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# Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Photon Correlation Spectroscopy (PCS)<sup>1</sup>

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 ( $\epsilon$ ) 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:<sup>2</sup>

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<sup>3</sup>

ISO 14488 Particulate Materials—Sampling and Sample Splitting for the Determination of Particulate Properties<sup>3</sup>

ISO 13321 Particle Size Analysis—Photon Correlation Spectroscopy<sup>3</sup>

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<sup>2</sup> 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.

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

## 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  $\mu\text{m}^2/\text{s}$ .

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.

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).<sup>4</sup>

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

<sup>4</sup> 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 (e.g., 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 (e.g., 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  $\lambda$  is the wavelength of light employed), then this intensity of scattering is proportional to  $r^6$  – or (volume)<sup>2</sup> or (relative molecular mass)<sup>2</sup>. 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 6nm 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 (e.g., 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  $\lambda/2$  ( $\sim 300$  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:

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

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),
- $\pi$  = the universal constant,
- $\eta$  = 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:

$$D_t = \frac{kT}{6\pi\eta R_h} \quad (2)$$

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

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

(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 $\mu\text{m}$	Diameter $\text{nm}$	$\rho$ (Material) $\text{kg/m}^3$	$\rho$ (Water) $\text{kg/m}^3$	$\eta$ (Water) 298K, Poise	Time to Settle 1 cm ( $1 \times 10^{-2}$ 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,  $\delta t$ , (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 (i.e., 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

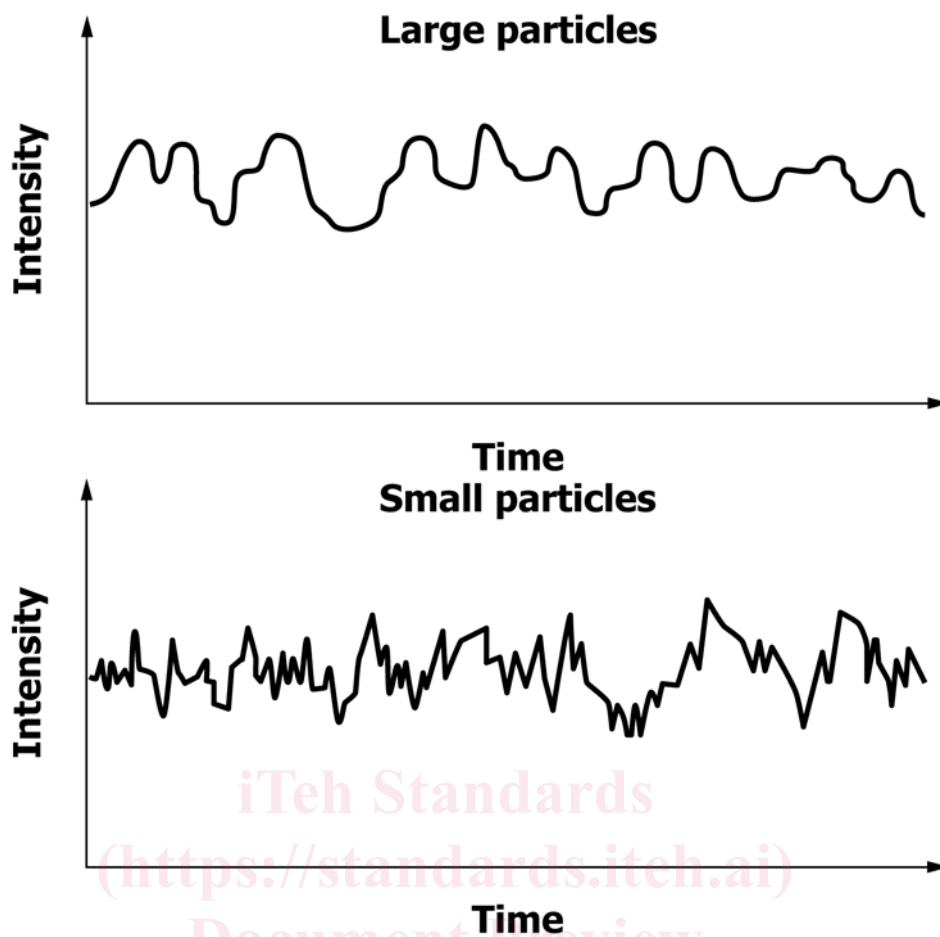


FIG. 1 Diagrammatic Representation of the Intensity Fluctuations with Small and Large Particles

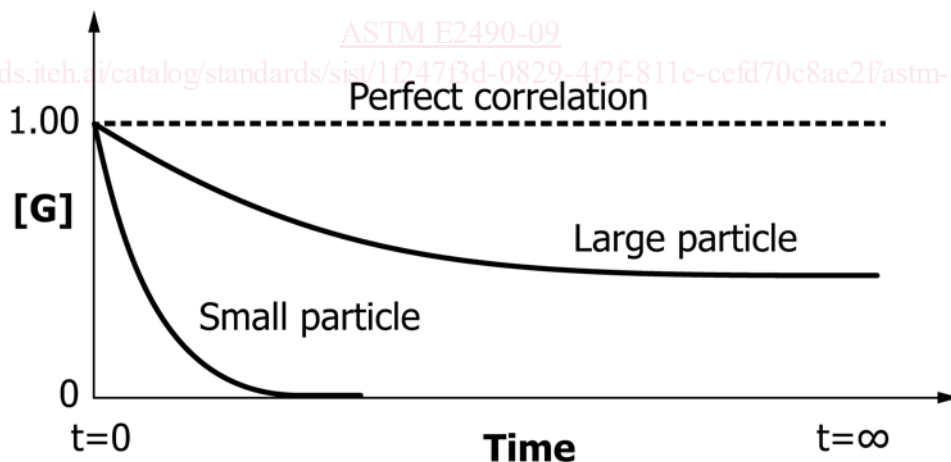


FIG. 2 Traditional PCS Measurement Indicating the Main Components of a Typical System

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