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Standard Guide for
Measurement of Particle Size Distribution of Nanomaterials in Suspension by Photon Correlation Spectroscopy (PCS)[1]

This standard is issued under the fixed designation E2490; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide deals with the measurement of particle size distribution of suspended particles, which are solely or predominantly sub-100 nm, using the photon correlation (PCS) technique. It does not provide a complete measurement methodology for any specific nanomaterial, but provides a general overview and guide as to the methodology that should be followed for good practice, along with potential pitfalls.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E1617 Practice for Reporting Particle Size Characterization Data

F1877 Practice for Characterization of Particles

2.2 *ISO Standards:*

ISO 13320-1 Particle Size Analysis—Laser Diffraction Methods—Part 1: General Principles[3]

ISO 14488 Particulate Materials—Sampling and Sample Splitting for the Determination of Particulate Properties[3]

ISO 13321 Particle Size Analysis—Photon Correlation Spectroscopy[3]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 Some of the definitions in 3.1 will differ slightly from those used within other (non-particle sizing) standards (for example, repeatability, reproducibility). For the purposes of this Guide only, we utilize the stated definitions, as they enable the isolation of possible errors or differences in the measurement to be assigned to instrumental, dispersion or sampling variation.

3.1.2 *correlation coefficient, n*—measure of the correlation (or similarity/comparison) between 2 signals or a signal and itself at another point in time.

3.1.2.1 *Discussion*—If there is perfect correlation (the signals are identical), then this takes the value 1.00; with no correlation then the value is zero.

3.1.3 *correlogram or correlation function, n*—graphical representation of the correlation coefficient over time.

3.1.3.1 *Discussion*—This is typically an exponential decay.

3.1.4 *cumulants analysis, n*—mathematical fitting of the correlation function as a polynomial expansion that produces some estimate of the width of the particle size distribution.

3.1.5 *diffusion coefficient (self or collective), n*—a measure of the Brownian motion movement of a particle(s) in a medium.

3.1.5.1 *Discussion*—After measurement, the value is be inputted into in the Stokes-Einstein equation (Eq 1, see 7.2.1.2*(4)*). Diffusion coefficient units in photon correlation spectroscopy (PCS) measurements are typically $\mu m^2/s$.

3.1.6 *Mie region, n*—in this region (typically where the size of the particle is greater than half the wavelength of incident light), the light scattering behavior is complex and can only be interpreted with a more rigorous and exact (and all-encompassing) theory.

3.1.6.1 *Discussion*—This more exact theory can be used instead of the Rayleigh and Rayleigh-Gans-Debye approximations described in 3.1.8 and 3.1.9. The differences between the approximations and exact theory are typically small in the size range considered by this standard. Mie theory is needed in order to convert an intensity distribution to one based on volume or mass.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

3.1.7 *polydispersity index (PI), n*—descriptor of the width of the particle size distribution obtained from the second and third cumulants (see 8.3).

3.1.8 *Rayleigh-Gans-Debye region, n*—in this region (stated to be where the diameter of the particle is up to half the wavelength of incident light), the scattering tends to the forward direction, and again, an approximation can be used to describe the behavior of the particle with respect to incident light.

3.1.9 *Rayleigh region, n*—size limit below which the scattering intensity is isotropic—that is, there is no angular dependence for unpolarized light.

3.1.9.1 *Discussion*—Typically, this region is stated to be where the diameter of the particle is less than a tenth of the wavelength of the incident light. In this region a mathematical approximation can be used to predict the light-scattering behavior.

3.1.10 *repeatability, n*—in PCS and other particle sizing techniques, this usually refers to the precision of repeated consecutive measurements on the same group of particles and is normally expressed as a relative standard deviation (RSD) or coefficient of variation (C.V.).

3.1.10.1 *Discussion*—The repeatability value reflects the stability (instrumental, but mainly the sample) of the system over time. Changes in the sample could include dispersion (desired?) and settling.

3.1.11 *reproducibility, n*—in PCS and particle sizing this usually refers to second and further aliquots of the same bulk sample (and therefore is subject to the homogeneity or otherwise of the starting material and the sampling method employed).

3.1.11.1 *Discussion*—In a slurry system, it is often the largest error when repeated samples are taken. Other definitions of reproducibility also address the variability among single test results gathered from different laboratories when inter-laboratory testing is undertaken. It is to be noted that the same group of particles can never be measured in such a system of tests and therefore reproducibility values are typically be considerably in excess of repeatability values.

3.1.12 *robustness, n*—a measure of the change of the required parameter with deliberate and systematic variations in any or all of the key parameters that influence it.

3.1.12.1 *Discussion*—For example, dispersion time (ultrasound time and duration) almost certainly will affect the reported results. Variation in pH is likely to affect the degree of agglomeration and so forth.

3.1.13 *rotational diffusion, n*—a process by which the equilibrium statistical distribution of the overall orientation of molecules or particles is maintained or restored.

3.1.14 *translational diffusion, n*—a process by which the equilibrium statistical distribution of molecules or particles in space is maintained or restored.

3.1.15 *z-average, n*—harmonic intensity weighted average particle diameter (the type of diameter that is isolated in a PCS experiment; a harmonic-type average is usual in frequency analyses) (see 8.9).

3.2 *Acronyms:*

3.2.1 *APD*—avalanche photodiode detector

3.2.2 *CONTIN*—mathematical program for the solution of non-linear equations created by Stephen Provencher and extensively used in PCS (**1**).[4]

3.2.3 *CV*—coefficient of variation

3.2.4 *DLS*—dynamic light scattering

3.2.5 *NNLS*—non-negative least squares

3.2.6 *PCS*—photon correlation spectroscopy

3.2.7 *PMT*—photomultiplier tube

3.2.8 *QELS*—quasi-elastic light scattering

3.2.9 *RGB*—Rayleigh-Gans Debye

4. Summary of Guide

4.1 This Guide addresses the technique of photon correlation spectroscopy (PCS) alternatively known as dynamic light scattering (DLS) or quasi-elastic light scattering (QELS) used for the measurement of particle size within liquid systems. To avoid confusion, every usage of the term PCS implies that DLS or QELS can be used in its place.

5. Significance and Use

5.1 PCS is one of the very few techniques that are able to deal with the measurement of particle size distribution in the nano-size region. This Guide highlights this light scattering technique, generally applicable in the particle size range from the sub-nm region until the onset of sedimentation in the sample. The PCS technique is usually applied to slurries or suspensions of solid material in a liquid carrier. It is a first principles method (that is, calibration in the standard understanding of this word, is not involved). The measurement is hydrodynamically based and therefore provides size information in the suspending medium (typically water). Thus the hydrodynamic diameter will almost certainly differ from other size diameters isolated by other techniques and users of the PCS technique need to be aware of the distinction of the various descriptors of particle diameter before making comparisons between techniques. Notwithstanding the preceding sentence, the technique is widely applied in industry and academia as both a research and development tool and as a QC method for the characterization of submicron systems.

6. Reagents

6.1 In general, no reagents specific to the technique are necessary. However, dispersing and stabilizing agents often are required for a specific test sample in order to preserve colloidal stability during the measurement. A suitable diluent is used to achieve a particle concentration appropriate for the measurement. Particle size is likely to undergo change on dilution, as the ionic environment, within which the particles are dispersed, changes in nature or concentration. This is particularly noticeable when diluting a monodisperse latex. A latex that is

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[4] The boldface numbers in parentheses refer to the list of references at the end of this standard.

measured as 60 nm in $1 \times 10^{-3} M$ NaCl can have a hydrodynamic diameter of over 70 nm in $1 \times 10^{-6} M$ NaCl (close to deionized water). In order to minimize any changes in the system on dilution, it is common to use what is commonly called the "mother liquor". This is the liquid in which the particles exist in stable form and is usually obtained by centrifuging of the suspension or making up the same ionic nature of the dispersant liquid if knowledge of this material is available. Many biological materials are measured in a buffer (often phosphate), which confers the correct (range of) conditions of pH and ionic strength to assure stability of the system. Instability (usually through inadequate zeta potential (**2**) can promote agglomeration leading to settling or sedimentation in a solid-liquid system or creaming in a liquid-liquid system (emulsion). Such fundamental changes interfere with the stability of the suspension and need to be minimized as they affect the quality (accuracy and repeatability) of the reported measurements. These are likely to be investigated in any robustness experiment.

7. Procedure

7.1 *Verification:*

7.1.1 The instrument to be used in the determination should be verified for correct performance, within pre-defined quality control limits, by following protocols issued by the instrument manufacturer. These confirmation tests normally involve the use of one or more NIST-traceable particle size standards. In the sub-micron ($< 1 \times 10^{-6}$ m) region, then these standards (for example, NIST, Duke Scientific- now part of Thermo Fisher Scientific) tend to be nearly monodisperse (that is, narrow, single mode distribution, PI < 0.1) and, while confirming the *x* (size) axis, do not verify the *y* (or quantity axis). Further, there is a lack of available standards for the sub-20 nm region and therefore biological materials (for example, bovine serum albumin–BSA, cholesterol, haem, size controlled dendrimers, Au sols) of known size (often by molecular modeling) can be utilized. Note that PCS is a first principles measurement and thus calibration in the formal sense (adjustment of the instrument to read a true and known value) cannot be undertaken. In the event of a "failure" at the verification stage, then the issues to check involve quality of the dilution water, state of dispersion and stability of the standard under dilution plus instrumental issues such as thermal stability, cleanliness and alignment of optical components. The raw correlogram data can be examined during and after acquisition. Such examination requires some experience and training. During data acquisition one looks for stable count level without jumps or leaps in the level of the scattering counts that could be produced by particles (of dust or contamination) falling through the measurement zone ('number fluctuations'). Ideally the form of the correlogram is an exponential decay to a flat baseline (approximating to the photon counts in the system without sample) and not rise again (again indicating number fluctuations in the data). Manufacturers also provide other means of assuring the reliability of the data and is recommended that these protocols are consulted, as appropriate.

7.1.2 Given the nature of the produced intensity distribution and the likelihood that the size standard has been certified by electron microscopy (number distribution) care needs to be exercised in direct comparison of the results. For a completely monodisperse sample, (every particle identical) then the number and intensity distributions are essentially identical. For the real-world situation where there is some polydispersity (width) to the distribution, then the number distribution is expected to be smaller than the produced intensity distribution; the greater the polydispersity, then the larger the differences between intensity, volume and number distributions. Note that verification of a system only demonstrates that the instrument is performing adequately with the prescribed standard materials. Practical considerations for real-world materials (especially 'dispersion' if utilized or if the distribution is relatively polydisperse) mean that the method used to measure that real-world material needs to be carefully evaluated for precision (repeatability).

7.2 *Measurement:*

7.2.1 *Introduction:*

7.2.1.1 The measurement of particle size distribution in the nano- (sub 100 nm) region by light scattering depends on the interaction of light with matter and the random or Brownian motion that particle exhibits in liquid medium in free suspension. There must be an inhomogeneity in the refractive indices of particle and the medium within which it exists in order for light scattering to occur. Without such an inhomogeneity (for example, in so-called index-matched systems) there is no scattering and the particle is invisible to light and no measurements can be made by the PCS or any other light scattering technique.

7.2.1.2 For particles < 100 nm, as considered in this guide, several facts hold true:

(1) The amount of scattering is weak in relative terms and depends highly on the size of the particle. In the Rayleigh approximation region (typically $d < \lambda/10$ in which d is the diameter of particle and λ is the wavelength of light employed), then this intensity of scattering is proportional to r^6 – or $(\text{volume})^2$ or $(\text{relative molecular mass})^2$. With a commonly utilized helium-neon (He-Ne) laser (632.8 nm), then this limit is approximately 60 nm. This means, in practice, that a 60 nm particle scatters 1 million times as much light as a 6 nm particle of the same composition. Thus, it is imperative that solutions are kept free of any contaminating particles, for example dust, that are often present in the local environment and is usually considerably larger than the material that requires measurement. This means filtering liquids used to contain or dilute the particles to a least the same level as the size of the particles that require characterizing. The very weak scattering means that conventional light detectors (for example, silicon photodiodes) as used in other light scattering technique (for example, laser diffraction) cannot be used. The technique of correlating the signal with itself combined with photon counting techniques is thus employed; the principle being that the noise is random while the Brownian motion is fixed. Constantly subtracting the noise from the overall signal leaves the retained Brownian motion signal.

(2) The intensity of scattering in the Rayleigh region is inversely proportional to the fourth power of the wavelength of light employed. Thus, if the wavelength of incident light could

be halved then the intensity of scattering that would be observed is increased by a factor of 16. It is common practice to use lasers of a lower wavelength than a He-Ne (632.8 nm) to increase the amount of scattering and, hence, signal. This is usually preferable to increasing the power of the laser with possible undesired effects (for example, heating, convection currents). However, note that lower wavelengths sometimes overlap an absorption edge for some molecular species leading to a loss of signal intensity. Potential fluorescence issues also need consideration, as the detectors used for photon counting are usually responsive to a wide wavelength range. Sometimes, narrow bandwidth filters can be employed to ensure that only light of the correct wavelength is detected. Such means usually reduce or compromise the actual signal seen by the detector. The detector is typically either a photon multiplier tube (PMT) or avalanche photodiode (APD) as both count individual photons.

(3) For spherical particles, there is limited (assumed to be no) angular dependence of the scattering in the Rayleigh region for unpolarized light. This effective isotropic (or equal) scattering means that only a single detector angle need be employed to measure the scattered light. For non-spherical particles, rotational motion will give angular dependence (even in the Rayleigh region). Above the Rayleigh region (> 60 nm) the light starts to be scattered towards the forward angle—in layman's terms it becomes egg-shaped with more forward than back-scatter—and up to λ/2 (~ 300 nm for a He-Ne laser at 632.8 nm) then the Rayleigh-Gans-Debye approximation works well as there is little structure to the observed polar pattern of scattering. Thus, in the < 100 nm region of interest, then approximations can be usefully employed and a full explanation of the interaction of light with matter (Mie theory) need not be invoked unless the information is required to be presented on a volume or number basis (see 8.9).

(4) The measurement of size in the sub-100 nm region relies on the measurement of the amount of Brownian motion (in particular the diffusion coefficient) of the particle as formulated in the Stokes-Einstein equation:

$$R_h = \frac{kT}{(6\pi\eta D)} \tag{1}$$

where:

R_h = the hydrodynamic radius,
k = Boltzmann's Constant (= *R/N* where *R* = Gas constant and *N* = Avogadro's number),
T = the absolute temperature (Kelvin),
π = the universal constant,
η = the viscosity of the medium, and
D = the (measured) diffusion coefficient.

(5) Note that, in Eq 1, the density of the particle plays no role in Brownian motion (although, of course, it does in settling; see Point 9 below), even though this appears to be counterintuitive to first instinct. Note also that a hydrodynamic radius (or diameter) is derived. This refers to an equivalent size in spherical terms to that of a particle moving with the same diffusion coefficient as the observed particle. Thus, for an irregularly shaped particle or one with significant external morphology (or both), then the derived diameter is not likely to correspond to any measured axis of the image of the particle. The viscosity refers to the medium that the particle is dispersed in. In a dilute system it is assumed that the particles do not interact, so the viscosity can be assumed to be that of the medium or diluent. In higher concentrations, particles are likely to be in regions of hindered mobility and the effective viscosity is thus higher than that of the particle-free suspension medium.

(6) Note the term diffusion coefficient. There are two types of diffusion to be considered for particles in free suspension:

(a) Translational, where the so-called Stokes-Einstein relationship given in Eq 1 applies. Rewriting with the diffusion coefficient on the left:

$$D_t = \frac{kT}{6\pi\eta R_h} \tag{2}$$

(b) Rotational, where the Stokes-Einstein-Debye relation applies:

$$D_r = \frac{kT}{8\pi\eta(R_h)^3} \tag{3}$$

(7) Association of particles (or molecules) leads to changes in the rotational diffusion coefficient, which also affects the translational diffusion coefficient. Hence, interactions between particles can complicate the interpretation of the observed diffusion coefficient, which for nonspherical particles, is a combination of the translational and rotational diffusion coefficients. These particle-particle interactions tend to be concentration rather than size dependent, and both translational and rotational diffusion coefficients are dependent on the viscosity of the surrounding fluid.

(8) The motion of the particles must be random. Nonrandom particle motion is the main reason for apparent failure or nonapplicability of the technique. Such nonrandom motion can occur through convection currents being present in the system or through particles (too large or dense for the technique) settling during the measurement sequence. Therefore, accurate temperature control and stabilization are mandatory. If settling/sedimentation occurs in the measurement, other than to a very minor extent, then the result is almost certainly compromised, as it will reflect a changing and unstable system. If visible settled solid is present at the bottom of a container, then it is very likely that the PCS technique is not recommended. In this case conventional laser light scattering (laser diffraction) is likely to be the preferred technique. If settling can be observed either in the measurement container or in the measurement cuvette, then it is certain that the original material being measured is not "nano" or is unstable during the measurement time frame.

(9) With respect to size and density, consider the calculations in Table 1 using Stokes' Law.

(10) It can be deduced from Table 1 that if a material is truly "nano" (that is, < 100 nm), it tends to remain in permanent suspension and exhibits little if any settling tendency. In many situations, for example a gel, then the particle density is significantly lower due to incorporation of water into the particle matrix and thus the settling time increased further.

(11) Sometimes it is thought that placing the particles in a material of higher viscosity reduces or even eliminates any settling tendency. This is true, but the Brownian motion is also

TABLE 1 Settling Calculations Based on Stokes' Law as a Function of Size and Density at Constant Temperature

Diameter μm	Diameter nm	ρ (Material) kg/m^3	ρ (Water) kg/m^3	η (Water) 298K, Poise	Time to Settle 1 cm (1 × 10^{-2} m) in Water		
					Minutes	Hours	Days
0.01	10	2500	1000	0.008905	1815494.39	30258	1261
0.1	100	2500	1000	0.008905	18154.94	302.58	12.61
1	1000	2500	1000	0.008905	181.55	3.03	0.126
10	10000	2500	1000	0.008905	1.82	0.03	0.001
100	100000	2500	1000	0.008905	0.02	0.00	0.000
0.01	10	3500	1000	0.008905	1089296.64	18154.94	756
0.1	100	3500	1000	0.008905	10892.97	181.55	7.56
1	1000	3500	1000	0.008905	108.93	1.82	0.076
10	10000	3500	1000	0.008905	1.09	0.02	0.001
100	100000	3500	1000	0.008905	0.01	0.00	0.000
0.01	10	4200	1000	0.008905	851013.00	14183.55	591
0.1	100	4200	1000	0.008905	8510.13	141.84	5.91
1	1000	4200	1000	0.008905	85.10	1.42	0.059
10	10000	4200	1000	0.008905	0.85	0.01	0.001
100	100000	4200	1000	0.008905	0.01	0.00	0.000
0.01	10	5500	1000	0.008905	605164.80	10086.08	420
0.1	100	5500	1000	0.008905	6051.65	100.86	4.20
1	1000	5500	1000	0.008905	60.52	1.01	0.042
10	10000	5500	1000	0.008905	0.61	0.01	0.000
100	100000	5500	1000	0.008905	0.01	0.00	0.000

reduced accordingly and no gain is achieved (in the same way that swimming in concentrated sucrose solution is no quicker or slower than in water).

(a) Most dry powder materials cannot be fully dispersed back to a primary size and thus size measurements from diffusion reflect the state of agglomeration of the system rather than to a primary size. Hence this Guide assumes that the reader has access to a well dispersed liquid suspension or preparation of nano-size particles for the measurement.

(12) Note from Eq 1 the obvious points that:

(a) As the size of particle increases, then the speed of Brownian motion decreases.

(b) As the viscosity of the medium increases, then the speed of Brownian motion decreases.

(c) As the temperature is increased, then the speed of Brownian motion increases correspondingly.

7.3 *Theoretical Background to the Correlation Function:*

7.3.1 It is necessary to measure the diffusion coefficient to input into Eq 1 in order to derive a particle size. Note that such a single input would only produce a single size value. This section deals with the measurement of the diffusion coefficient and the objective of providing a particle size distribution from the measured data.

7.3.2 In viewing the intensity of scattered light from a group of suspended moving particles, there is a temporal fluctuation of this light intensity (the "speckle" pattern) in the same way that the leaves of a tree, in windy conditions, attenuate the light of the sun and give light fluctuations over a short period of time, but the overall light intensity is not altered. Small particles diffuse quickly and thus exhibit more rapid fluctuations on a short time frame than larger particles, which diffuse more slowly. Over a very short time frame, δt, (typically units of nanoseconds or milliseconds), then the instantaneous signal intensity correlates well with the signal at time = 0. Light fluctuations that change more rapidly (small particles) lose this correlation more quickly than larger particles. If the instantaneous signal intensities are stored then it is possible to compare the values of the received signals over time with those at the start of the experiment (or indeed with that at any other period of time). The degree of comparison between 2 signals or 1 signal with itself is represented by the correlation coefficient, usually given the symbol [G], which can range from 1 (perfect correlation, the signal is identical to the signal it is being compared against) down to zero (no correlation). It can easily be shown **(2)** that this correlation coefficient decays exponentially with time for monodisperse particles (that is, all the particles are identical in size). See Fig. 1. The decay in correlation is more rapid for a small particle in comparison to a larger one (see Fig. 2).

8. Interpretation of the Correlation Function

8.1 *Introduction:*

8.1.1 There are a number of ways to interpret the correlation function and this section describes the more commonly utilized techniques.

8.2 *Linear Analysis:*

8.2.1 In the simplest analysis of the plot of the correlation coefficient against time, a straight line is fitted to the exponential decay by taking logarithms. Thus a monodisperse sample generates a straight line for the Log[G] versus Time plot. The slope of the plot is related to the reciprocal of the mean size of the particle system and the constant represents the noise in the system. We note that such an analysis only provides a mean size and no width of distribution is assumed or calculated. Clearly this assumption is only valid for narrow distributions—ideally monodisperse. A genuinely bimodal sample produces a single mean value when the cumulants analysis is used because the fitting of a straight line to the log[G] data set is not appropriate. This *z*-average mean value is then intermediate between the 2 separate mean values of the each of the

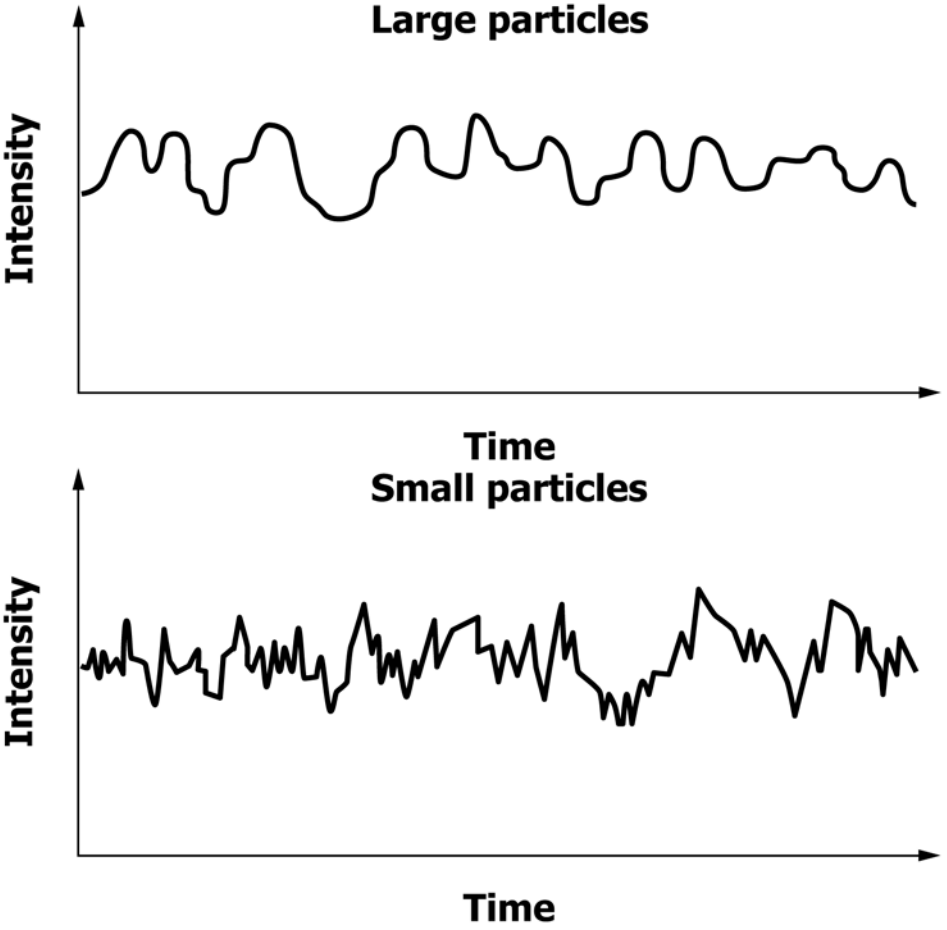

FIG. 1 Diagrammatic Representation of the Intensity Fluctuations with Small and Large Particles

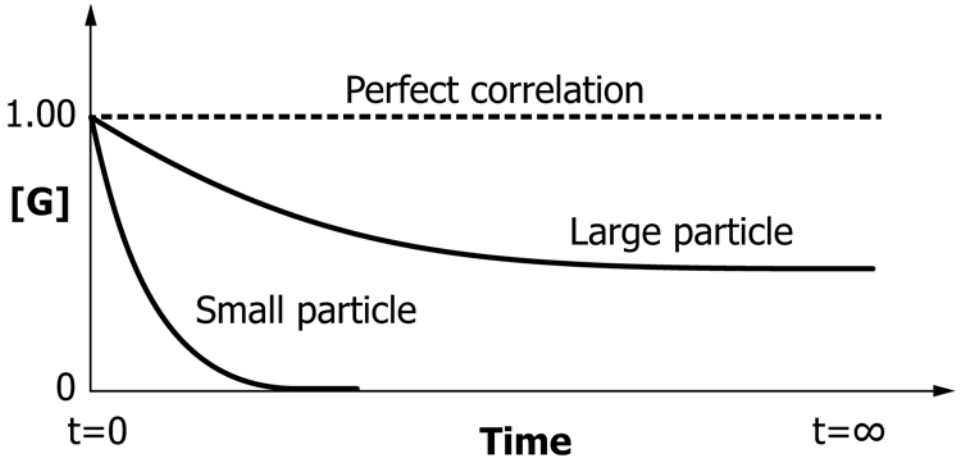

FIG. 2 Traditional PCS Measurement Indicating the Main Components of a Typical System

components of the bimodal. For the general case situation in which the log[G] versus Time plot is not linear (that is the norm!), then see 8.3.

8.3 *Polydisperse Samples—Cumulants Analysis:*

8.3.1 First, note the important point that many of the techniques discussed below relate to situations where there is likely to be material > 100 nm present in the sample (and thus the distribution is broader than "monodisperse"). The situation is likely to be simpler (smaller values of polydispersity index) for samples that are 100 % < 100 nm, although polydisperse characterized standards in this region are non-existent and thus, this point is difficult to verify in practice.

8.3.2 For samples that exhibit some width to the distribution (that is, contain a range of sizes), then the logarithmic decay plot of the correlation function is not linear. This curve can be fitted by a polynomial of any desired number of terms or indeed can be fitted by any sum of any type of simple or complex curves. Therefore, we need to take extreme care in this region. While computers can calculate the number of terms based on an arbitrary number of terms, the end-user needs to decide whether it is a reasonable and sensible process to undertake since more decimal places or numbers in the end result imply nothing about accuracy or resolution or sensitivity. All the preceding assessments of the quality of the instrument and result need to be verified for the system being measured.

8.3.3 In the simplest (Taylor/Maclaurin's series type) expansion of the non-linear form of the Log[G] decay, then we can express the form of the curve as:

$$\mathrm{Log}[\mathrm{G}] = a + b\tau + c\tau^2 + d\tau^3 + f\tau^4 \ldots \tag{4}$$

where a, b, c, d, etc. are empirically fitted constants to the experimental curve.

8.3.4 The term b corresponds to the mean size (strictly speaking, the intensity weighted z-average mean) and the second cumulant ($c\tau^2$) can be shown to be related to the variance (standard deviation^2) or width of a hypothetical Gaussian distribution as follows:

$$\text{Polydispersity Index (PI)} = \frac{2c}{b^2} \tag{5}$$

where the term c is identical to the standard deviation in a Gaussian distribution and the b value is the Gaussian mean (identical, of course, to the mode and median for such a distribution).

8.3.5 The deconvolution of an single (measured) exponential decay curve to a set of exponential curves, each corresponding to a single particle size, that sum to give the measured exponential is clearly an ill-conditioned problem and taking further terms beyond the fifth power (which would exactly fit six points or histogram bins if these were assumed) is usually meaningless as this degree of information is not inherent in the raw plot. Normally we do not go beyond the third term (**3**). The corollary to this is that information such as x_{90} (from diffraction—the 90 % undersize percentile; 90 % by volume less than this value), that the end-user is likely to be familiar with, become meaningless if only six channels of information are the maximum possible from a 5th order deconvolution. Any noise in the signal creates uncertainty in the derived solution. Worse still, with noise, the number of possible solutions tends to infinity and errors in these solutions are mathematically unbounded. In particular, more peaks can always be added in and thus give better and closer fits between the observed plots and those calculated. This does not mean that extra peaks provide a better solution—they only deal with the vagaries of any variation in the measured and calculated correlation curves. Note that fitting the measured data and deconvoluting within prescribed and predetermined experimental error limits is not guaranteed to yield a correct answer. This is disturbing to the uninitiated!

8.3.6 Johnsen and Brown (**4**) list the following ways of analyzing the raw correlation data: cumulants, Marquardt, S-exponential sums, Lambda depression, linear programming with sequence statistics (Zimmermann I, Zimmermann A, Zimmermann B, Jakeš), z-transform with spike recovery, exponential sampling, profiled singular value, histogram, CONTIN, RILIE, REPES, MAXENT and so on. Finsy (**5**) also deals with these analytical tools.

8.3.7 In addition other schemes exist. In all the cases indicated in 8.3.6, the authors show that the above algorithms can be "fooled" in pre-defined situations and that different particles size distributions arise as a result. Chapter VII in Chu's standard text (**6**) deals with similar issues. Stephen Provencher terms this deconvolution an "apparently hopeless problem" (Lines 8 and 9 of p. 93 in Ref (**7**)).

8.3.8 Notwithstanding the above caveats, the most common ways of deriving a distribution from the non-linear logarithmic correlation plot involve first constraining the solution to give positive sizes (x axis) and positive percentages (y axis) in the NNLS (Non-Negative Least Squares approach). This minimizes the differences between the calculated and observed data (on the basis of the lowest difference between the modulus of the sets) and allows only positive values of size and quantitiy. A further mathematical treatment is then invoked to isolate a distribution:

8.3.8.1 *CONTIN*—This is a (free) mathematical program designed by Dr. Stephen Provencher (**1, 7, 8, 9**) while working at EMBI, Heidelberg, Germany—the Max-Planck-Institut für Biophysikalische Chemie.[5] The (originally Fortran 66) program was formulated primarily to deal with inversion of noisy linear equations including Fredholm and Lotka-Volterra equations. The use of CONTIN in PCS relates to the general analysis of multi-exponential decay (Laplace inversion). CONTIN has the ability to accept pre-conditions (for example, negative particle sizes and negative percentages of components gives mathematically feasible solutions but can be ruled out in advance with CONTIN) that is probably (but not definitely!) likely to improve the accuracy and resolution of the mathematical solutions. The program does not provide a single, unique solution, although a preferred solution is indicated. Rather a number of possible solutions are given and the user is given the opportunity to inspect these and use auxiliary information in order to select the user's preferred solution. The indicated preferred solution is generated on the basis of the best-fit/smoothest solution (it dislikes—"rejects" is not the correct term—solutions with sharp boundaries) with the minimum number of peaks—what is called "parsimony". This assumption is clearly in error for mixtures of more than one (possibly monodisperse) component (sharp not smooth distributions and multiple peaks), so, as with other deconvolution methods, it can produce solutions that are not correct for a known system and thus scenarios can always be found to 'defeat' such algorithms. Thus, any auxiliary information (especially with respect to the amount of noise on the original signal, something that is not easy to define!), is essential in deciding whether any given result is reasonable or not. In Ref (**5**), Provencher also shows areas where CONTIN has problems and also points out deficits of other deconvolution algorithms (MAXENT, for example). Consult the literature for these less used approaches. Many manufacturers of PCS equipment provide a CONTIN implementation within their software. It is unsure how any

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[5] See a list of references and original manuals on the website: http://s-provencher.com/pages/contin/shtml.

proprietary implementation resembles or differs from Provencher's original implementation. Indeed, the computer language may be different for a start. Thus, reading of the equipment supplier's manual followed possibly by a telephone call to the manufacturer may be needed.

8.3.8.2 *Multi-Exponential Analysis or Eigenvalue Analysis of the Laplace Transform*—This was the first method that was used to obtain particle size distribution information from correlation coefficient decay curves and is mainly of historical interest now. It is credited to Pike-Ostrowski and involves taking a number of histogram size channels and fitting iteratively the sums of discrete logarithmically spaced exponentials (24 or 32). Thus, the set of predicted exponentials are summed to construct a final exponential, which is compared against that observed in the experiment. The exponentials are then adjusted to optimize the fit and minimize the residual:

$$\text{Residual} = \text{Square Root}\left[(\text{Observed} - \text{Theoretical})^2\right] \tag{6}$$

as in the standard least-squares approach mentioned earlier while describing NNLS.

8.3.8.3 *Multi-Angle Information*—In terms of larger systems (>> 100 nm and therefore not relevant to this Guide) there is then a variation in scattering intensity with angle (the scattering is non-isotropic in contrast to the sub-100 nm (approximate) regime. Any angular variation in scattering can be used (along with the known optical properties of the particulate system), in theory at least, to obtain particle size distribution information. This area (0.1 µm and higher) is now the preserve of "laser diffraction" (for example, see ISO 13320-1) where light scattering is involved and a range of other non-optical techniques (for example, sedimentation, sieves, electrical sensing zone) dependent on the size range of the system.

8.4 *Carrying Out the Measurement:*

8.4.1 A generic diagram is shown in Fig. 3.

8.4.2 Fig. 3 shows the "classic" design where scattered light is detected at a variable angle (often 90°), although for dilute small systems (< 100 nm) there is little angular dependence.

8.4.3 Clearly, over the history of the technique, a number of technical modifications and developments of the above traditional format are evident in the literature and from reading manufacturers specification sheets. The suitability of a particular instrument should be carried out in line with the needs of the application and it is recommended that samples be run in order to examine such factors as resolution and sensitivity.

8.4.4 Light (normally of fixed wavelength, coherent, possibly polarized and of relatively high intensity, that is, a laser) illuminates the sample, and the scattered light is detected and analyzed. The signal is stored within a correlator (hardware or software) and the computer processes this raw signal data with the parameters (laser wavelength, particle refractive index and so forth; analytical model) that the operator has predefined. A particle size distribution result is then produced either in frequency or histogram format. The user needs to check the derived distribution for reasonableness and repeat consecutive measurements are advised to ascertain the stability of the final answer (dependent both on the stability of the material and the mathematics in the deconvolution). Replicate samples allow the sample-to-sample variation to be ascertained.

8.5 *Sampling:*

8.5.1 Preparation of a representative sample in stable and dispersed state is vital to an accurate and meaningful analysis. To obtain the material in this state is not a trivial matter. Useful guides are to be found in the NIST Practice Guide Special Publication 960-1 Particle Size Characterization (**10**) and the first chapter of T. Allen's Particle Size Measurement (**11**) as well as a large number of ASTM standards, only a limited number being relevant to nano systems (for example, Practice C322). The now defunct Part 1 of the BS3406 series dealt with

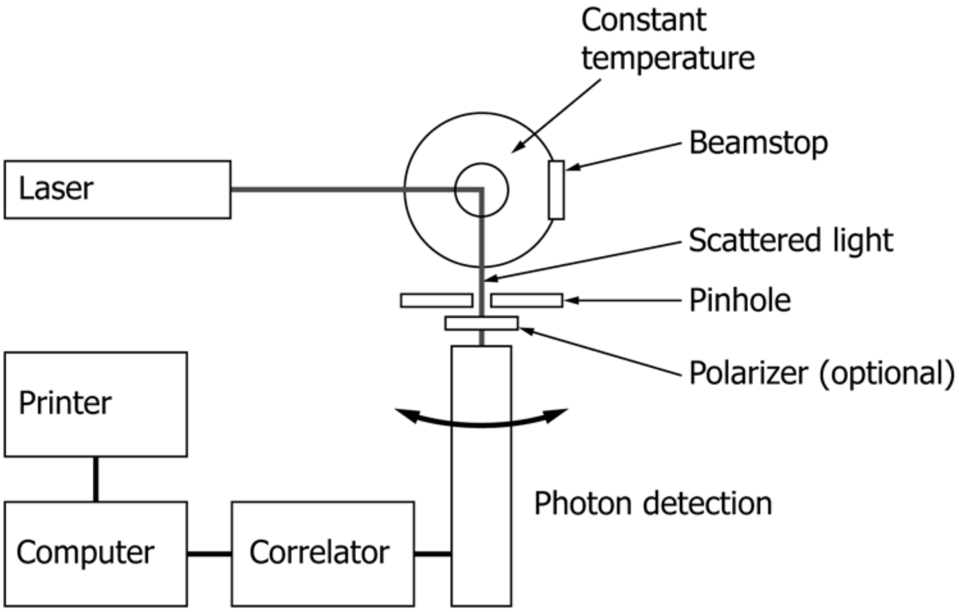

FIG. 3 Traditional PCS Measurement Indicating the Main Components of a Typical System

sampling and this has been partially used within ISO 14488. The examination of the time trend (size with time, consecutive measurements, input energy–sonication) for the particle size distribution in a repeatability study is vital to ensure stability and confidence in the final reported results. Sample-to-sample reproducibility can be assessed by the taking of replica aliquots or subsamples from the same bulk lot.

8.5.2 To take a representative aliquot of material, the material needs to be moving when the sample is extracted. With a slurry or suspension, sampling is normally carried out by pipetting the required amount of sample from a stirred beaker containing the primary material. If the sample has settled or is settling, and material is extracted only from the supernatant, it is clear that a smaller answer than the bulk material is obtained. Slurry sampling is notoriously difficult to carry out correctly and the use of a Burt sampler (the slurry equivalent of a spinning riffler for powders) is recommended.

8.5.3 The wider the particle size distribution, then more problems are likely to be encountered throughout the sampling and measurement especially if a "distribution" is sought. Statistically, 10 000 particles are required in the last size band for a standard error of 1 %, as the standard error is proportional to $1/n^{0.5}$, where n is the number of particles. Wider particle sizes are subject to greater possibilities of segregation or settling which complicate the sampling and measurement issues although again these are likely to be minimal for truly sub-100 nm systems.

8.5.4 With tiny amounts of sample then subsampling is not likely to be statistically admissible for the desired degree of accuracy and the entire amount should be required for the analysis. Note that similar constraints apply to electron microscopy where, unfortunately too often, relatively few particles (= little mass, picograms or nanograms) are sampled.

8.6 *Dispersion:*

8.6.1 Before the experiment is started, it is important to ascertain the purpose of the measurement. Unfortunately, the tendency is to seek the "smallest possible answer" without regard to whether the real problem is (say) a plant rheology or filtration problem—controlled by the bulk particulate size—or whether information is required relating to the primary particle size (dissolution, reactivity, take up of ions from solution, and so forth). The needs and objective dictate the amount of energy needed prior to the analysis. The use to which the end results are put is also crucial especially if economic values are at stake (for example, batch control, incoming goods check). We need to consider the implications of what an 'out-of-specification' result will mean in monetary terms. Note that this statement implies that we have a specification in place and test against this specification.

8.6.2 Dispersion for small systems often involves the use of large amount of input (sonication) energy, especially if the material is in a powdered state to start. Some materials (especially biological or those of high aspect ratio) are not likely to withstand huge amounts of energy input. Given that we are to be measuring the Brownian motion of the particulate system, then co-joined (aggregated or agglomerated or both) groups of primary particles behave as a single larger particle. This fact needs to be borne in mind, even with microscopy, where judgment as to what can be considered a single particle, or whether it is strongly bound to its neighbor, is certain to give interpretation difficulties.

8.6.3 Note also that on a mass basis a single 100 nm particle is equivalent to 1 million 1 nm particles. On an intensity basis, as there is a d^6 dependence of scattering on size, a single 100 nm particle is equivalent to 10^{12} (or a thousand billion) 1 nm particles. Thus the technique is especially sensitive to any larger or agglomerated particles in the system. It is often desirable to remove (by filtration or centrifugation) even small amounts of any larger material present, recognizing the fact that this is altering the particle size distribution, in particular, on a mass basis. It is to be noted that as the measurement relies on the interpretation of a correlogram and that an intensity distribution (normalized to 100 %) is produced, then a background subtraction 'count' of the solvent is not possible or feasible. Thus cleanliness of the background solvent is essential and filtration to 20 nm is usual.

8.7 *Particle Concentration:*

8.7.1 A certain concentration of particles is required in the system in order that sufficient scattering can be "seen" by the system—in other words, adequate signal to noise. This is a complex situation with the particle size, relative refractive index and volume concentration all playing a role. However, note that very low concentrations of poorly scattering materials (for example, proteins) are not likely to generate adequate signal for reasonable measurement, in a number of situations.

8.7.2 Commensurate with the requirements of sufficient particle concentration are those ensuring that the concentration is not so high as to cause multiple scattering. This is when light scattered from a single particle interacts with another particle before it reaches the detector and is also dependent on the optical system used. While this is not likely to prevent the taking of a measurement (unless the solution is so concentrated that light cannot get in and out of the system), multiple scattering, if present, is likely to confuse the interpretation. Thus, it is normal to carry out a concentration ladder at three or more dilutions in order to ascertain the areas where the particle size is stable. In areas where it is difficult or impossible to control the dilution stage and measurements are still desired, note that the particle concentration needs to be controlled and recorded. Note that comparative measurements can still be obtained in this situation. Accuracy for this system is not definable but repeatability obviously is. The experimental set-up can also reduce multiple scattering effects and a variety of experimental set-ups are stated to deal with this issue. Again samples related to the application in question are the best means of ensuring suitability or otherwise of a system for its intended use.

8.8 *The Measurement:*

8.8.1 In order that random Brownian motion is to be measured then the system needs to be in complete thermal stability (± 0.1°C or better) with no thermal/convection currents present in the measurement cell or cuvette. Follow the manufacturer's guidelines in terms of warm-up and sample equilibration times. Select the conditions for the measurement ensuring that the correct parameters are set within the instrument and that the sample is described correctly in the documentation. Parameters that need setting and recording are likely to include (see Practices E177 and F1877):

8.8.1.1 The wavelength of the laser utilized in the experiments.

8.8.1.2 The duration of the experiment and any sub-experiments carried out. Normally it is advisable to take measurements as a consecutive set of replicate experiments of identical duration. In this way any changes with time (agglomeration, de-agglomeration, dissolution, settling) can be followed.

8.8.1.3 The analytical model to be employed in the analysis (see 8.3.6 and 8.9.2).

8.8.1.4 The set or measured temperature that the measurements were taken.

8.8.1.5 The viscosity appropriate to the liquid used in the study.

8.8.1.6 The refractive index (real and imaginary) of the particle and the liquid suspension medium if the conversion to volume is to be undertaken.

8.8.1.7 The name of the operator.

8.8.2 It is more than desirable to take repeated consecutive measurements (five minimum—rather than the often used three) to assess the stability of the system. In this manner, a system undergoing settling or other untoward effects can be seen. It is generally better to run a series of short duration measurements (runs) and then average these, rather than a single long duration measurement. Intermediate noise, for example, from dust particles, is not likely to affect all runs and in this way 'bad' runs can be excluded and do not contribute to the correlation function or baseline determination. The total measurement duration needs to correspond with manufacturer's recommendations. Extended measurement times are likely to be needed with larger particles (less Brownian motion) or smaller particles (poor scattering) or those of relative refractive indices approaching unity (poor scattering). The count rate (number of photons recorded per unit time above background) may be useful in assessing the extent of scattered light during and after the experiment and is important to record. In some cases, it is possible to adjust the concentration or the pinhole through which the light enters the sample region (or both) to compensate for a too high or too low count rate.

8.8.3 Examine the cell/cuvette before and after measurement for evidence of settling. At the end of the measurement, a particle size distribution is produced in graphical and tabular form. Repeated measurements give confidence in the stability of the material over time. Averaging several measured distributions reduces the importance of occasional spikes or other spurious peaks in a distribution. Essentially these are averaged out which is essentially another form of regularization. In some cases it is possible (within the constraints of the system and material) to follow kinetic events.

8.8.4 Utilized modeling parameters such as fit or residual are often useful in assessing the quality of the obtained result and record these, as appropriate.

8.8.5 The two main calculated/derived parameters that are most useful are the following:

8.8.5.1 *z*-average (or cumulants mean), and

8.8.5.2 Polydispersity index (PI).

8.8.6 Other information can be displayed, but the assumptions in obtaining this derived information need to be carefully understood and the implications of reporting this information also carefully evaluated. As such, volume and number distributions derived from dynamic light scattering measurements are best used for comparison purposes or for estimating the relative amounts of multimodal (multiple size peaks) samples, and therefore are never to be considered absolute.

8.9 *Interpretation and Comparison with Other Techniques:*

8.9.1 The mean result that is derived is an intensity-weighted mean (commonly known as the *z*-average) that corresponds to how the particle moves within the liquid and interacts with light. Particles, which are not spherical, move in different ways and an equivalent sphere on the basis of diffusion is thus generated. The *z*-average is weighted towards the higher end of the particle size distribution, as the intensity is proportional to d^6 (or V^2 or MW^2). In simple terms if we have one 100 nm particle it is equivalent in intensity weighting terms to 10^{12} (1000 billion) 1 nm particles. This differs from say a classic laser diffraction experiment where the volume weighting would have 10^6 (or 1 million) 1 nm particles equivalent to the single 100 nm particle, which is absolutely correct in mass terms (for constant density of particle). Note that with any form of microscopy the 1nm particle has equal equivalence (or weighting) to the 100 nm particle, even though they differ massively in mass (and therefore value). Thus, conversions to other distributions to which the reader is likely to be familiar (for example, volume, number), are fraught with dangers (see 8.10). In general terms derived percentile parameters such as x_{10} and x_{90} (the diameters below which 10 and 90 % of the distribution exist) are not sensible to report or to use for quality control (pass or fail) purposes.

8.9.2 The accuracy of the width of the presented distribution (polydispersity) is related to the model employed—CONTIN, NNLS, cumulants, coated particle, etc.—and selected by the user before the experiment is commenced. Thus the user needs to have prior information as to the nature of the sample in order to conclude whether a reported answer is reasonable or not and comparison with other techniques is necessary to generate confidence in a set of results. In all cases, some form of visualization (electron microscopy only is possible < 100 nm) of a statistically valid number of particles, is desirable. This can be combined with the particle size information to give a fuller characterization picture of the system. Note that it is often very difficult to get images for a number of biological systems, for example, micelles or proteins or other materials such as emulsions usually require specialized techniques (for example, freeze-fracture).

8.9.3 For narrow distributions (polydispersity index < 0.2), there is little inherent problem in the deconvolution of the raw scattering information to particle size information. For wider distributions (polydispersity index 0.1–0.7), then distribution algorthims are likely to be useful. At higher polydispersity indices (> 0.7) then the sample is unlikely to be suitable for PCS and is not likely to give a stable distribution with time.

8.10 *Conversion of the Intensity Distribution to Other Particle Size Distributions:*

8.10.1 In mathematical terms, this deconvolution is termed ill-posed or ill-conditioned that means, in practical terms that it is ill advised. Small changes in collected data can give rise to enormous changes in derived result and as such treat any derived result with caution and skepticism. To convert from intensity to volume distribution would involve the manipulation of perfect noise-free experimental data with accurately measured refractive indices using Mie theory. *A further conversion to number should never be attempted.* If a number distribution is desired then an instrument that collects such information should be used in the first place.

8.10.2 The wider the initial distribution the more serious are the errors in the conversion, and we have previously shown that the given solution(s) are derived from ill-posed mathematical problems and thus possibly subject to unbounded errors.

8.10.3 Notwithstanding the above caveats and cautions, conversion to a volume-weighted distribution can often provide an indication of the relative importance (prominence) of two or more reported peaks. A common situation is to see an apparently dominant large-size peak virtually disappearing and a low-intensity smaller-sized peak becoming the primary mode after conversion to volume weighting. This conversion tends to be relatively insensitive to the refractive index (the additional parameter required for the conversion) except when the particle and medium have very similar values for the real refractive index (~ < 0.03).

9. Report

9.1 See Practice E1617.

9.2 As a minimum the following need reporting in addition to graphical and tabular information:

9.2.1 The instrument type and manufacturer and serial number. Version of software employed.

9.2.2 Date and results of the last verification. Details of the traceability of the standards employed.

9.2.3 Date of measurement together with analyst's name and affiliation.

9.2.4 Details of the sample including chemical composition. Shape information if obtained by SEM is helpful.

9.2.5 Details of the dispersion conditions (concentration of material, liquid used, ultrasound time, frequency and power, surfactants and stabilizing agents, if used, and their concentration) and evidence that full dispersion or primary particle size has been reached.

9.2.6 *Measurement Conditions*—Time of measurement, wavelength of laser (if employed), stabilization period prior to measurement, temperature. Number of measurements.

9.2.7 Minimum of 5 replicate consecutive measurements. This demonstrates the stability of the material (especially) and the instrument during the duration of the measurements. The use of a relative standard deviation (RSD) or coefficient of variation (CV) is highly recommended. (Note: $100 \cdot \sigma / \text{Mean} = \text{RSD}$.)

9.2.8 Minimum of 3 separate aliquots/samples from the same bulk lot measured under identical conditions and with each having a minimum of 5 replicate consecutive measurements as above. This demonstrates the homogeneity or otherwise of the bulk lot and the aliquot-to-aliquot reproducibility.

9.2.9 *Deconvolution Conditions:*

9.2.9.1 Viscosity of medium (for Stokes-Einstein equation). Note that this is the viscosity that the particle(s) experience in their interaction with the medium and thus would tend to be higher the more concentrated the suspension or sample is. It is equivalent to a viscosity at zero stress.

9.2.9.2 Temperature (absolute) of the sample.

9.2.9.3 Concentration. Ideally a concentration 'ladder' experiment of 3 or more dilutions needs to be explored in order to determination the correct concentration for stable results. In general terms: *(1)* the lower the concentration, then the greater the danger of poor signal-to-noise (and therefore poor repeatability) and *(2)* the greater the concentration, then the higher the probability that multiple scattering is occurring leading to a reduction in the apparent particle size. There is likely to be a happy medium (usually over 3 decades or more) for the concentration where there is adequate signal-to-noise combined with no multiple scattering. This region is best found by empirical measurement.

10. Precision and Bias

10.1 The precision of this test method is based on an interlaboratory study of Guide E2490, Measurement of particle size distribution of nanomaterials in suspension by Photon Correlation Spectroscopy (PCS), conducted in 2008. Results in this study were obtained from 26 participating laboratories, testing various combinations of five different materials. Every "test result" reported represents an individual determination. Each participating laboratory reported between one and four replicate test results for every material. Except for the occasional reporting of fewer than two replicates or the analysis of data from less than six laboratories testing a particular material and analysis combination, Practice E691 was followed for the design and analysis of the data; the details are given in ASTM Research Report[6]

10.1.1 *Repeatability Limit (r)*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the "r" value for that material; "r" is the interval representing the critical difference between two test results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory.

10.1.1.1 Repeatability limits are listed in Table 2 through Table 8.

10.1.2 *Reproducibility Limit (R)*—Two test results shall be judged not equivalent if they differ by more than the "R" value for that material; "R" is the interval representing the critical difference between two test results for the same material, obtained by different operators using different equipment in different laboratories.

10.1.2.1 Reproducibility limits are listed in Table 2 through Table 8

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[6] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:E56-1001. Contact ASTM Customer Service at service@astm.org.

TABLE 2 DLS z-Average Mean Diameter (nm) from Cumulants Analysis of Data

NOTE 1—All data from Labs 11 and 22 were excluded from the DLS z-average diameter calculations after being identified as consistent outliers.

Material	Average[A]	Standard Deviation of the Lab Averages	Repeatability Standard Deviation	Reproducibility Standard Deviation	Repeatability Limit	Reproducibility Limit	Number of Reporting Laboratories
	$\bar{x}$	$\sigma\bar{x}$	σ_r	σ_R	r	R	n
Sample A-combined	15.8	4.2	2.0	4.7	5.7	13.1	13
Sample B- combined	31.2	3.6	2.0	4.1	5.7	11.5	13
Sample C- combined	59.8	5.0	5.0	6.8	13.9	19.2	13
Sample D- combined	8.0	2.4	0.9	2.6	2.6	7.2	12
Sample E- combined	6.7	1.8	0.9	2.0	2.6	5.6	12

[A] The average of the laboratories' calculated averages.

TABLE 3 DLS Polydispersity Index (Unitless) From Cumulants Analysis of Data

NOTE 1—All data from Lab 11 were excluded from the DLS polydispersity index calculations after being identified as consistent outliers.

Material	Average[A]	Standard Deviation of the Lab Averages	Repeatability Standard Deviation	Reproducibility Standard Deviation	Repeatability Limit	Reproducibility Limit	Number of Reporting Laboratories
	$\bar{x}$	$\sigma\bar{x}$	σ_r	σ_R	r	R	n
Sample A-combined	0.289	0.224	0.148	0.264	0.414	0.740	12
Sample B- combined	0.212	0.170	0.058	0.178	0.163	0.499	12
Sample C- combined	0.165	0.139	0.019	0.140	0.053	0.392	12
Sample D- combined	0.159	0.177	0.076	0.191	0.214	0.535	12
Sample E- combined	0.176	0.098	0.054	0.110	0.152	0.309	12

[A] The average of the laboratories' calculated averages.

TABLE 4 DLS Intensity Mean Diameter (nm) from Distribution Analysis

Material	Average[A]	Standard Deviation of the Lab Averages	Repeatability Standard Deviation	Reproducibility Standard Deviation	Repeatability Limit	Reproducibility Limit	Number of Reporting Laboratories
	$\bar{x}$	$\sigma\bar{x}$	σ_r	σ_R	r	R	n
Sample A-combined	15.21	3.30	1.02	3.44	2.87	9.64	8
Sample B- combined	32.74	3.15	1.37	3.41	3.85	9.54	8
Sample C- combined	63.05	3.65	1.09	3.79	3.06	10.61	8
Sample D- combined	8.51	2.17	0.93	2.34	2.61	6.55	9
Sample E- combined	6.74	2.31	0.63	2.39	1.78	6.69	9

[A] The average of the laboratories' calculated averages.

TABLE 5 DLS Volume Mean Diameter (nm) from Distribution Analysis and Conversion of Intensity Mean Diameter

Material	Average[A]	Standard Deviation of the Lab Averages	Repeatability Standard Deviation	Reproducibility Standard Deviation	Repeatability Limit	Reproducibility Limit	Number of Reporting Laboratories
	$\bar{x}$	$\sigma\bar{x}$	σ_r	σ_R	r	R	n
Sample A-combined	10.80	5.56	1.86	5.84	5.22	16.34	7
Sample B- combined	27.37	2.33	4.51	4.87	12.63	13.64	7
Sample C- combined	55.37	3.43	3.57	4.82	10.00	13.50	7
Sample D- combined	7.66	1.89	0.89	2.07	2.48	5.79	7
Sample E- combined	5.88	2.38	0.78	2.49	2.17	6.98	7

[A] The average of the laboratories' calculated averages.

10.1.3 The above terms (repeatability limit and reproducibility limit) are used as specified in Practice E177.

10.1.4 Any judgment in accordance with statements 10.1.1 and 10.1.2 would normally have an approximate 95 % probability of being correct, however not all of the precision statistics obtained in this ILS may be treated as definitive mathematical quantities, applicable to all circumstances and uses. The limited number of replicates tested and laboratories reporting results guarantees that there will be times when differences greater than predicted by these ILS results will arise, sometimes with considerably greater or smaller frequency than the 95 % probability limit would imply. The

TABLE 6 TEM Mean Diameter (nm)

Material	Average[A]	Standard Deviation of the Lab Averages	Repeatability Standard Deviation	Reproducibility Standard Deviation	Repeatability Limit	Reproducibility Limit	Number of Reporting Laboratories
	$\bar{x}$	$\sigma\bar{x}$	σ_r	σ_R	r	R	n
Sample A	9.15	1.01	0.00	1.01	0.00	2.84	11
Sample B	27.47	2.18	0.00	2.18	0.00	6.10	11
Sample C	52.21	12.28	1.96	12.35	5.49	34.59	11

[A] The average of the laboratories' calculated averages.

TABLE 7 SEM Mean Diameter (nm)

Material	Average[A]	Standard Deviation of the Lab Averages	Repeatability Standard Deviation	Reproducibility Standard Deviation	Repeatability Limit	Reproducibility Limit	Number of Reporting Laboratories
	$\bar{x}$	$\sigma\bar{x}$	σ_r	σ_R	r	R	n
Sample A	9.68	NA	NA	1.70	NA	4.77	6
Sample B	29.30	NA	NA	3.70	NA	10.36	6
Sample C	60.68	NA	NA	2.85	NA	7.98	6

[A] The average of the laboratories' calculated averages.

TABLE 8 AFM Mean Maximum Profile Height (nm)

Material	Average[A]	Standard Deviation of the Lab Averages	Repeatability Standard Deviation	Reproducibility Standard Deviation	Repeatability Limit	Reproducibility Limit	Number of Reporting Laboratories
	$\bar{x}$	$\sigma\bar{x}$	σ_r	σ_R	r	R	n
Sample A	7.63	1.06	0.04	1.06	0.11	2.98	8
Sample B	25.56	1.80	0.02	1.80	0.06	5.04	8
Sample C	54.55	1.74	0.24	1.75	0.66	4.89	8

[A] The average of the laboratories' calculated averages.

repeatability limits and the reproducibility limits should be considered as general guides, and the associated probability of 95 % as only a rough indicator of what can be expected.

10.2 *Bias*—At the time of the study, there was no accepted reference material suitable for determining the bias for this test method, therefore no statement on bias is being made.

10.3 The precision statement was determined through statistical examination of 1723 results, from 26 laboratories, on five materials. These five materials were described as the following:

10.3.1 Sample A: Nominal 10 nm Gold Nanoparticles (NIST RM8011).

10.3.2 Sample B: Nominal 30 nm Gold Nanoparticles (NIST RM8012).

10.3.3 Sample C: Nominal 60 nm Gold Nanoparticles (NIST RM8013).

10.3.4 Sample D: G6 PAMAM-DAB Dendrimer, OH terminated.

10.3.5 Sample E: G6 PAMAM-DAB Dendrimer, NH2 terminated.

To judge the equivalency of two test results, it is recommended to choose the material closest in characteristics to the test material.

11. Keywords

11.1 DLS; dynamic light scattering; PCS; photon correlation spectroscopy; nano; QELS; quasi-elastic light scattering

APPENDIX

(Nonmandatory Information)

X1. DISCUSSION/COMMENTARY

X1.1 Discussion of Interlaboratory Results

X1.1.1 At the outset it must be noted that this is an interlaboratory study and thus the reported figures (precision and bias) reflect the total performance of the laboratory in terms of sample handling and reporting and may not be completely indicative of the variability of the technique itself. Further, it is to be noted (Annex B of the full report RR:E56-1001[6]) that a number of different instrument types, laser wavelengths and analytical models (as well as different temperatures) were used for the measurements and subsequent analyses. While the influence of many of these parameters is likely to be small (for example, laser wavelength) or corrected for (temperature/viscosity) it may be that certain differences do exist. For example, a forward scattering measuring angle is generally more sensitive to larger size particles and thus, in theory at least, there may be minor angular dependence on the reported results. All of these factors are mitigated by the fact that the gold colloid and dendrimers samples are of narrow distribution (close to monodisperse) and also that the size is within the Rayleigh regime ($d < \lambda/10$) where there is little or no angular variation, so the interlaboratory study should be reasonably robust to variation of this sort. Similarly, the analytical model utilized is likely to be of minor significance given the narrowness of the distributions for the 5 test materials.

X1.1.2 Notwithstanding the preceding discussion, the three gold colloid test materials (RM8011, RM8012, and RM8013) may be an excellent indicator of potential bias within a laboratory as carefully measured size values are available within the report of investigation provided by NIST and publicly accessible at http://ts.nist.gov/measurementservices/referencematerials/index.cfm. The nominal size of a G6 dendrimer is also available in the literature (6.7 nm hydrodynamic diameter; D A Tomalia Birth of a new macromolecular architecture: dendrimers as quantized building blocks for nanoscale synthetic organic chemistry. Aldrichimica Acta, Vol. 37, No. 2, pp. 39 – 57 (2004); the size is given within a table on page 44), and this also provides a benchmark to which laboratories can aspire. Laboratories that did not achieve close agreement (± 3 σ) with the certified results and stated tolerances should examine both their measurement technique and also whether the possibility of sample damage (for example, by freezing) could have occurred in practice. The big advantage for such laboratories is that the gold test materials are available through NIST for remeasurement should they wish. This is also true of the G6 dendrimers, which represent a much greater measurement challenge for PCS.

X1.1.3 The main objective of the interlaboratory study was to provide a bias and precision statement for a standard; the secondary objective was to collect information related to implementation of the PCS technique in the nanoscale materials community. However, as many laboratories possess other complementary techniques that can corroborate PCS results, it was felt sensible to ask participants to use these techniques (when available) and provide corollary data. In terms of the microscopy techniques included in this study, this was particularly valuable for providing visualization of the test materials (Note: only the gold test materials were included in the corollary portion of this study).

X1.1.4 The two basic measurands that arise from a PCS measurement are the z-average size and polydispersity index (PI), where the latter is a metric for distribution width. The other reported parameters (intensity and volume means) are to an extent equipment and analytical model dependent. Conversion of intensity to volume mean is an ill-posed mathematical problem where the input of optical properties is also needed. Again, given the narrow nature of the distribution, these effects are likely to be minimal in the present study.

NOTE 1—Laboratories 11 and 22 excluded from the ILS average calculation for z-average. Laboratory 11 is excluded from the ILS average for polydispersity index. The NIST data refer to the 3 RM samples only and are stated with 95 % confidence limits approximately ± 2 standard deviations.

Sample	z-Average (nm)	Uncertainty (±)	Polydispersity Index	Uncertainty (±)
RM8011	13.5	0.1	Not stated	
ILS Average	15.3	4.4	0.289	0.224
RM8012 (173 deg)	28.6	0.9	Not stated	
RM8013 (90 deg)	26.5	3.6	Not stated	
ILS Average	30.7	4.0	0.212	0.17
RM8013 (173 deg)	56.6	1.4	Not stated	
RM8013 (90 deg)	55.3	8.3	Not stated	
ILS Average	58.9	5.7	0.165	0.139
G6 (Sample D)	6.7 (see text)	Not stated		
ILS Average	7.8	2.4	0.159	0.177
G6 (Sample E)	6.7 (see text)	Not stated		
ILS Average	6.4	2.0	0.176	0.098

X1.1.5 Within the appropriate caveats described above, we can compare the results obtained by laboratories against the reference values provided by NIST. It should also be noted that the NIST reference values were obtained from very careful measurements on a single measurement platform for each scattering angle under the best possible conditions. This can obviously be carried out for the measurements on other techniques, but this discussion limits itself to the light scattering measurements. Obviously, too, each individual laboratory can compare its own results against the group mean and the NIST results.

X1.1.6 For the gold test materials the grouped average results are close to but larger than those values stated by NIST. This is expected as all potential errors (contamination, insufficient equilibration time) are likely to result in an increased size result. This is also reflected in the spread of results from the ILS study, which tend to be wider than those quoted by NIST.

X1.1.7 The number of decimal places for the z-average stated in the table above has been restricted to one . It is highly unlikely that PCS measurements could provide better precision or that additional decimal places would be meaningful given the underlying uncertainties in the technique and data analysis.

X1.1.8 It appears, as a general rule that improvements were made on the second and subsequent aliquots when these were reported. This may reflect the added experience and the potential for increased cleanliness on washing out the first sample with subsequent samples. In terms of the (hemolysis and cytotoxicity) interlaboratory studies (ILS# 211, ILS #202) run concurrently with the present study, and utilizing the same test materials, it was concluded that education and training play a large role in the 'tightness' of obtained results. The same may be true here, but to a much lesser extent.

X1.1.9 The obvious and expected observation is that the smallest materials (RM 8011 and the two G6 dendrimers) are the most difficult. Another not wholly unexpected result is how well laboratories actually performed on the two very small but highly uniform and monodisperse G6 dendrimer test materials. Theoretically, there the polydispersity index associated with the G6 materials should approach zero, given that they are well defined molecular species. Thus any value obtained for a polydispersity for a perfect measurement of these materials is an indication of the ill-conditioned nature of the deconvolution of the correlogram and is therefore a math discussion. The presence of noise in the correlograms or large particle contamination in the tested sample will likely contribute to apparent polydispersity.

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