurement uncertainty and expanded with a factor of 2 to obtain an expanded combined