

Good Practice Guide No. 119

Good Practice Guide for the Determination of the Size and Size Distribution of Spherical Nanoparticle samples

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Measurement Good Practice Guide No.119

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ABSTRACT

The aim of this guide is to introduce the reader to some of the key metrological aspects in nanoparticle sizing. The critical role of traceability and uncertainty analysis in obtaining meaningful measurements is highlighted. Several common techniques routinely used in the analysis of nanomaterials are reviewed and example of uncertainty calculation given for each technique. These techniques are the high-resolution techniques of electron and scanning probe microscopy, which can resolve individual particles, and the ensemble methods of dynamic light scattering and small angle X-ray scattering that can analyse many thousands of particles simultaneously. A list of existing relevant standards is also provided.

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Contents

Introduction	1
Metrological concepts	3
Measurement uncertainty	5
Sample preparation for nanoparticle measurements	7
Ensuring a good dispersion	9
Deposition onto a solid substrate.	11
Other factors affecting measurement uncertainty	13
Selected current nanoparticle sizing techniques.....	15
Electron Microscopy	19
Type of electron microscopes	20
Calibration of electron microscopes	22
Image analysis.....	23
Case study I: STEM measurement of 30 nm diameter gold nanoparticles.....	24
Case study II: Traceable TSEM Size measurement.....	27
Scanning Probe Microscopy	31
SPM measurement procedure	33
SPM image analysis.....	34
Source of uncertainties.....	37
Case study III: SPM measurement of polymer particles	39
Dynamic Light Scattering	41
Results interpretation	43
Case study IV: Uncertainty budget for Dynamic Light Scattering measurement	45
Small Angle X-ray Scattering.....	49
SAXS standard procedure.....	51
Sources of uncertainties	54
Conclusions.....	55
Appendix - Existing standards.	57
References	66

List of Figures

Figure 1: Accuracy vs precision	4
Figure 2: Errors in nanoparticle measurement.....	5
Figure 3: Standard measurement flow diagram	8
Figure 4: Standard deviation of the sample mean.in individual measurement.....	14
Figure 5: High-energy electrons interaction with a thin sample.....	20
Figure 6: Transmission detector for SEM.....	22
Figure 7: Selected TEM calibration standards.....	23
Figure 8: Gold nanoparticles lattice spacing.....	25
Figure 9: Size distribution comparison for various TEM modes.....	26
Figure 10: TEM images of 15 nm gold particles in various modes.....	27
Figure 11: Leading edge distortion in SEM.....	29
Figure 12 Measured and simulated signal profile in electron microscopy	29
Figure 13: Threshold level at particle boundary for various particles	30
Figure 14: Height vs lateral measurement using SPM.....	35
Figure 15: Image analysis for SPM.	36
Figure 16: Packing density effect on lateral measurement.	37
Figure 17: SPM height measurement.....	40
Figure 18: Particle size distribution from SPM	41
Figure 19: SPM lateral size measurement	41
Figure 20: Comparison between height and lateral measurement.	41
Figure 21: Contribution of scattering angle to uncertainty in DLS	79
Figure 22: SAXS scattering curve for 30 nm gold nanoparticles	79

List of Tables

Table I: Common techniques used in nanoparticle sizing applications.....	17
Table II: Comparison of nanoparticle size measurements using STEM in various modes.	27
Table III: SPM results for a sample of spherical 200 nm polymer reference particles.	40
Table IV: Simulating the effect of agglomeration on the mean particle size	44
Table V: Uncertainty budget for 100 nm latex nanoparticles as measured by DLS.....	48

Glossary

AFM	Atomic Force Microscopy
DLS	Dynamic Light Scattering
EDS	Energy Dispersive X-ray Spectroscopy
GUM	Guide to the Expression of Uncertainty in Measurement
HAADF	High Angle Annular Dark Field
ISO	International Standards Organisation
NND	Nearest neighbour distance
SAXS	Small Angle X-ray Scattering
SEM	Scanning Electron Microscopy
SI	International System (from the French <i>Système International</i>)
STEM	Scanning Transmission Electron Microscopy
SPM	Scanning Probe Microscopy
TEM	Transmission Electron Microscopy
TSEM	Transmission Scanning Electron Microscopy
UKAS	United Kingdom Accreditation Service
VIM	International Vocabulary of Metrology — Basic and general concepts and associated terms

1

IN THIS CHAPTER

- The importance of nanoparticle size measurements.
- Key metrological concepts.

Practical applications of nanoparticles are becoming ever more varied. For example, gold nanoparticles can be readily modified with biologically active agents and used as new bio-diagnostics agents [1,2]. Semiconductor nanoparticles can self-assemble into monolayers which can form the basis of novel photovoltaic, light emitting and sensing devices [3]. Nanoparticles display a series of novel properties:

- Electronic: when the nanoparticle size is smaller than the mean free scattering path, electron transport is controlled by ballistic interactions rather than diffusion [4,5].
- Optical: changing the size of particles down to the nanoscale has a strong influence on their optical properties, for example in quantum dots [6] and gold nanorods [7]
- Catalytic: The large surface area to volume properties of nanoparticles make them ideal for catalysis [8].

As the raw materials for nanostructured materials, nanoparticles have also applications in nano-composites; examples are in enhanced barrier properties for packaging applications and better conductivity for electromagnetic field shielding applications [9]. Applications in emerging energy sources are also numerous, nanostructured thermoelectric devices are significantly more efficient than their macroscale counterparts [10, 11] and solar cells could be produced at a lower cost.

Whilst nanotechnology is a relatively new field, the production and use of nanoparticles is quite ancient. In the Middle Age, glass makers used colloidal gold to produce the distinctive red coloured glass seen in cathedrals and churches [12]. In 1905, Zsigmondy described the formation of a red gold sol, which is now understood to comprise of particles in the 10 nm size range. Michael Faraday reported the presence of colloids as far back as 1857 and described them as ‘divided metals’.

What has changed during the past few decades is the number of potential applications and the sophistication of the production processes. Particles with specific size, shape and composition are now readily available [13]. However, many of the previous applications are highly dependent on the nanoparticles size [14,15]. In addition, recent concerns have been expressed with regard to the short and long term health effects of exposure to such materials [16].

Techniques used to measure the size of nanoparticles are often based on different principles and measure different properties – or measurands, in metrological language. This creates a certain degree of divergence between laboratories or production centres.

Whilst much metrology activity has been focused on measuring the average particle size accurately, there has not been much effort in determining the size distribution of nanoparticle samples. Uncontrolled size distribution will cause adverse effects in nanocomposites for example [18,19] This distribution is, in-turn, affected by the agglomeration of the core particles [17] and may influence the measured nanoparticle size.

To ensure continuing innovation and the safe utilisation and development of nanoparticle-enhanced products, it is thus imperative that the size distribution of nanoparticles is measured accurately. The aim of this guide is to provide a sound basis for measurement in this advancing area.

Metrological Concepts

In this section the basic concepts of metrology, the science of measurement and its application, are briefly introduced. A more detailed exposition can be found elsewhere: for example in “The Expression of Uncertainty and Confidence in Measurement” - document M3003 produced by UKAS [21]; “A Beginner’s Guide to Uncertainty of Measurement” by S. Bell [22] or “Introductory Guide to Nanometrology” by P.E. Hansen and G. Roebben’ [23].

Measurand

A measurand is defined as the ‘quantity intended to be measured’ [24]. Whilst this may sound trivial, a poorly defined measurand can lead to much confusion when comparing analyses. For example, the phrase “average particle diameter” may refer to many different measurands: the ‘Feret’ diameter - the largest single dimension - will be different from the ‘spherical equivalent diameter’ and the ‘hydrodynamic diameter’, often reported incorrectly as the physical diameter, will be different again.

Traceability

A measurement is traceable if there is an unbroken line of calibrations from the measurement through to the relevant SI unit. For example, atomic force microscopy topography images can be made traceable by the use of interferometers - which can be traceable directly to the wavelength of light - or via the use of calibrated transfer standards [25]. Each of these calibrations should be documented and will contribute to the overall uncertainty of the measurement. Traceability allows different measurement, made at different times and locations, to be meaningfully compared with each other.

Measurement precision and accuracy

There is sometimes a great deal of confusion with regard to these terms; how can a precise measurement be inaccurate and vice-versa? To understand this, let us consider the following definitions taken from the International Vocabulary of Metrology (VIM) [24].

Measurement error: measured quantity value minus a reference quantity value of a defined material or device. The latter value will be defined or calibrated beforehand and gives a reference point to compare new measurements. Measurement errors consist of several components:

Systematic error: a component of measurement error that in replicate measurements remains constant or varies in a predictable manner. The **measurement bias:** is an estimate of a systematic measurement error

Random error: component of measurement error that in replicate measurements varies in an unpredictable manner. A **reference quantity value** for a random measurement error is the average that would ensue from an infinite number of replicate measurements of the same measurand (Figure. 1).

Trueness: Closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value. Measurement trueness is then inversely related to the systematic measurement error estimated by the ‘bias’.

Precision: Closeness of agreement between measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. Precision depends only on the distribution of results and it is not related to the true value. Usually precision is expressed numerically by measures of imprecision such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.

Accuracy: Closeness of agreement between a measured quantity value and a true quantity value of a measurand. A measurement is said to be more accurate when it offers a smaller measurement error.

So, when talking on individual measurements, we can see if one value is more accurate than other by checking the closeness to the true value. After replicate measurements, we get an average value with an offset (bias) from the true (reference or conventional) value, this average value centred on a certain distribution whose narrowness, estimated by its standard deviation, characterizes the precision of the measurement. But precision does not play any role in the accuracy, only the position of the average value of the distribution of values.

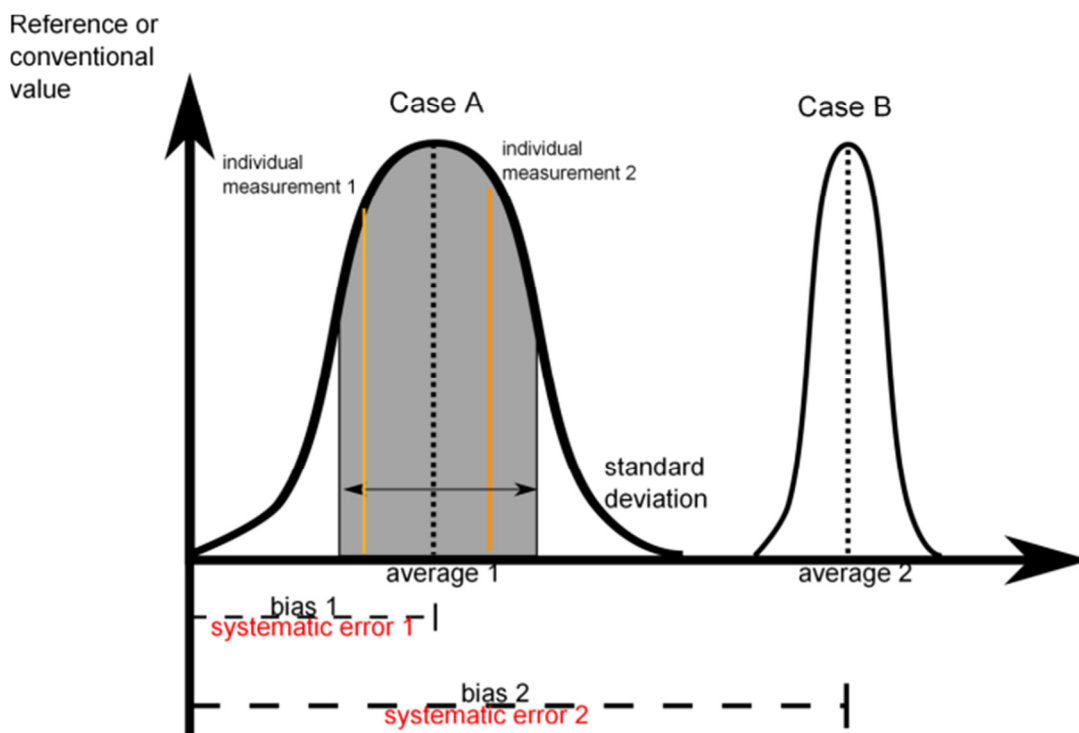


Figure 1: Accuracy vs precision.

- Individual measurement 1 is more accurate than individual measurement 2.
- When taking many repeated measurements, only the average value plays a role with respect to accuracy, independently of the precision: Case A is more accurate than Case B and shows a smaller bias. Case B is more precise than Case A.

Measurement uncertainty

Measurement uncertainty is defined as a non-negative parameter characterising the dispersion of the quantity values being attributed to the measurand [24].

The **standard measurement uncertainty** is the measurement uncertainty expressed as a standard deviation.

The **combined standard uncertainty** is obtained using the individual standard uncertainties associated with the input quantities in a measurement model.

Although the combined standard uncertainty can be used to express the uncertainty of a measurement result, in some commercial, industrial, and regulatory applications, and when health and safety are concerned, it is often necessary to give a measure of uncertainty that defines an interval about the measurement result that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand.

The **coverage factor** is a number larger than one which when multiplied by the measurement uncertainty gives the **expanded uncertainty**. Typically a coverage probability of 95 % is stated, which is normally reported as the standard uncertainty multiplied by the coverage factor $k = 2$, assuming that the final distribution is normal or Gaussian [21]

To properly quantify a measurement result it is thus essential to report

- 1) the measurement result with an uncertainty figure
- 2) a statement of the coverage factor with a level of confidence if appropriate
- 3) how the uncertainty was estimated.

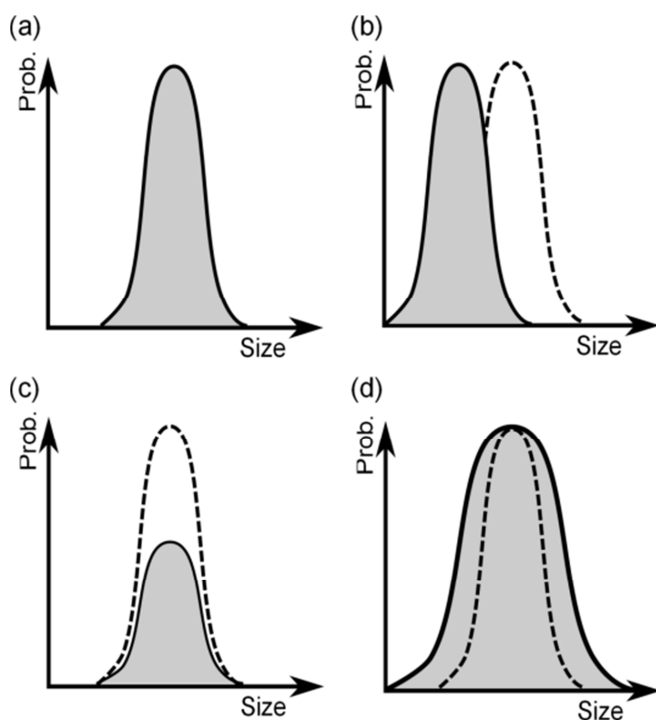


Figure 2: Errors in nanoparticle measurement.

(a) is the 'true' nanoparticle size distribution.

(b) error in determining the mean particle size.

(c) error causing the probability of the average particle size to drop and

(d) broadening of the measured nanoparticle size distribution from the true value.

This guide will provide practical examples on how to determine measurement uncertainty when nanoparticle size is measured by using a model approach (Type A) in accordance to the international document ‘Guide to the expression of uncertainty in measurement’ (GUM) [100].

Application to nanoparticle measurements

Applying these metrological concepts to nanoparticle measurements is made more complicated by the presence of a particle size distribution within the nanoparticle sample. Practically, this means that a range of particle sizes will be measured and errors will affect both the average particle size (Figure 2(b)) and the size distribution (Figure 2(c-d)). Ideally, these errors should be investigated along with the accuracy to determine the mean particle size.

2

IN THIS CHAPTER

- Description of factors affecting the dispersion of nanoparticles in a liquid.
- Recommendations for the preparation of nanoparticles deposited onto a solid substrate.
- Review of selected factors affecting measurement uncertainty such as the number of particles measured.

The correct handling and preparation of samples is essential to obtain the correct size distribution. Production batches of nanoparticles are normally supplied in the form of powders or in suspension. In most cases, even nanoparticles that are supplied as a powder must be dispersed into a liquid at one stage of their handling. This section provides guidelines to dilute nanoparticles to the correct concentration for direct analysis or subsequent deposition onto a flat substrate as described in Figure 3.

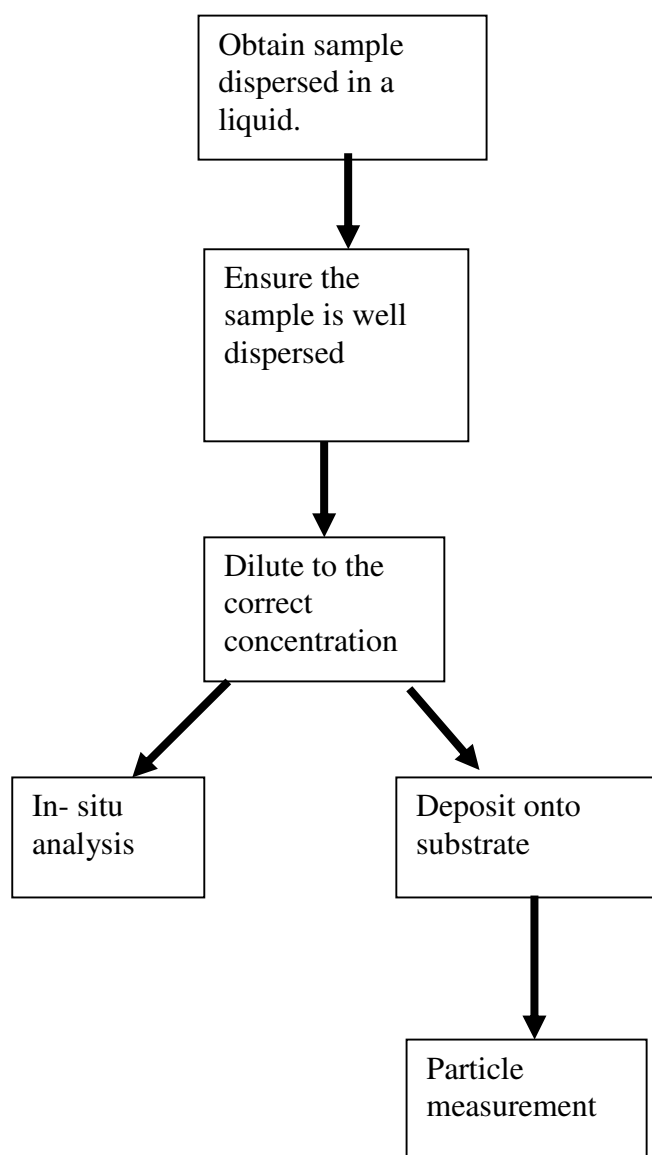


Figure 3: Flow diagram showing the sample preparation procedure with minimum contribution to the uncertainty.

Ensuring a good dispersion

Dispersing nanoparticles into suspension may be a difficult task: many nanoparticles tend to agglomerate over time unless stabilized [26]. The most common method to achieve a stable suspension is to control the interaction forces between the particles. This is done either by altering the nature of the dispersing medium or by coating the nanoparticles with a surfactant. In general, there is an abundance of resources and experience from the field of colloidal chemistry and details of the correct medium and procedure can often be obtained from the producer. If this approach is unsuccessful, the next option is to determine the correct conditions [27,28].

The interaction between particles of all sizes in a liquid is usually separated into an attractive component and a repulsive one. Minimizing the attractive component and maximizing the repulsive one will permit to avoid agglomeration.

The attractive component

Attractive forces between particles are made up of Van-der-Waals interactions. There are many different components to these interactions, including Debye, Keesom and the London dispersion force [29] with the latter being the major component under normal conditions. At short distances (less than 30 nm) the attractive force between two spheres with diameters a_k and a_i can be approximated to:

$$F \approx - \frac{(A\bar{a})}{12h^2} \quad (\text{Eq. 1})$$

where h is the separation distance, A the Hamaker constant, and $\bar{a} = 2 a_k a_i / (a_k + a_i)$. An important parameter is the Hamaker constant, which is dependent on the dielectric properties (related to the polarisability) of both the particles and the medium. Hence by changing the dielectric properties of the medium the attractive forces between the particles can be controlled. The Hamaker constant has been widely studied and values for various materials are reported in the literature [30].

The repulsive component

This repulsive component depends on the surface charge density of the particles. Surface charges arise due to dissociated groups, adsorbed ions or polyelectrolyte. The repulsive force is controlled by the electrostatic potential of the particles, the dielectric constant of the medium and the Debye length, itself influenced by the temperature.

The electrostatic potential is usually determined using zeta potential measurement and is mainly influenced by the pH of the medium. Therefore, changing the pH and ionic content of the medium is a standard way to modify the repulsive component and control the aggregation of nanoparticles.

Use of a surfactant

The addition of small amount of surfactant (e.g. 0.1 % w/v) into the medium has been extensively used to stabilize nanoparticle distributions [31]. Surfactants consist of a charged head and a hydrophobic polymer tail. Either the polymer or head will adsorb onto the particles leading to steric repulsions between the particles. Great care must be taken to choose the correct agent. The correct degree of surface coverage by the surfactant is an important factor as too low a coverage may lead to flocculation. The affinity of the polymer for the dispersion medium is also critical: if the tail will not extend from the particle surface but will instead fold up near the surface, good steric repulsion may not be achieved or the apparent hydrodynamic size of the nanoparticle be modified.

A few recommendations are given to ensure that the particles are well dispersed and any agglomerates are broken:

- 1) Make sure the suspension appears clear with no sign of a cloudy appearance.
- 2) If agglomerates are known to exist or are expected they must be broken up prior to analysis. Common methods of doing so including milling [32] or ultrasound [33].
- 3) For all treatments, care must be taken to not alter the nature of the core particles; either by local heating or by break-up of the constituent elements. It is recommended to measure the particle size as a function of treatment time to evaluate possible adverse effect.

Dilution of a suspension

Most liquid based nanoparticle characterization techniques operate with an optimum concentration range. This normally requires dilution of the suspension. This should be done with great care; any change in the nature of the liquid medium can cause the particles to agglomerate. If the material was supplied in suspension then it must be diluted with a fluid that matches the native medium as closely as possible. . It is always recommended to measure the particle size for different concentration to evaluate the possible influence of the concentration the measurement result.

Deposition onto a solid substrate.

Many of the techniques described in this document require the nanoparticles to be attached to a substrate prior to analysis. However, most surfaces (substrate and nanoparticle) tend to be negatively charged and a simple drop and dry approach cannot be used. This section describes deposition techniques on two commonly used substrates. These procedures should be modified for other materials.

Deposition on mica

Mica is a layered inorganic mineral that can be easily cleaved producing large atomically flat regions. They are therefore often used as a substrate for nanoparticle deposition. A typical procedure will be as follows.

- Cleave mica either by fracturing by applying pressure parallel to the mica planes using a sharp object (such as a pair of tweezers) or by using an adhesive tape to remove the top layer.
- Freshly cleaved mica is negatively charged, it can be used directly if a monolayer of particles is needed (see the section on scanning probe microscopy).
- If well dispersed nanoparticles are required, the surface charge must be changed from negative to positive. A simple way of doing this is to coat with a thin layer of poly-L-lysine via the immersion of the mica for 15 seconds in 0.1% w/v poly-L-lysine suspension followed by washing four times with distilled water.
- The nanoparticles are deposited onto the substrate by immersing the coated mica with a small volume of the nanoparticle suspension from a few minutes to several hours. The actual incubation time is dependent on the concentration of the nanoparticles. A series of trial and error is usually required to determine the correct time. If a long immersion time is required, the sample should be place in a hydration chamber to avoid dry out.
- Many particles are stabilised with ionic salts which could precipitate onto the substrate. In order to prevent this washing with distilled water five times and drying under a stream of nitrogen or filtered air is sufficient.

Deposition on silicon wafers

The main drawback of mica is that it is non-conducting. A suitable alternative substrate is a silicon wafer. These are widely available and are suitably flat. However they cannot be easily cleaved and the procedure has to be modified [35].

- The silicon wafer surface should be cleaned using a strong cleaning agent such as piranha or a sodium hydroxide/ethanol mixture. Standard precaution should be observed during this step [36].

- The surface charges needs to be also changed from negative to positive. The previous poly-l-lysine approach can also be used, however the use of silanizing agents is more common[37] [38]
 - 3-aminopropyltriethoxysilane (APTES) or
 - 3-glycidoxypropyltrimethoxysilane
 are commonly used. Typically, a 1% solution of the active agent is diluted in acetone, toluene or similar. The cleaned substrate is immersed in the solution for 15 minutes after which the substrate is washed at least five times in the solute, and then blown dry with nitrogen or filtered pressurised air.
- The procedure can proceed as with modified mica.

Deposition on TEM grids

TEM grids are commercially available with various coating, including carbon and silicon monoxide. TEM grids coated with carbon films are the most commonly used. If the nanoparticles are sufficiently well dispersed in a non-aqueous or low salt concentration medium, pipetting a small (5 µl) amount of the suspension onto the carbon coated grid and allowing the grid to dry is generally sufficient.

The strong surface forces associated with water means that as the sample dries out, the passing air/water interface can cause the particles to agglomerate. Another problem occurs when the particles are negatively charged: they will not interact strongly with the substrate and tend to agglomerate. A simple way to overcome this is to suspend the particles in a less polar solvent (for example ethanol and chloroform). If this is not possible, an alternative procedure [39] based on the deposition of a hydrophilic film on the TEM grid has to be used. Carbon coated TEM grid can be:

1. treated with a plasma,
2. exposed to aminopropyltriethoxysilane (APTES)
3. exposed to 3-glycidoxypropyl-trimethoxysilane (GOPS)
4. exposed to amino-propyl-dimethyl-ethoxy-silane (APDMES) [39].
5. Place 1-10 µl of the suspension onto the grid and seal in a hydration chamber.
6. After 1 hour, remove the grid and dip once in water and twice in ethanol.

In this procedure the particles adsorb onto the film rather than being dried onto it. Washing with water removes any non-adsorbed particles and the ethanol means there is no passing water to air interface.

Other factors affecting measurement uncertainty

Common sources of systematic error and uncertainty in dimensional measurements are described in this section, whilst those relevant to specific techniques will be described later on. For more information see the UKAS document ‘The expression of uncertainty and confidence in measurement’, p.45 (21).

Temperature

Variations in the temperature can have both direct and indirect effect on the measured value. An example of a direct effect is the measurement of the hydrodynamic radius by dynamic light scattering where the measurement is temperature dependent. Some indirect effects are the changing nature of the particles with temperature including expansion or degradation and changes in the suspension medium such as viscosity. Controlling the temperature during the whole measurement cycle is then vital. Uncertainties may be associated to both the control and the recording of the temperature.

Cosine and geometric errors

These derive from

- internal misalignments in the measuring instrument
- external misalignments between the probe (stylus, beam, cantilever, electron beam etc.) and/or the imaging or detection systems with the object under measurement.
- incorrect image analysis procedures.

In dimensional metrology, cosine errors always lead to a lower value of the measurand. Geometric errors may cause either lower or higher values when affecting the magnification. In microscopic methods on which the dimensions of the particles are measured individually, these errors may cause relatively high measurement errors.

Number of particles measured

When using microscopic techniques to determine the size distribution of particulate samples, choosing the number of particles to sample and measure is critical. This number will obviously represent only a very small fraction of the real population. The actual number required is in turn influenced by the size distribution of the sample, the degree of confidence required and, in practical situations, the resources available.

Assuming that the particles diameter are distributed along a known distribution with mean size (u), the ISO standard 13322-1:2004 gives the number of particles (n) that need to be measured for a given confidence limit :

$$\log n = -2 \log u + K \quad (\text{Eq. 2})$$

where K depends on the confidence limit and the particle distribution function.

In the special case of a number based particle size distribution distributed along a log-normal the equation becomes:

$$\log n = -2 \log u + \log \left(\sigma^2 \left(\frac{\sigma^2}{2} + 1 \right) \right) \quad (\text{Eq. 3})$$

where σ^2 is the variance of the distribution.

An alternative approach is to use Monte Carlo computer simulations. A random sample of a log-normal distribution of nanoparticles - with the experimental mean size and variance - can be created to estimate the standard deviation of the sample mean (*SDSM*):

$$s(x) = s(x_i) \left(\frac{1}{\sqrt{N_s}} \right) \quad (\text{Eq. 4})$$

where N_s are the sample size and $s(x_i)$ is the standard deviation of the sample data.

Examples for scanning probe microscopy (SPM) and transmission electron microscopy (TEM) measurements are shown in Figure 4. In this figure, the experimental standard deviation of the mean is compared to a theoretical SDSM calculated using the Monte-Carlo method, validating the latest approach. Note that TEM and SPM have different associated uncertainties and, as a result, require different number of particles to be measured. For example, for SPM, after measurement of 1000 particles, the SDSM is about 0.1 nm; whereas for TEM, for the sample number of particles measured, it is in the region of 0.5 nm.

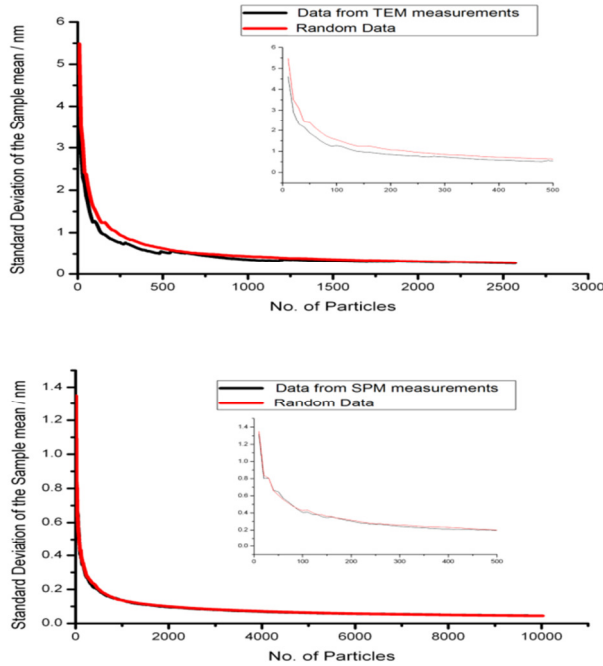


Figure 4:
Comparison between the standard deviation of the sample mean with sample size for real and random generated data for the analysis of silica colloidal nanoparticles using TEM and SPM.

3

IN THIS CHAPTER

- Description of the principles underlying nanoparticle-sizing techniques.
- Brief review of the selected techniques.

Techniques capable of determining the average size of nanoparticle production batches are many and varied. Descriptions of the most common are given in Table I, although this list is not exhaustive. The majority of the techniques fall within one of two types given below:

- **Single Particle Technique.** These techniques are able to take an image of an individual particle. Typical examples are electron and scanning probe microscopies. Such techniques are now well established and accurate calibration standards are available. In many cases, they can provide additional information such as internal structure or chemical composition. They suffer however from a relatively high cost, long analysis time and the requirement for highly trained personnel. When used correctly, their relative uncertainty is mostly limited by the number of particles that can be measured.
- **Ensemble Technique.** In this type of technique many thousands of particles are analysed simultaneously. Common examples are dynamic light scattering (DLS) and Small angle X-ray scattering (SAXS). These techniques are usually indirect in the sense that they do not measure the size of the particles directly, but rather the modelled effect of size change on another property. Ensemble techniques are generally cheaper and easier to use compared to microscopic techniques and better suited to quality control applications.

Table I: Summarising the most common techniques used in nanoparticle sizing applications.

Technique	Size Range in nm	Ensemble or single particle	Sample condition	Additional Comments	Refs	Standards
Transmission electron microscopy	1 – 1000	Single Particle	Deposited onto a film	Provides additional information on the shape, internal structure and chemical composition of individual nanoparticles. Due to this it is often seen as the ultimate technique of nanoparticle measurement. However the high set up cost, the requirements for highly trained personnel and issues such as particle agglomeration means that this technique cannot be used for routine analysis	[40-45]	[46]
Scanning electron microscopy	10 – 10000	Single Particle	Deposited onto a film	Similar to TEM except with lower overall resolution (but with a higher lower limit) but can successfully resolve size larger particles. In addition there are less sample preparation issues.	[42][44][47]	[46]
Scanning probe Microscopy	0.5 – 3000	Single Particle	Deposited onto a film	The major advantages over TEM and SEM are the lower setup and running costs and offers a direct route to traceability via the use of a metrological instrument. In addition particle agglomeration is not as a big an issue. Image artefacts mean that little or no shape information can be obtained.	[42][48][49]	
Dynamic light scattering	0.5 – 1000	Ensemble	In suspension	Well-established technique, which provides reliable measurements of monodispersed, non-agglomerated particles in suspension. Confusion often arises as it measures the hydrodynamic radius and not the physical size. For poly dispersed samples the analysis is weighted towards larger particles and/or agglomerates leading to misleading results.	[20][42][45] [47][50-52]	[53]
Nanoparticle tracking analysis	30 – 1000	Single particle	In suspension	Based on the same principle as DLS but tracking the behaviour of individual nanoparticles, which can be related back to the hydrodynamic radius. Overcomes the problems of polydispersity and agglomeration that	[54-56]	

				are seen with DLS. However there are issues with the relatively small number of particles analysed and low particle concentrations.		
Centrifugal liquid sedimentation	40 – 300	Ensemble	In suspension	Uses the principle of liquid phase sedimentation and light absorption to determine a particle size distribution. Provides information on the spherical equivalent diameter of mono and poly dispersed nanoparticle suspensions. Generally works better with a larger particle size.	[20][45][57]	[58][59]
Small angle X-ray scattering (SAXS)	2 - 300	Ensemble	In suspension	Versatile technique can work with a range of samples, analyses both mono and poly dispersed sample and obtains size and shape information. Major drawbacks are the initial set-up costs (some systems are synchrotron radiation based) and complicated data analysis.	[60-63]	
Scanning mobility particle sizer	3-1000	Ensemble	Aerosols	Measures the size distribution and concentration of submicron aerosols using electrical mobility detection. Due to this it has become the industrial standard for environmental nanoparticle analysis. Measures the aerodynamic diameter rather than the physical size.	[42][64-66]	
Field Flow Fractionation plus DLS or SAXS	1-100	Ensemble	In suspension	The addition of asymmetric flow field flow fractionation (A4F) is a method to gently fractionate nanoparticle suspensions. This has been combined with nanoparticle characterisation techniques to extend their capabilities, for example DLS to analysis polydisperse samples or SAXS to allow online determination of particles shape and size.	[42][67][68]	
Ultra centrifuge	4-100	Ensemble	In suspension	Overcomes the limitations of CLS by operating at higher centrifugal forces. Can successfully analyse poly as well as mono dispersed samples. Measures the hydrodynamic radius.	[42][47][69]	
X-ray Disc Centrifuge	10 – 100	Ensemble	In suspension	This technique has been used for many years and is particularly well adapted for inorganic powders, which tend to be strong absorbers of X-rays. More sensitive to small particle sizes than the CLS.	[20][42][45]	[58,70]

4

IN THIS CHAPTER

- Types of electron microscopes.
- Calibration of electron microscopes.
- Image analysis procedures.
- Two Case studies.

Type of Electron Microscopes

Electron microscopes use a beam of electrons accelerated to high energies (typically 1 – 400 keV) to form high resolution images and are now routinely used to measure the size distributions of nanoparticles.

The electron wavelength is related to the accelerating voltage:

$$\lambda = \frac{h}{\left[2m_0 eV \left(1 + \frac{eV}{2m_0 c^2} \right) \right]^{1/2}} \quad (\text{Eq. 6})$$

where h is the Plank constant, m_0 is the electron mass, e is the charge on an electron and V the accelerating voltage. Electrons accelerated to 200 keV have a wavelength of 0.00273 nm for example. However, the practical resolution of most electron microscopes is lower due to the fact that the quality of electron lenses is not nearly as good as optical lenses in terms of aberrations. Another major source of error is due to the interaction between the electrons and the object under study and the precise determination of the surface of the object.

The potential interactions between a beam of high energy electrons and a thin sample are summarised in Figure 5.

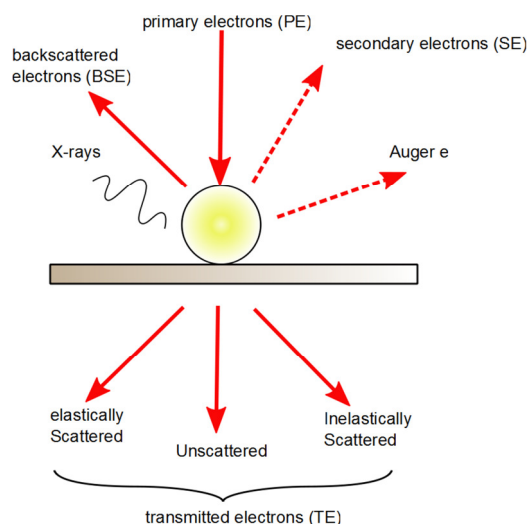


Figure 5: High-energy electrons interaction with a thin sample

Transmission Electron Microscopy

The transmission electron microscope, first demonstrated in 1931 by Ruska and Knoll, and is currently a widely available instrument that can provide high-resolution images (~ 0.1 nm resolution). A thin sample is subjected to a highly focused and coherent beam of electrons; the electrons transmitted through the sample are then projected onto an imaging device - photographic film or CCD camera - to form an image. The electrons can be transmitted through the sample elastically (electrons deflect but do not lose any energy) or inelastically (electrons lose energy). To contribute to the TEM image contrast, the nanoparticles must scatter electrons, which depend on the sample composition and crystalline phase orientation.

Scanning Transmission Electron Microscopy

Scanning transmission electron microscopy (STEM) collects the transmitted electrons as the beam rasters across the specimen. The resolution of the image is controlled by the diameter of the electron beam; state of the art non-corrected electron sources have a diameter around 0.2 nm. Initially STEM were first commercialised as add-on to existing TEM instruments. However, the development of STEM dedicated instruments has led to significantly simpler design compare to conventional TEM (CTEM). As a result they are easier to align and operate and this translates to a significant reduction in training and analysis time.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) uses a highly focussed electron beam incident onto the specimen. As the beam moves across the sample, the backscattered or secondary electrons are collected and their intensity measured. The latest SE microscopes offer a resolution of better than 1nm. Whilst earlier instruments operate at the upper limit of their acceleration voltage, modern instruments can now obtain good images with beam energy of only a few hundred eV, reducing sample charging. However it is always recommended that SEM measurements of nanoparticles should be conducted on a flat conducting substrate - such as a silicon wafer. While conventional SEM images may be used for size measurements of nanoparticles, a number of disadvantages have to be noted. A precise determination of the particle boundary is difficult due to edge effects for secondary electrons. For very small particles – the precise size being dependent of the transmission of the imping electrons in the materials, the signal to noise quite be quite poor as more secondary electrons are being produced in the substrate and less in the particle itself.

Transmission Scanning Electron Microscopy.

For thin samples or nanoparticle, edge effects and influence of the interaction volume can be minimized if transmitted electrons are used for imaging rather than the emitted secondary electrons, see Figure 6. In the transmission scanning electron microscopy (TSEM) mode, the resolution approximately equals the spot size of the electron beam. This allow to use higher acceleration voltage, resulting in smaller spot size, higher beam current and reduced

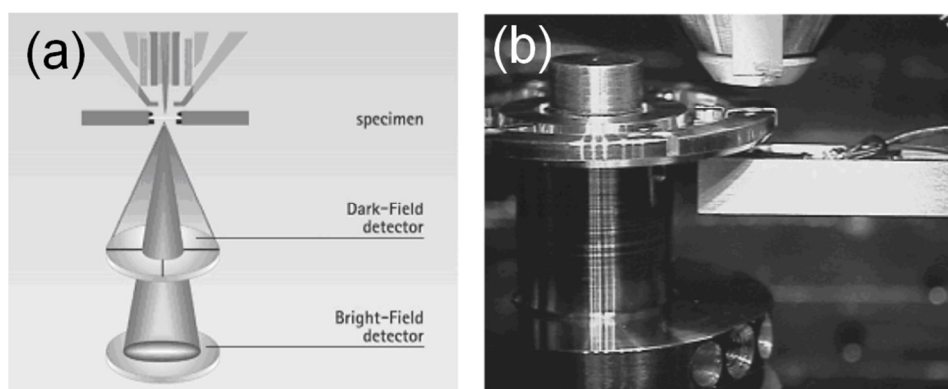


Figure 6: (a) Transmission detector capable of bright field and dark field imaging, by courtesy of Zeiss. (b) The sample holder is able to hold up to six samples which may be chosen by rotating the sample stage.

Calibration of Electron Microscopes

A variety of calibration standards are available for electron microscopes, see Figure 7. Two-dimensional calibration standards are available, Figure 7(a), and astigmatism and orthogonality can also be tested using these standards. For higher resolution, stained catalase crystals, Figure 7(b), with lattice spacings of 8.75 nm and 6.86 nm [71] are commonly used. Latex or gold nanoparticles, Figure 7(c), are now available with a mean particle size defined to nanometre accuracy [34]. The exact magnification of the TEM instrument is however dependent on the position of the specimen within it, which will vary from specimen to specimen. It is this therefore recommended, where possible, to calibrate using the lattice fringes from a known crystalline material, Figure 7(d).

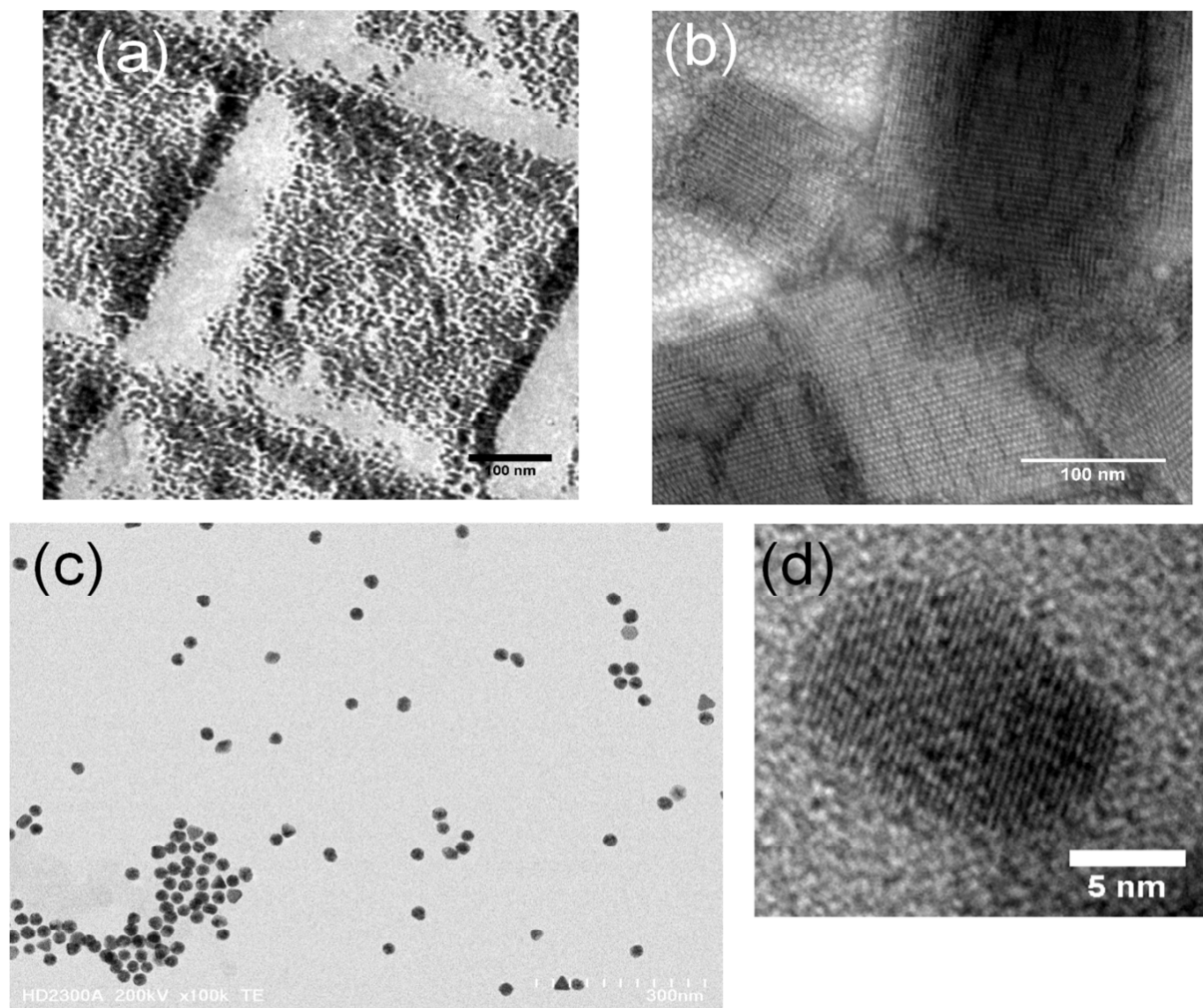


Figure 7: Calibration standards used for TEM. (a) 2160 lines per mm grating (b) Catalase crystals (c) Gold nanoparticles reference materials and (d) Lattice fringes from a zinc oxide nanoparticle.

Image Analysis

The determination of the particle boundaries is the essential requirement for precise particle size measurement. Because manual measurement is both tedious and a source of considerable non-reproducible errors; digital image processing is preferred whenever possible. Thresholding techniques are often used to separate objects from the background, see Sezgin *et al* [72] for a review. Thresholding techniques rely however only on the distribution of grey scale values across the image and the resulting size distribution is highly dependent on the chosen thresholding algorithm [73].

To reduce uncertainties, other information such as the image acquisition parameters and details of the scattering process can be used. For size measurements, threshold levels of the nanoparticles can be calculated using Monte Carlo simulations of the image formation process. The signal level of the transmitted electrons at the particle edge is then calculated by taking into account all relevant parameters of the instrument (e.g. electron energy, probe diameter, detector acceptance angle and energy sensitivity) and of the specimen (material, density, estimated particle size). Frase *et al* [74] gives an overview of program packages used for scanning electron microscopy. Some of these packages are also able to model transmitted electrons and their detection.

Once the nanoparticles are separated from the background, particle analysis routine [75] can be used to calculate the desired diameter: equivalent spherical, Feret or other. When image analysis is done automatically, artefacts like dried chemicals or touching particles are falsely included as identified objects. Depending on the size of the data set their effect may be removed by hand or automatically via limits of some geometrical parameters, limits for the minimum and maximum size or circularity when analysing nearly spherical particles. It is recommended that such limits are set with great care and to verify that they do not alter the size distribution significantly. The use of watershed algorithm, which works by assuming that an image is a topographical surface and then modelling the flow of water upon this surface [76], often leads to a systematic underestimation of the size. If high precision measurements are required, it is therefore advised that touching and overlapping particles should not be included in the measurement.

Sources of uncertainties

There are multiple sources of errors that contribute to the uncertainty associated with the determination of the mean particle size.

Calibration of the pixel size: The uncertainty related to the calibration of the pixel size is due to imperfect reproducibility, drifting instrument conditions, etc. The uncertainty of the stated pitch values of the transfer standard has to be included as well.

Digitization leads to errors because a round object is converted to a number of square pixels. By interpolating image regions of interest the impact of digitization may be decreased.

Pixel noise: Depending on the instrument performance and the image quality, image noise at the boundary of the particle may lead to erroneous inclusion or exclusion of pixels

Case study I: STEM measurement of 30 nm diameter gold nanoparticles.

In this section, scanning transmission electron microscopy operated in secondary electron (SE) bright field transmission (TE), and HAADF imaging on the same set of nanoparticles is presented. One of the aims in this study is to demonstrate how the various modes affect the observed particle size.

The sample of 30 nm colloidal gold (NIST RM 8012); a 3 μ l aliquot was deposited onto a carbon film coated TEM grid (Agar Scientific, UK) and allowed to dry in air. All images were taken with a Hitachi 2300A instrument operated at 200kV with an emission current of 79 μ A. All images were acquired using the ultra-high resolution mode of the instrument with a nominal probe size of 0.2 nm; HAADF images were collected with the annular detector spanning an angular range from 80mrad to 260mrad. The instrument was aligned using the recommend procedure with fine adjustments using Ronchigrams

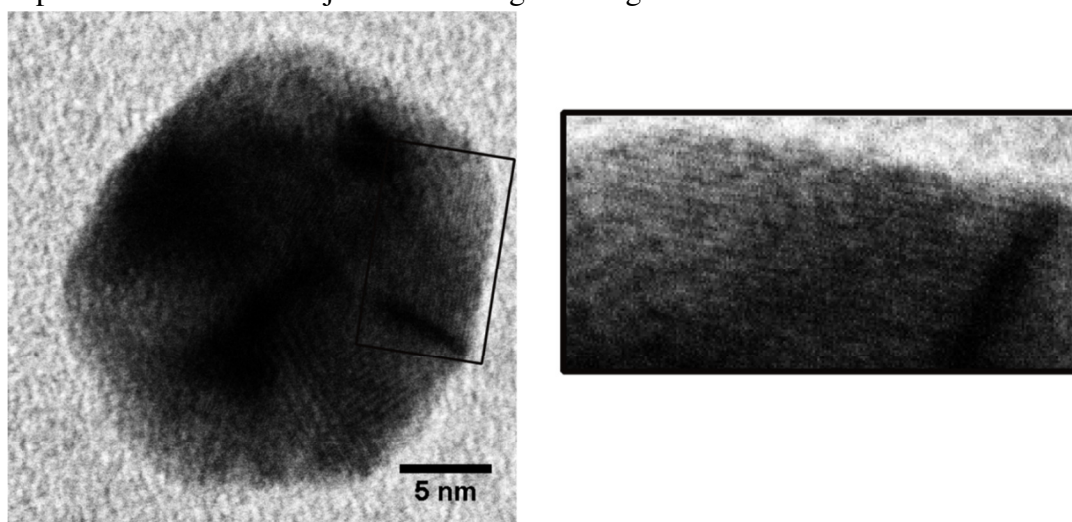


Figure 8: Bright field STEM image with visible lattice spacing (inset).

Gold has a well-defined lattice spacing of 0.204 nm and the calibration of the instrument can be done by measuring the lattice obtained for a high resolution image, see Figure 8.

Secondary electron (SE), transmitted bright field (TE) and high angle annular dark field (HAADF) images were acquired, see Figure 10. An automatic threshold level was applied to the images using the ImageJ program and isolated particles Feret's diameter was measured. Table II summarizes some of the statistical properties of the resultant size distributions shown in Figure 9.

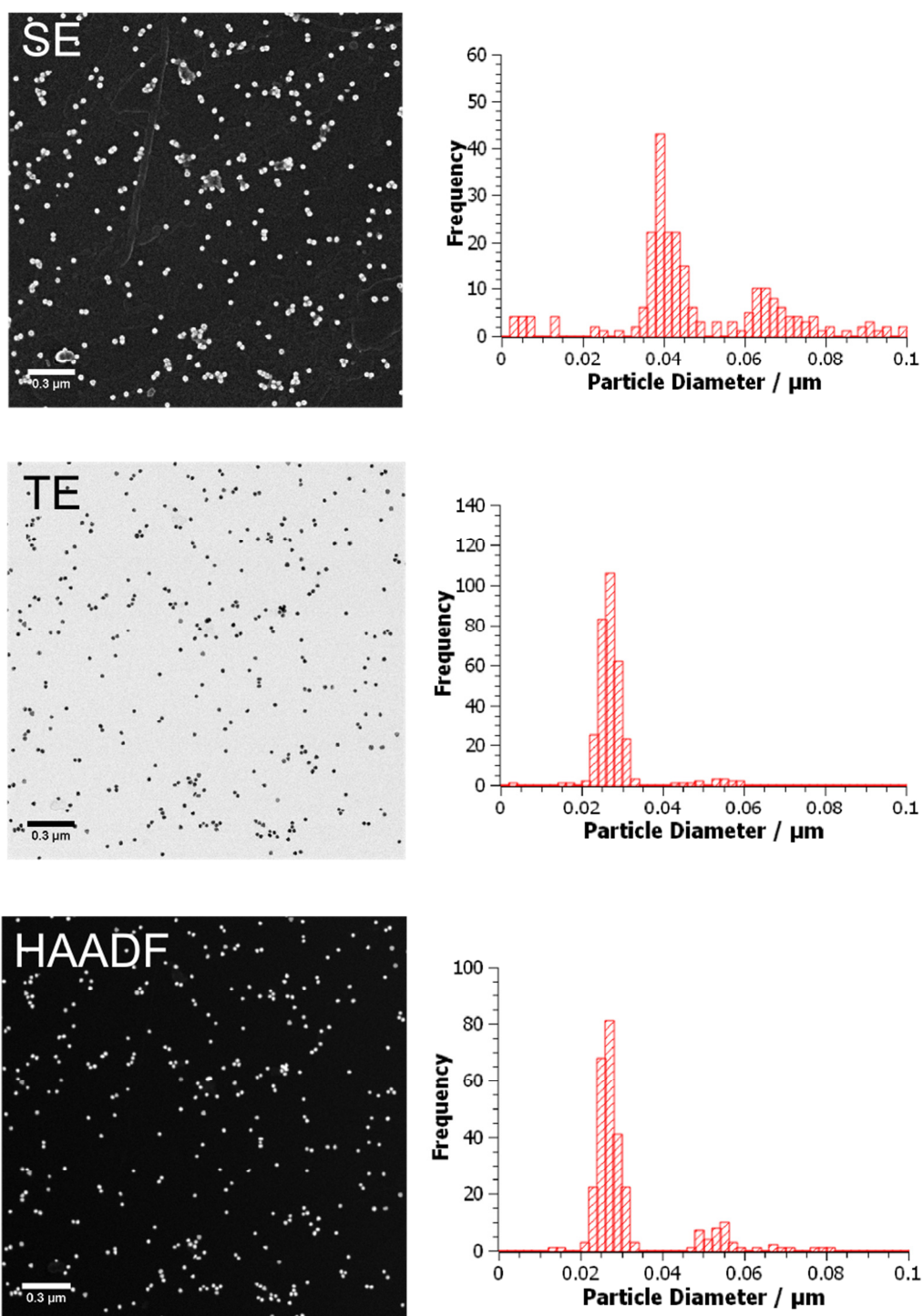


Figure 9: Comparison between the size distributions obtained from the same set of nanoparticles when imaged by SE, TE and HAADF modes.

The mean size of nanoparticles measured in the SE mode is by far the largest due to the edge effect. The TE mode detects more particles than the HAADF mode, with the latter detecting more agglomerates. Even if the mean particle sizes is identical for the TE and HAADF mode, the number of particles identified is larger for the TE mode. The measurement error, estimated by the standard deviation of the mean, is smaller for the TE mode.

Technique	Number of particles detected	Mean of major mode / nm	Standard deviation / nm	Standard deviation of the mean / nm
SE	147	40.3	4.5	0.4
TE	304	26.7	2.2	0.1
HAADF	241	26.6	2.4	0.2

Table II: Comparison of nanoparticle size measurements taken using a dedicated STEM instrument operated in the various modes.

To further understand these results, the line profiles through two neighbouring particles are shown in Figure 10. For the SE image, there is only a small drop in contrast between the particles. The particles appear brighter around the edges, where more secondary electrons are emitted, giving the particles a broader appearance. The line profile shows that it is only for the TE image that the image intensity between the particles is the same as that outside the particles.

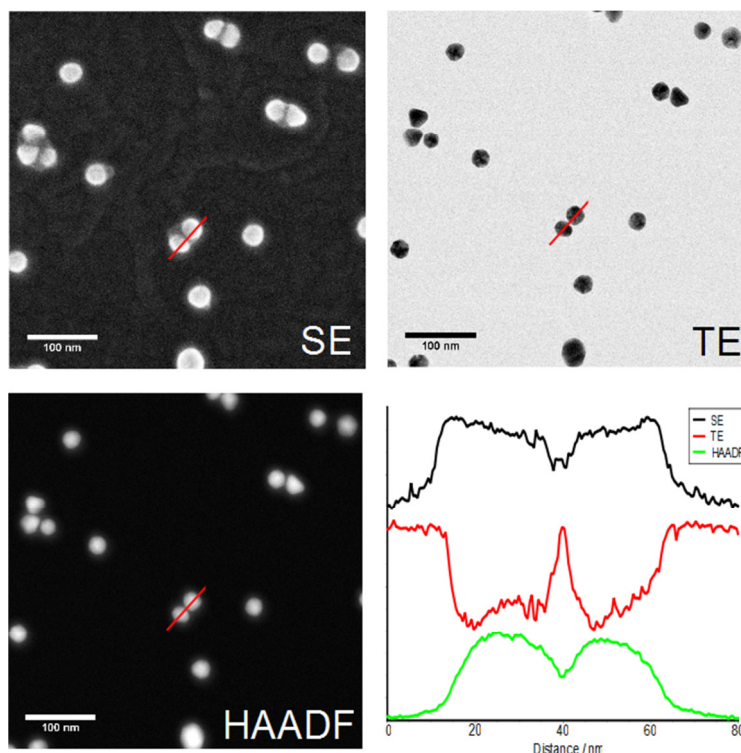


Figure 10: SE, TE and HAADF images on the same set of 15 gold colloidal particles and resultant line profiles from the indicated two particles.

Whilst this study is not a ‘true’ inter-comparison of the three methods, it presents an exemplary cautionary tale when comparing different measurement techniques for nanoparticles

Case study II: Traceable TSEM Size measurement

Traceable size measurements of latex spheres nanoparticles using the TSEM technique are described, further details can be found in Klein *et al.* [79].

A commercial Zeiss Leo Supra 35 VP scanning electron microscope equipped with a transmission detector system (K. E. Developments Ltd - Cambridge, UK) was used. The transmission detector system consists of five solid state electron detectors, four of which are used as dark field detectors and the fifth placed underneath a small pin hole (approximately 140 μm diameter) used for bright-field imaging.

Calibration

For calibration, a two dimensional grating made of Al bumps with a mean pitch of about 144 nm, (2D-150 from Advanced Surface Microscopy Inc) was used. Traceability to the SI unit is ensured by deep UV laser diffraction which yields the mean pitch in x and y directions with uncertainties well below a tenth of a nanometre [78,79].

The settings of the SEM parameters used during the recording of the images of the particles and of the calibration artefact were the same (electron energy, magnification, working distance, scan speed, image shift, etc.). For each SEM parameter set, a number of images of the 2D grating were taken at various positions on the grating. Since the grating is not electron-transparent, the SE detector is used for imaging.

The (x/y)-positions of the individual aluminium bumps in the images were determined using ImageJ [75]. This image analysis software offers straightforward automation thanks to a macro editor and the possibility of recording manual operation of the software as a macro script. The distance between two bumps may be determined by the distance between their centres of gravity. It is therefore not necessary to determine the edge position of the bumps exactly.

As long as the background illumination stays homogenous a simple global thresholding algorithm is sufficient to separate the bumps from the background. The positions of the bumps were further processed using a MatLab routine to determine the pixel distances between adjacent aluminium bumps in both the x and y directions. Ensuring sufficient counting statistics, the mean distance was then compared to the pitch of the transfer standard obtained by optical diffraction and the pixel size was calculated separately for x and y directions.

Because the electron beam is usually scanned along the x direction and has to be repositioned in y direction after every line scan, there is a slight difference of the pixel size in both directions. Therefore, the reproducibility, and thus uncertainty, in the y direction is worse than in the x direction. Most instruments also show an effect called ‘leading edge distortion’. The electron beam deflection has to be accelerated at the beginning of every line scan which leads to a slightly different scan speed at the beginning of the line and thus to deviating pixel sizes at the beginning of the image, see Figure 11. The respective pixels were therefore omitted for both calibration and subsequent measurements. Another possibility would have been to raise the uncertainty of the pixel size to a level that includes the deviating sizes.

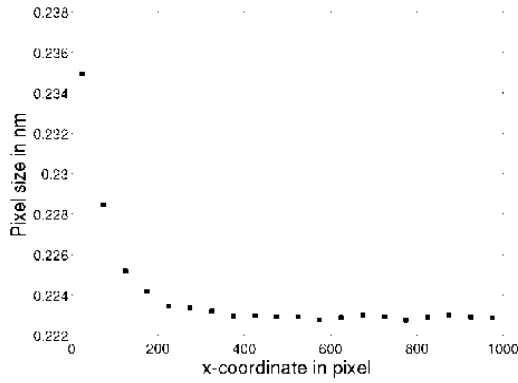


Figure 11 Leading edge distortion leads to deviating pixel sizes at the left part of the image.

The SEM was operated using an acceleration voltage of 30 kV at a working distance of 3 mm. The detector was positioned 5 mm beneath the sample leading to an acceptance half-angle of 16 mrad for the bright field detector. The nominal magnification was set to 50,000 times corresponding to a pixel size of approximately 2.2 nm and an average area of 120 pixels per particle. Recorded TSEM signals were digitized resulting in 16 bit tif-format images with a size of (1024 x 768) pixels. At least 200 TSEM images from at least two different TEM grids were taken to obtain a sufficient number of individual and separated particles. Most of the TSEM images were recorded in an automatic mode without the need for an operator.

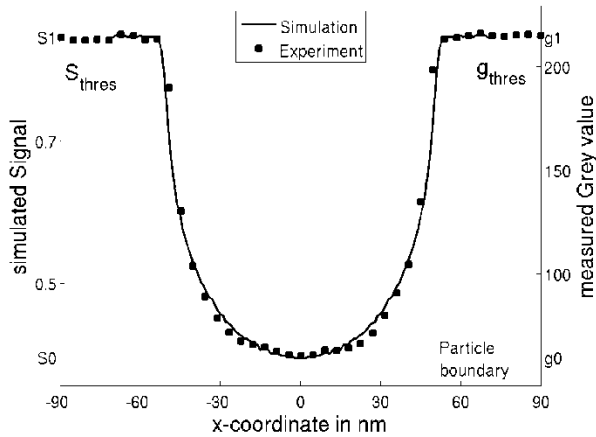


Figure 12: Real threshold level can be determined using simulation.

To achieve high accuracy measurements Monte Carlo simulations of the image formation process were carried out so the detection of the particles boundaries could be modelled, see Figure 12. The simulation program package MCSEM which takes into account the properties of the electron beam, the particle and the detector [78] was used.

As can be seen in Figure 13, simulations show a high dependence of the threshold signal level at the particle boundary on both particle size and material. In the limiting case of a large particle composed of a heavy material the normalized signal at its boundary will be 0.5 because half of the electrons of the Gaussian beam run alongside the particle and hit the detector while the second half is prevented from transmission by scattering and absorption inside the particle. The smaller the particle and the lighter its material, the more electrons are able to transmit through the particle and contribute to the signal. Thus the signal level at the

particle boundary is raised for small and light particles. Taking into account the additional thin supporting film the signal level at the boundary is somewhat lower.

Because the threshold level at the particle boundary is size dependent, the boundary of every particle had to be detected individually by an iterative procedure which adjusts the threshold level according to the size estimated in the previous step. For this purpose a data set of threshold values for various particle sizes has been calculated in advance. Data evaluation of the TSEM images was performed using a special Matlab (Mathworks, MA) program developed at PTB which uses the particle analysing routine of the open source software package ImageJ [75]. The image analysis consisted of several steps:

1) Global thresholding.

A given TSEM image was analysed using a global threshold level derived from simple histogram analysis using the minimum or mode algorithm [81]. Analysing the threshold image gives the (x/y)-positions of potential particles. A region of interest (ROI) is defined around every particle.

2) Iterative determination of threshold (and size).

For each particle of the ensemble a first rough estimate of its size based on the global threshold value was carried out. Since the threshold depends on particle size, a better estimate of the threshold is obtained from the first guess of the diameter. This procedure was iterated until the diameter and threshold were stable. The conversion of simulated threshold levels S_{thres} to grey scale values g_{thres} needed for image analysis can be done using the following equation:.

$$g_{thres}(d) = \frac{S_{thres}(d) - S_0(d)}{S_1 - S_0(d)} \cdot (g_1 - g_0) + g_0 \quad (\text{Eq. 8})$$

Therein the simulated signal level and measured grey scale value at the background are S_1 and g_1 , respectively. Similarly, S_0 and g_0 are determined from the darkest region at the middle of the particle. The dependence from the diameter d is explicitly stated.

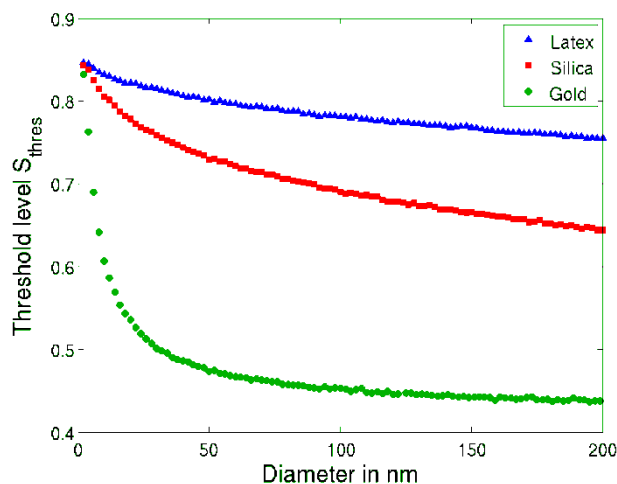


Figure 13: The threshold level at the particle boundary depends on both the size and material of the particle.
NB: The data shown only holds for the specific experimental conditions

3) Interpolation of the image and final size determination.

The image of each identified particle, including its surrounding (ROI), was expanded by interpolation with a factor of 5 to improve determination of the particle boundary. The particle diameter was determined from the number of pixels belonging to the particle under test, i.e. pixels with grey values below the threshold level determined in step 2. The diameter is defined as the diameter of a circle with the same area, i.e. number of pixels.

4) Removal of artefacts.

Some objects have to be removed in order to eliminate artefacts like particle clusters or contamination. The selection of features for removal is controlled by three geometric parameters. Firstly, particles are identified simply by their size. Objects whose sizes do not fit in a certain range are omitted. Two parameters have to be set: the minimum and maximum size. The remaining parameter is the “circularity” of the objects under test. Only particles with circularity above a certain threshold (e.g. 0.8 or 0.85) were analysed. These parameters have to be set carefully because they directly influence the size distribution and thus the mean size. Our software provides visual feedback: The outlines of all objects are displayed superimposed on the original image with their size and circularity. The outlines of analyzed particles are white while those of rejected objects are black. In this way a quick inspection is possible to determine if the geometrical parameters have been set to meaningful values.

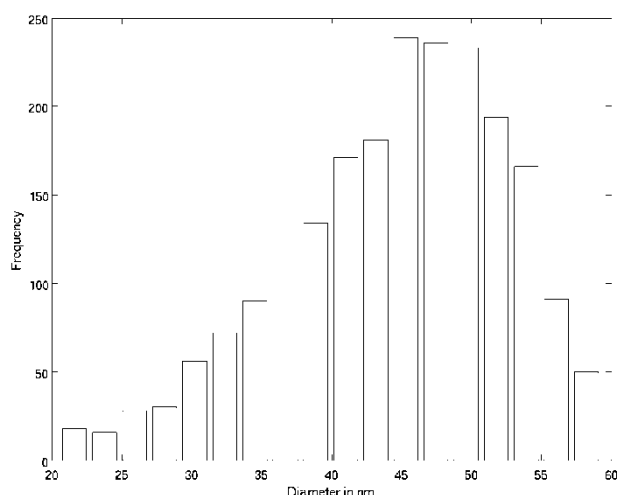


Figure 14: Size distribution of the measured latex nanoparticles.

Results

During the measurement 271 images were taken which showed 2113 particles that could be analyzed using the automated image analysis based on the iterative threshold approach. The size distribution of the latex particles is shown in Figure 14. It leans towards smaller particles. The mean particle diameter is 44.5 nm. The combined expanded measurement uncertainty (coverage factor $k = 2$, 95 % confidence interval) is 2.6 nm. The standard deviation of the particle size, which characterizes the spread of the size distribution, is 7.9 nm. The median and mode of the size distribution are 45.7 nm and 45.3 nm respectively.

5

IN THIS CHAPTER

- The various approaches to nanoparticle size measurement using SPM.
- How to set-up the instrument and measure nanoparticle size distribution.

Using the scanning probe microscopy (SPM) technique the mean diameter and size distribution of spherical particle (1-2000 nm in diameter range) samples can be determined in a non-destructive way and under ambient conditions. There are two different possible methods how the particles size can be determined from SPM measurements. Firstly, the height of individual particles, spread out on an atomically flat surface, can be measured. Secondly, for a close-packed particle monolayer, one can determine the particle diameter from lateral distances between particles. We strongly recommend the first method, as the second maybe a source of large errors.

The SPM instrument itself should provide results traceable to the definition of the SI unit metre. Preferably, metrology SPMs with interferometer position measurements should be used [82,83]. Powerful SPM image evaluation methods are necessary, as many particles on many images have to be analysed to obtain a meaningful average size and size distribution.

The SPM technique involves scanning a sharp fine tip, usually made of silicon, across the surface to be measured. Detecting the tip-sample interaction force and varying the height of the sample or tip the distance between tip and surface is maintained at a constant value and thereby a height map of the sample is acquired. There are many different scanning probe microscope instrument providers, and a large number of instruments on the market, most of which are capable of imaging nanoparticles. However, accurate measurement requires the following:

- Intermittent or non-contact mode imaging [84,85] to reduce the lateral forces. In contact mode imaging, the high lateral forces generated may displace any weakly attached particles.
- Closed loop x and y scanning [86].
- The resolution of the z piezo needs to be optimised: on some instruments, a low voltage mode can reduces the voltage required to produce maximum movement of the z piezo.

SPM measurement procedure

1) SPM calibration: Before starting particle size measurements it is essential to calibrate the SPM instrument using certified gratings and step height standards. It is important to use similar parameters with respect to, amongst other things, SPM mode, image size, resolution, scan speed and z-piezo position as for the planned particle measurements. For commercial instruments, procedures given in the literature [92] can be applied. Metrological SPMs provide usually direct traceability to the definition of the SI unit, the metre. [49]

2) Approach: Particles tend to stick onto the AFM tip due to surface forces leading to tip artefacts. Using a high set point and a slow initial scanning rate with moderate feedback parameters is recommended for the first image. Always start with a small image range to minimise tip crashes with particles.

3) Scan range setting: The maximum SPM range should be adjusted according to the number of pixels in the image, the particle diameter, the SPM tip radius and the requested relative height accuracy.

4) Parameter optimisation: Initial tests using different SPM operation parameters for scan size, scan speed, oscillation amplitude set point and with various PID feedback settings should be performed before starting the final measurements. The results of these tests are important for the uncertainty estimation.

5) Number of particles: The higher the number of particles measured and evaluated, the lower the uncertainty in the average size estimate. This contribution scales roughly inversely to the square root of the number of particles.

6) Reference surface and validation: It is always a good idea to measure a sample without nanoparticles with and without pre-treatment in order to verify the scanner flatness or bow, the cleaved mica surface quality, the film homogeneity, roughness and noise. If available, known particle reference samples of about the same type and size should be used for validation.

SPM image analysis

Powerful image evaluation tools are required, as many particles on many images have to be analysed. Because there are many different tools available it is not possible to give a step-by-step guide here. Usually several diameter parameters such as mean, median, mode, standard deviation and size distribution histograms are determined from the measured SPM images. The evaluation process often includes manual interactions for selecting areas of interest, setting threshold values, applying filters, selecting fitting functions, etc. It is therefore strongly recommended that the evaluation process is verified using simulated SPM reference data sets.

The SPM can be used to measure the height of individual particles or the lateral distance of touching particles. The two methods are to some extent independent; however we strongly recommend using the height to improve the accuracy of the measurement (Figure 15).

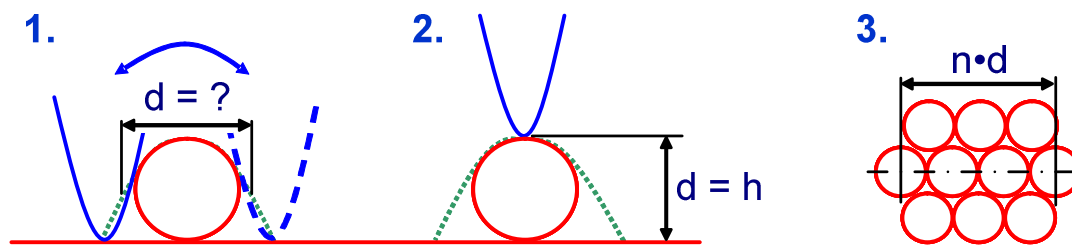


Figure 15: Particle diameter measurement using SPM. 1) Lateral diameter measurement on a single particle is strongly influenced by the SPM tip. 2) Particle diameter measurement using its height. 3) Diameter determination using the lateral distance of a close-packed particle surface layer will be strongly influenced by the packing density.

Height measurement

The height method [48] works on single or lightly agglomerated particles (Figure 16) and appears simple and straightforward; however, the measured height corresponds to the particle diameter only if the interaction distance of the SPM tip is the same on top of the particle as on the flat reference surface. It is recommended that certain experimental conditions are verified.

- no surface layer, where the particles can be raised or “embedded”, is present on the surface
- the interaction forces cannot deform the particles [91].

Typically tests with different SPM imaging parameters such as different scan sizes, scan speeds, oscillation amplitude, set points and PID feedback parameters should be performed to test variability related the measurement conditions.

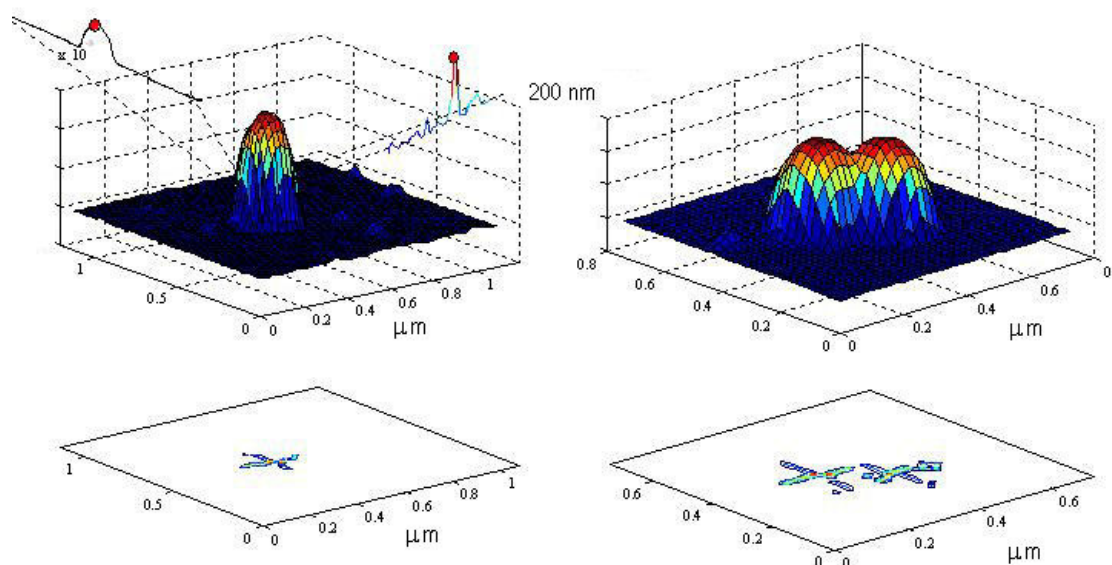


Figure 16: the height maximum is found using slices along two axes. This approach can also be applied to the analysis of agglomerated particles.

Lateral measurement

When nanoparticles form monolayer islands of close packed particles, **lateral measurements** [49] would appear to be more accurate to height measurements because x-y calibration standards and closed loop x-y scanners are generally more accurate and widely available than their z-axis counterparts. The issues regarding shape of the SPM probe and surface interactions are also overcome. Depending on the possibilities of the image evaluation tool the average particle distance can be determined from single particle rows, from nearest neighbour distances or by an estimation of the “grating periods” through Fourier analysis.

However, the lateral method is intrinsically limited, meaning for most circumstances, height measurements are by far the most reliable approach. Using simple geometrical considerations the ideal close packed arrangement is only possible for perfect spherical particles of one single size. Even a small size distribution of a few percent - generally the variation for calibration grade reference particle samples - disturbs the regular pattern. As the size distribution becomes larger, more irregularities in the close-packed particle monolayer occur with gaps forming that then affect the average particle distance - see Figure 17. Generally larger particle agglomerates tend to accumulate more defects than smaller clusters consisting of only a few particles.

Typically the particle diameter determined from nearly perfect particle rows is increased by about 1/3 to 1/2 of the standard deviation of the particle size distribution. The only way to correct for this effect is by numerical simulations of particle agglomerates on flat surfaces. Modelling additionally the SPM tip convolution in the simulation gives SPM data sets which allow the verification of the entire evaluation process. Besides these geometrical effects, there

are also further influences on the lateral particle distances to be considered such as interface layers between particles due to adsorbed water or surfactants.

An example illustrating the importance of correcting lateral measurement is given in Figure 18.

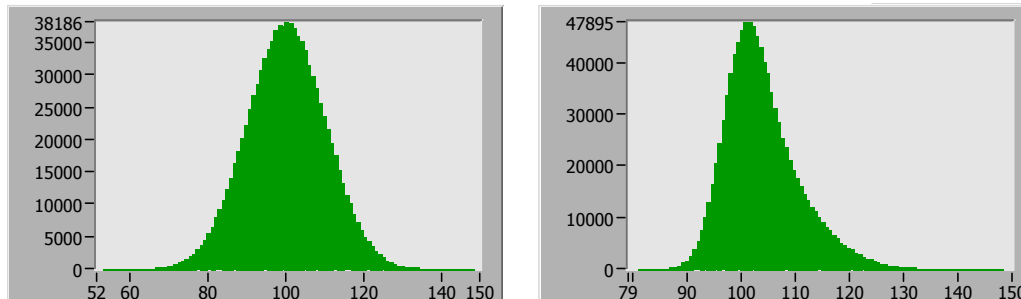


Figure 17: Left: Ideal Gaussian distribution of 100 nm diameter nanoparticles with a 10 nm standard deviation.

Right: Lateral measurement of simulated AFM images: mean is larger by a third of the original standard deviation (103.6 nm) and the standard deviation is reduced to 6.7 nm.

When simulation of the complete measurement process is available, it is possible to obtain accurate measurement using the lateral measurement method - see case study at the end of this section. However, this method is more involved than the height measurement methods and required a-priori assumption about the nanoparticle size distribution.

Source of uncertainties

In the case of particle size measurements, the uncertainty depends not only on the SPM instrument used but also on many additional influence factors that can lead to systematic offsets. The following list of influence factors can serve as a checklist for the uncertainty estimation.

Instrument:

- Lateral and height calibration
- Scanner flatness and noise
- Scanner hysteresis effects
- Pixel size or image resolution
- Tapping set point
- Scanning speed
- Feedback parameters
- Evaluation procedure and correction factors

The SPM relies on piezo scanners to control the movement of the sample. In normal operation, it is also used to record the height as a feedback loop is normally employed to keep the deflection of the cantilever constant. Errors arise, as the movement response of the scanner with voltage is not linear. These uncertainties can be reduced or even removed completely by using a closed loop z-scanner. If this is not a practical option, then the instrument should be calibrated using reference materials with dimensions as close as possible to those of the nanoparticle sample being measured

Sample:

- Substrate flatness and roughness
- Sampling of particle material
- Particle shape and size distribution
- Number of particles measured
- Packing factor due to particle size distribution
- Particle embedding in a surface layer
- Particle deformation due to particle-particle interaction forces
- Interface layers between particles due to adsorbed water or surfactants films

Size measurement of nanoparticles by SPM is only truly accurate if the particles are perfectly spherical. In the vast majority of cases, this is obviously not true; hence the manner in which the particle attaches to the substrate will affect the size measurement. It would be expected that only the shortest dimension would be recorded.

Interaction:

- Different interaction distances of the SPM tip on top of the particle and on the reference surface
- Particle deformation due to tip-particle interaction forces

SPM intermittent contact mode is unique as there is actual physical contact between the measurement device (SPM tip) and the particle. The large forces that occur between tip and surface can deform soft particles and even displace those that are weakly adhered to the surface. In order to determine if this is fact occurring the measurement should be repeated with varying forces between the probe and sample, which can normally be adjusted by varying the set point..

In the example described in the following case study, the uncertainty budget had nine contributions and the largest ones were the tapping set point and the estimated background flatness (scanner and sample). An international comparison, where various methods such as SEM, TEM and SAXS were applied to identical samples, indicated the presence of an additional uncertainty contribution for all methods in the order of $u_i = 2.2$ nm due to unknown effects.

Case study III: SPM measurement of polymer particles

The following example is based on measurements made at METAS using a metrological SPM. The instrument has a head with parallelogram flexures and uses capacitive sensors for the tip displacement measurement. The capacity sensors are traceable to the definition of the metre through interferometric z-calibration and laterally by using reference gratings calibrated by laser diffraction. Additional verification measurements were performed with a 3D-SPM with 3 differential interferometers. The reference particle sample was obtained from Thermo Scientific (3200A) and consisted of 200 nm polymer spheres in suspension. The polymer particles formed hexagonal close packed areas on freshly cleaved mica surfaces that were not pre-treated (Figure 18). Locations with narrow stripe-like depositions were used for the measurements. Lateral distances and particle height values were evaluated from the same images.

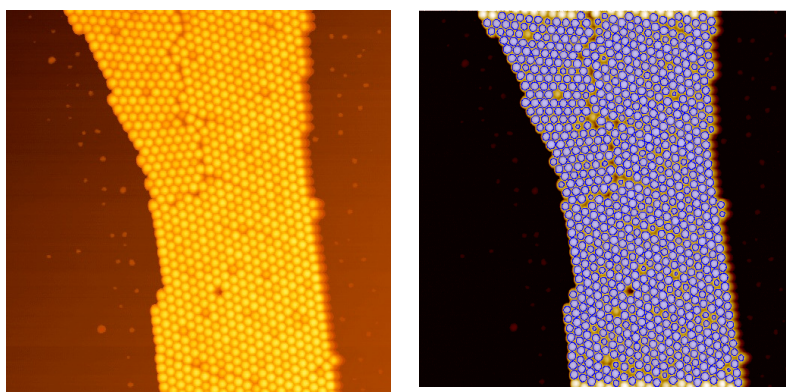


Figure 18: Left: SPM height image with (1024 x 1024) pixels (8 μm x 8 μm). Right: Particle height evaluation on the flattened image using a threshold for the separation of the individual particles.

No of particles	Mean /nm	St. dev. /nm	St. dev. of mean /nm	Median /nm	Mode /nm
1282	194.40 $\pm$ 4.2	5.92	0.17	195.83	196.78

Table III: Summary of results for a sample of spherical 200 nm polymer reference particles.

The defect density in close packed areas was rather high. Lateral distances were estimated from profiles across short rows of nearly perfectly arranged particles. The lateral measurements were corrected using a model for the packing of particles with the same size and size distribution as determined from height evaluation. The lateral spacing of the 3200A sample was 196.1 nm before and 194.0 nm with packing correction. The height value was at 194.4 nm very close to the corrected lateral distance (Figure 21 and Table III).

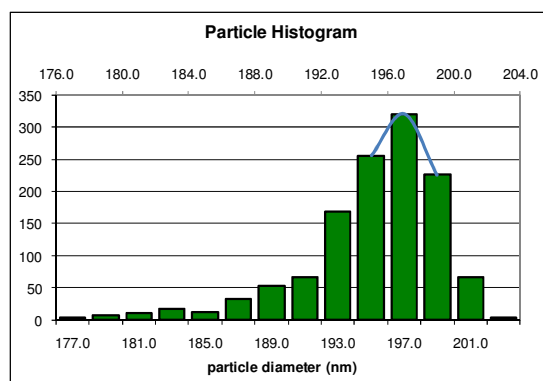


Figure 19: Particle size distribution with particle diameters acquired from several images. The mode diameter (highest probability) was determined by fitting a normal distribution to the top 3 points in the histogram (blue line).

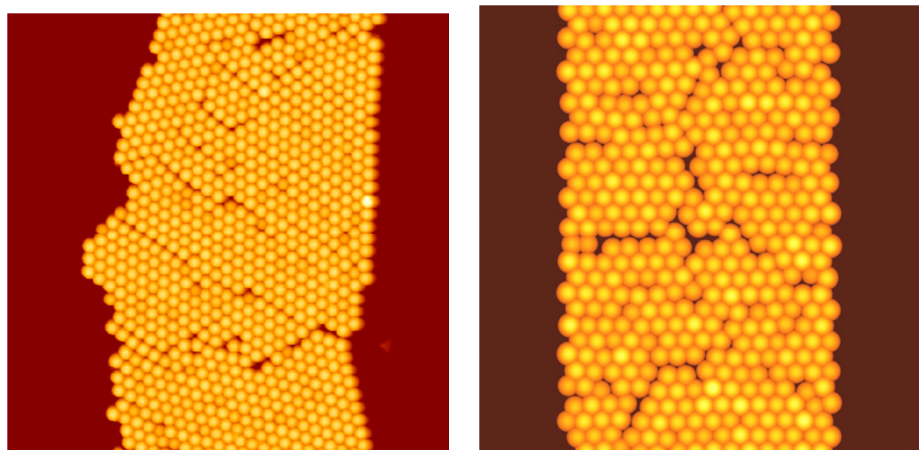


Figure 20: Left: SPM measurement, image $7\ \mu\text{m} \times 7\ \mu\text{m}$. Right: Simulated particle arrangement and SPM tip convolution with assumed particle data used for the estimation of lateral distance correction factors.

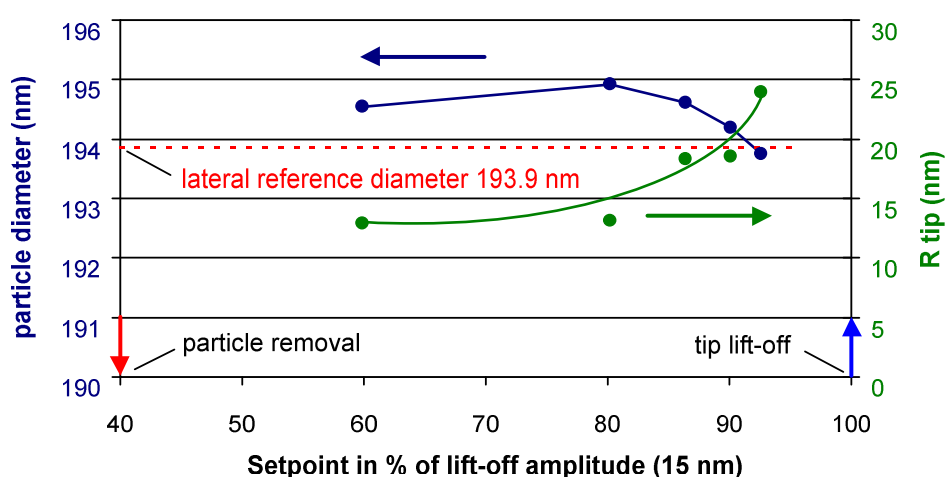


Figure 21: Comparison of the height and the lateral particle size evaluation method using identical polymer particles. The corrected lateral reference diameter and particle height values obtained for various AFM tapping mode set points are shown.

6

IN THIS CHAPTER

- Comparing Dynamic Light scattering nanoparticle measurements with other methods.
- Using the GUM approach to uncertainty evaluation.

When coherent light is shone on a suspension of nanoparticles, variations or ‘speckles’ in the intensity of the scattered light are seen which are caused by the variation in phase of the waves scattered by different particles. By recording these speckles it is possible to tell how fast the scattering particles diffuse over a distance equivalent to the wavelength of scattered light. Dynamic light scattering uses this principle to determine first the diffusion coefficient, and then the mean hydrodynamic diameter and size distribution of nanoparticles suspension [93].

To determine the diffusion coefficient the correlation function (97) of the intensity of the scattered light ($g^{(2)}(\tau)$) for a given time τ needs to be determined using equation 9:

$$g^{(2)}(\tau) = \frac{\langle I(t)I(t+\tau) \rangle}{\langle I(t) \rangle^2} \quad (\text{Eq. 9})$$

where $I(t)$ and $I(t+\tau)$ are the intensities of the scattered light at times t and $t+\tau$ respectively and the braces indicate averaging over t . This function measures the correlation in time of the intensity of the scattered light. For monodispersed particles in solution, the correlation function is characterized by exponential decay with a decay rate (Γ) given by equation 10.

$$\Gamma = Dq^2 \quad (\text{Eq. 10})$$

with D the diffusion coefficient of the particles and q (the scattering vector) defined in equation 11:

$$q = \frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right) \quad (\text{Eq. 11})$$

where λ is the wavelength of the laser, θ the scattering angle and n the refractive index of the suspension liquid. Hence by measuring the decay rate of the correlation function, the diffusion coefficient can be determined. The **Stokes-Einstein** equation (equation 12) is then used to determine the Hydrodynamic diameter (d):

$$d = \frac{kT}{3\pi\eta D} \quad (\text{Eq. 12})$$

with k being the Boltzmann constant, η the solvent viscosity and T the absolute temperature.

Results interpretation

Using DLS, only the average size of nanoparticles in suspension can be determined. Evaluation of the size distribution requires a more complicated analysis such as the Cumulants approach [95].

As mentioned in the previous section DLS is an ensemble technique providing the intensity weighted size distribution for nanoparticles in suspension. There are several sources of misinterpretation of DLS data that should be kept in mind when comparison is made with other techniques:

Hydrodynamic diameter

DLS measures the hydrodynamic diameter, which is the effective diameter of a particle moving in a liquid. The relationship between hydrodynamic and physical diameters is complex and depends on the particle surface structure and the type and concentration of ions in the suspension medium. There is no accepted way to correct for hydrodynamic effect in DLS to recover physical size.

Polydispersity

The role of polydispersity in dynamic light scattering measurements cannot be understated. The light intensity (I) being scattered from an individual particle is proportional to the sixth power of the particle diameter (d)

$$I \propto d^6 \quad (\text{Eq. 13})$$

Hence a 50 nm particle will scatter 10^6 as much light as a 5 nm one and will most likely dominate the measurement. Table IV shows the effect even a small amount of polydispersity has on the measured particle size [96]. Increasing the concentration of dimers in a suspension (i.e only 2 particles per agglomerate) has a minimal effect on the mean particle size, but a disproportional effect on the intensity weighted mean particle size (the latter being measured by DLS). Accurate analysis by DLS can therefore only be achieved using particle samples with a narrow size distribution that have been properly dispersed with little or no agglomerates present.

	<i>Mean Size / nm</i>	<i>Intensity Weighted Mean Size / nm</i>
No agglomeration	97.8	100.2
3 % agglomeration	98.2	102.1
5 % agglomeration	98.7	103.6
10 % agglomeration	100.2	106.7
15 % agglomeration	101.9	109.6

Table IV: Simulating the effect of agglomeration on the mean particle size and the intensity weighted mean size for spherical nanoparticles.

The effort of controlling both the suspension medium (especially if the material has to be diluted prior to analysis) and the amount of agglomerates (which are expected to increase with time) means that standard reference materials for DLS are more difficult to produce and control.

The effect of the scattering angle

The scattering angle can potentially have an important effect on the overall uncertainty in the DLS measurement as demonstrated in the case study at the end of this section. Figure 22 shows that relative contribution to the uncertainty for the scattering angle decreases exponentially with increasing scattering angle. It is then recommended that a scattering angle greater than 90 degrees be used.

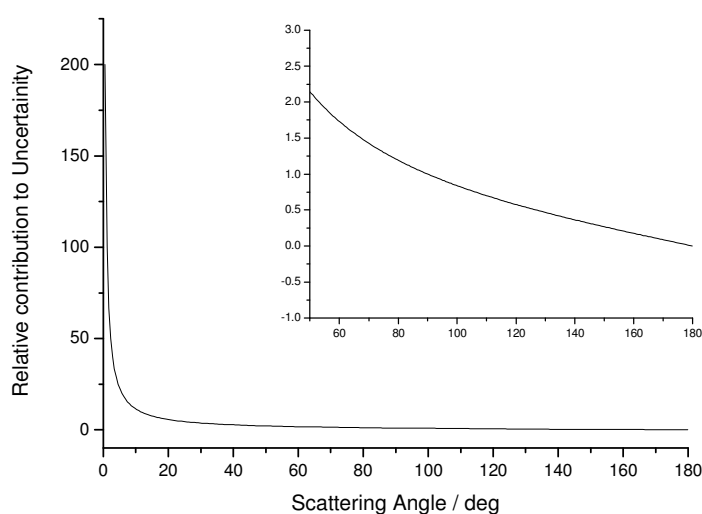


Figure 22:
Contribution of the scattering angle to the overall measurement uncertainty.

Scattering model

For small particles (approximately $d < \lambda/10$ or 60 nm for a He-Ne laser) the Rayleigh-Gans-Debye scattering approximation applies. In this approximation, the phase does not change across the particle and the spectral behaviour is essentially independent of the particle shape and internal structure. For larger particles or particle with a high index of refraction, the complete Mie scattering model predicts that the light scattered will have a complicated three dimensional distribution with, in general, scattering being more likely in the forward direction. Therefore, results measured at high scattering angles (160 degrees and greater) will be more sensitive to the smaller particles than those recorded at low scattering angles.

The most up to date and internationally recognized procedure for the nanoparticle size measurements using dynamic light scattering is covered by the standard BS ISO 22412:2008. With this well-established procedure and a straightforward mathematical model for the measurement, DLS is good example where a complete evaluation of the uncertainty can be established following the recommended international methods described in the GUM [100].

Case study IV: Uncertainty budget for Dynamic Light Scattering measurement

In this section each factor influencing the measurement will be examined and quantified with the uncertainty value, divisor, sensitivity coefficient and effective degrees of freedom stated. As mentioned before, this is the uncertainty related to the hydrodynamic diameter and not to the true physical size.

Using the equations described in the previous section then the hydrodynamic radius (d) is given by equation 14.

$$d = f(K, T, \eta, \Gamma, q) = \frac{kT}{3\pi\eta(\Gamma/q^2)} \quad (\text{Eq. 14})$$

These five parameters (Boltzmann's Constant, temperature, viscosity, decay rate and the scattering vector) should be included in the estimation of the combined uncertainty $u(d)$:

$$u^2(d) = \left(\frac{\partial f}{\partial k}\right)^2 u^2(k) + \left(\frac{\partial f}{\partial T}\right)^2 u^2(T) + \left(\frac{\partial f}{\partial \eta}\right)^2 u^2(\eta) + \left(\frac{\partial f}{\partial \Gamma}\right)^2 u^2(\Gamma) + \left(\frac{\partial f}{\partial q}\right)^2 u^2(q) \quad (\text{Eq. 15})$$

Similarly for the scattering vector, q :

$$u^2(q) = \left(\frac{\partial f}{\partial n}\right)^2 u^2(n) + \left(\frac{\partial f}{\partial \lambda}\right)^2 u^2(\lambda) + \left(\frac{\partial f}{\partial \theta}\right)^2 u^2(\theta) \quad (\text{Eq. 16})$$

Combining these equations one obtains the final expression for the standard uncertainty $u(d)$ of a DLS measurement:

$$u^2(d) = f^2 \left[\frac{u^2(k)}{k^2} + \frac{u^2(T)}{T^2} + \frac{u^2(\eta)}{\eta^2} + \frac{u^2(\Gamma)}{\Gamma^2} + 4 \left\{ \frac{u^2(n)}{n^2} + \frac{u^2(\lambda)}{\lambda^2} + \frac{u^2(\theta)}{4 \tan^2(\theta/2)} \right\} \right] \quad (\text{Eq. 17})$$

1. **The Boltzmann constant, k** , is estimated according to the committee on Data for Science and Technology (CODATA, 2010) to be: $k = 1.380\,6488 \times 10^{-23} \text{ J K}^{-1}$ with a standard uncertainty, $u(k)$ of $0.000\,0013 \times 10^{-23} \text{ J K}^{-1}$, ($k = 1$); i.e., 9.1×10^{-7} . The sensitivity coefficient, $c_i = 1$ with effective degrees of freedom, $\nu = \infty$ (type B uncertainty, rectangular distribution).
2. The **temperature, T** , sensitivity should be given by the instrument manufacturer. If not available, it needs to be measured using traceable thermometer. If possible, the temperature of the nanoparticle suspension when situated within the instrument with the laser beam turned on and the system given time to reach thermal equilibrium (normally at least 30 minutes) should be measured. Traceable thermometers will be supplied with documentation stating their associated uncertainties which can be inputted directly into the uncertainty analysis. Unless explicitly stated temperature measurement is a systematic error, assuming the measurement bias is equivalent for each reading made with an equal likelihood of occurring anywhere in the defined uncertainty region, and its contribution to

the uncertainty analysis will have a rectangular distribution, a sensitivity coefficient, $c_i = 1$ and effective degrees of freedom, $\nu = \infty$ (type B uncertainty).

3. The **solvent viscosity, η** , uncertainty has two contributions. The first arises from the uncertainty of the viscosity of pure water, reported in ISO 3666 at 20.0 °C to be 0.000 85 mPa s. The second from uncertainty in temperature other than 20 °C. This can be calculated using following equation [97]:

$$\eta = A \exp \left[\frac{1 + BT}{CT + DT^2} \right] \quad (\text{Eq. 18})$$

where: $A = 0.01257187$ mPa s; $B = -5.806436 \times 10^{-3} \text{ K}^{-1}$; $C = 1.130911 \times 10^{-3} \text{ K}^{-1}$; $D = -5.723952 \times 10^{-6} \text{ K}^{-2}$ and T = Temperature in Kelvin.

For a temperature of $(26 \pm 0.1)^\circ\text{C}$ then the viscosity varies between 0.8681 and 0.8720 mPa s with an assumed rectangular distribution of half-width 0.0020 mPa s. Therefore the combined uncertainty of viscosity at 26 °C is determined by:

$$u(\eta) = \sqrt{0.00085^2 + \left(\frac{0.0020}{\sqrt{3}}\right)^2} = 0.0014 \text{ mPa} \cdot \text{s}$$

Sensitivity coefficient, $c_i = 1$. Effective degrees of freedom, $\nu = \infty$ (type B uncertainty).

4. The **scattering angle θ** , uncertainty is determined from the geometry of the system. Sensitivity coefficient, $c_i = 1$, Effective degrees of freedom, $\nu = \infty$ (type B uncertainty).
5. The **wavelengths of the laser λ** , and their related uncertainties for many laser systems can be measured. The popular Helium Neon laser has wavelength of 632.816 nm in air within ± 0.001 nm due to thermal expansion and contraction of the cavity.
6. The **refractive index n , of the liquid medium** uncertainty depends on the temperature, wavelength of the laser and its composition. For temperatures between 20 °C and 30 °C and with a wavelength of laser of 632.816 nm, the refractive index of water varies between 1.3324 and 1.3346. Considering this range as the width of a rectangular distribution, the relative standard uncertainty $u_r(n)$ is:

$$u(\eta n) = \frac{0.0011}{\sqrt{3}} = 6.3509 \times 10^{-4}$$

Sensitivity coefficient, $c_i = 2$. Effective degrees of freedom, $\nu = \infty$ (type B uncertainty).

7. The uncertainty associated with the **decay rate of the correlation function Γ** is related to both the decay and the measurement time and can be calculated using the following formula:

$$\frac{u(\Gamma)}{\Gamma} = 4 \sqrt{\frac{\tau}{t}} \quad (\text{Eq. 19})$$

where τ is the decay time (defined as the time taken for the correlation function to fall by a factor of $1/e$) and t is the measurement time. This relationship was determined by work

carried out in the early days of DLS (101). In most commercial DLS systems several correlation functions are combined. In this case we have modified the above equation to:

$$\frac{u(\Gamma)}{\Gamma} = 4 \sqrt{\frac{\tau}{t_m}} \sqrt{\frac{1}{n}} \quad (\text{Eq. 20})$$

where t_m is the acquisition time per run and n is the number of runs. Sensitivity coefficient, $c_i = 2$. Effective degrees of freedom, $\nu = \infty$ (type B uncertainty).

8. The **repeatability** of the measurement (u_r) is the final contribution to the uncertainty that needs to be considered. This is determined by the standard deviation of the mean. Sensitivity coefficient, $c_i = 1$. This is a type A uncertainty, hence the effective degrees of freedom are one less than the number of repeated measurements.

The particular example given here is of 100 nm latex particles supplied by Duke Scientific. All DLS measurements were made using a commercial instrument (Nanosizer Malvern, UK). This instrument is fitted with a He-Ne laser and uses a scattering angle of 173°. All measurements were made at $(20 \pm 0.1)^\circ\text{C}$. 500 μl of each sample was measured in a single-use polystyrene half-micro cuvette with a path length of 10 mm. All measurements were made at a position of 4.65 mm from the cuvette wall with an automatic attenuator. For each sample 15 runs of 20 s were performed with ten repetitions. The software to collect and analyse the data was the Dispersion Technology Software (v 6.01). The mean diameter was calculated with the Dispersion Technology Software using the Cumulant analysis method (ISO 13321 and ISO 22412). The mean size recorded was 106.1 nm with a standard deviation of 0.91 nm. The various contributions to the uncertainty are given in Table V.

Input quantity x_i	Distrib.	$u(x_i)$ unit	ν_i	$c_i = \partial d / \partial x_i$	$u_i(d) / \text{nm}$
Boltzman Constant	normal	$1.3806 \times 10^{-23} \text{ J}\cdot\text{K}^{-1}$	∞	1	1.7673×10^{-5}
Temperature	rect	0.057 74 K	∞	1	0.0209
Viscosity	normal / rect	0.0014 mPa s	∞	1	0.1752
Wavelength of laser	normal	0.001 nm	∞	2	3.352×10^{-4}
Refractive Index	rect	6.3509×10^{-4}	∞	2	0.1011
Scattering Angle	rect	$2.5192 \times 10^{-4} \text{ rad}$	∞	1	0.0016
Decay rate	normal	-	∞	1	0.3465
Repeatability	normal	0.2881 nm	9	1	0.2881

Table V: Uncertainty budget for 100 nm latex nanoparticles as measured by DLS

Combined standard uncertainty: $u_c(d) = 0.9338 \text{ nm}$

For all type B uncertainties the effective degrees of freedom can be considered as infinite, therefore only the decay rate contribution need to be considered: In this case::

$$\nu_{eff}(d) = \frac{\frac{u_c^4(d)}{\frac{u_r^4(r)}{9}}}{9} \quad (\text{Eq. 21})$$

Effective degrees of freedom: $\nu_{eff}(d) = 1028 (>>100)$

Expanded uncertainty: $U_{95}(d) = 1.8675 \text{ nm}$

It can be seen that an uncertainty of less than 2 nm can be achieved, however the major contributors are the decay rate (which increases with particle size), viscosity and repeatability.

7

IN THIS CHAPTER

- Small angle X-ray scattering standard operating procedures.
- Source of uncertainties.

Small-Angle X-ray Scattering is a method to analyse the average dimensions of particle ensembles. The physical principle of SAXS is elastic or Rayleigh scattering. Electrons illuminated by X-ray photons oscillate with the same frequency and produce coherent waves that interfere to produce scattering pattern. An electron density difference is needed between the particle material and the surrounding medium.

This section is concerned with the application of SAXS to suspension of spherical particles with a narrow size distribution. Due to the random orientation of the particles, the scattering pattern consists of concentric ring like structures (see Figure 24, left). This guide will focus only on the so-called Fourier region, where the rings are located. The Guinier method, where the scattering curve is analysed in the range of lower q , contains more uncertainties. The diameter of the rings in the Fourier region of the scattering image depends on the particle parameters, the photon energy E and the distance L between the sample and the detector recording the scattering image. To easily compare scattering patterns from different experimental setups one uses the momentum transfer q

$$q = \frac{4\pi E}{hc} \cdot \sin\left(\frac{\alpha_o}{2}\right) \quad (\text{Eq. 22})$$

where α_o is the scattering angle, given by:

$$\alpha_o = \arctan\left(\frac{r}{L}\right) \quad (\text{Eq. 23})$$

with r being the distance from the direct beam position at the image (Figure 23, left).

This momentum transfer q is used to convert the intensity distribution into reciprocal space. For small particles, the scattering rings have larger diameters, and vice versa.

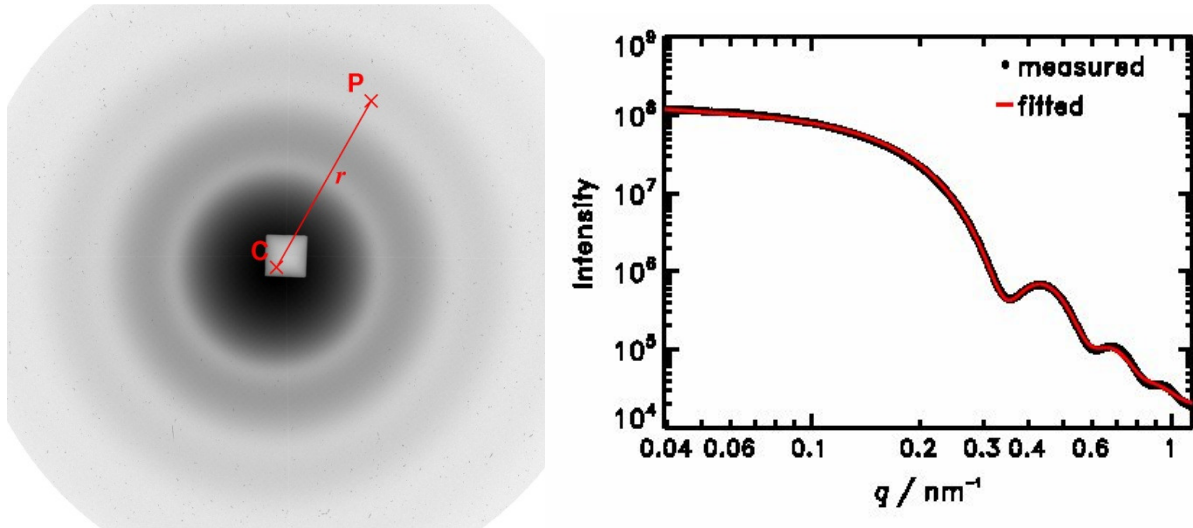


Figure 23: Scattering image of gold spheres with a nominal diameter of 30 nm (left) and the corresponding scattering curve with a fitted model curve (right). The centre C is the position where the direct beam, which is blocked by the quadratic beam stop, would hit the detector.

In analysis, scattering curves of model functions (the so called form factors) are fitted to the scattering curve by adjusting their particle parameters (see Figure 23, right).

SAXS standard procedure

A sample container with thin walls and small diameter is filled with a particle solution with a particle fraction of less than 1 w/v in to allow the X-ray beam to pass through with negligible absorption. A possibility is to use a flow-through setup as often found in tabletop SAXS instruments. Another possibility is to use glass capillaries of a diameter of 1 mm and a wall thickness of 0.01 mm. One issue that often occurs is that an air bubble can remain at the bottom of the capillary after placing the sample into the capillary with a medical syringe with a long thin injection needle. This bubble can be removed by carefully centrifuging the capillary. After that, the capillary must be welded shut if it is used in vacuum. Samples that contain larger particles should be measured quickly (within a few days) after being loaded into the capillary to avoid sedimentation effects.

Measurement

For measurement, the sample capillary is placed in the X-ray beam; this should have a smaller diameter than the capillary to avoid diffraction effects at the sides of the tube. For each sample the transmission must be determined and a measurement of a capillary filled with pure solvent must be made with the same experimental setup for reference. For measurements with an X-ray source, where the intensity changes with time (for example due to the decreasing current in synchrotron radiation facilities) for each measurement the mean photon flux must be noted. The transmitted primary beam must be blocked by a beam stop. There are several different factors to consider for good measurements:

- To be able to resolve small and narrow rings of big particles as well as wide rings of small particles, a large detector with a good spatial resolution is needed.
- The experimental setup needs to be adjusted for specific samples, i.e. for small particles the sample-detector distance should be smaller than for larger particles, because their rings have a larger opening angle.
- If the radiation source can be adjusted in this way, it is recommended to choose the photon energy with respect to the sample size, i.e. use higher photon energy for smaller particles, and for larger particles use lower photon energies.

A dark image must also be obtained with the 2D detector for the elimination of readout or thermal noise during the analysis.

Analysis

To generate a scattering curve, several different steps need to be carried out:

1. Subtraction of the dark image from all measurement images.
2. Division of every 'subtracted' image by:
 - sample transmission
 - measurement time duration
 - photon flux
3. Subtraction of the 'divided' image of the pure solvent from the 'divided' image of the sample.

4. Determination of the centre of the scattering pattern on the image.
5. Correction for spherical projection, i.e. normalize the intensity of the single pixels to their solid angle.
6. Angular integration around the centre and calculate the corresponding q values for the radii. one should mask the beam stop and, especially for longer exposure times, mask the footprints due to cosmic radiation to reduce the noise in the scattering curve significantly. Masking a pixel should not be carried out in such a way that it is treated as a pixel with a more proper value (in fact this procedure was found at some places) but the pixel should be treated as not existing at all.
7. Fitting of the scattering curve with a model curve from a solid or shell particle. This can be carried out successfully using the program SASfit written by Joachim Kohlbrecher and Ingo Bressler, which is available at <http://kur.web.psi.ch/sans1/SANSSoft/sasfit.html>.

Choice of particle form model:

Different particle structures lead to different scattering curves. Curves obtained from non-spherical particles cannot be fitted with those assumed for spherical particles. The uncertainty of the form is hard to estimate, because it is superimposed with the size distribution width. To avoid this, it is recommended to verify the spherical shape of the particles with a microscopic method.

Choice of size distribution form:

In SAXS the size distribution model has to be chosen. In case of a number weighted Gaussian size distribution, the mean diameter is the peak position. In case of other size distribution functions, the mean diameter can differ from the peak position. Additionally, the mean diameter differs for number weighted, intensity weighted, or volume weighted distributions.

Number weighted means that one determines the fraction of particles with the same radius.

Volume weighted means that one determines the fraction of the total particle volume represented by particles of the same radius. The volume weighted mean diameter for a not perfect monodisperse particle ensemble is shifted towards bigger particles compared to the number weighted.

Intensity weighted means that one determines the fraction of scattering intensity caused by particles of the same size. Since the scattering intensity is the square of the total of all wave amplitudes coming from a particle, it is proportional to the square of the particle volume, so the intensity weighted mean particle diameter is shifted towards bigger particles compared to the volume weighted mean diameter.

For better comparability with single particle methods it is recommended to use the mean diameter of the number weighted distribution.

Simulations and fits show that for size distributions with a FWHM of less than 10 % of its mean diameter, the mean diameters (as distinguished from the peak positions, which are the main diameters) are almost the same for different size distribution functions and because of this the choice will not influence the uncertainty of the results.

Choices of start parameters of fit:

As in every fitting analysis, the choice of start parameters can influence the result, but tests with different start parameters within a realistic range show the same fitting results. Because of this the uncertainty of the resulting particle diameter does not depend on the start parameters of the fit.

To determine the mean particle diameter, there is no need to calibrate the intensity scale of the scattered radiation, as only the momentum transfer calibration is required. Often, a reference material with a 'known' structure like Ag-behenate is used to calibrate the momentum transfer axis. This is a useful control measurement, but for traceable size determination, the independent quantities should be determined directly.

Centre estimation:

The uncertainty of the assumed centre in the scattering pattern affects the size distribution width but leaves the mean diameter unchanged. This is because the scattering curve is the result of a circular integration around the centre. For small errors (e.g. less than a quarter of ring distance) the intensity profile on one side is shifted by Δq to higher q values while on the opposite side of the centre the same intensity profile is shifted by the same Δq to lower q values. For the circular integral, which is the scattering curve, the maxima and minima stay at their correct positions. Because these positions define the mean particle diameter, it will remain unaffected. The effect of curve flattening caused by a misplaced centre will lead to a wider size distribution.

Distance between sample and detector

The sample detector distance should be determined e.g. with an incremental length measurement. If this is not possible, e.g. the exact sample position is unreachable in a vacuum chamber, it can be determined by taking two images of a sample with a well-structured scattering ring pattern in two different distances. The distance difference must be determined with an incremental length measurement. By aligning the rings of these two measurements the sample detector distance can be triangulated.

Pixel size of detector

To measure the pixel size of the 2D detector, images of the size reduced direct beam are made at different positions of the 2D detector. This movement of the detector between the images is done perpendicular to the beam along a well-known distance determined with an incremental length measurement. With the difference of the beam position in pixels at the images, the pixel dimension in the direction of movement can be determined. This should be done for x and y directions at the detector.

Sources of uncertainties

The particle diameter d determined by SAXS is the result of fitting a model scattering curve to the measured data. This makes the determination of measurement uncertainty difficult because there is no direct algebraic connection between measurement uncertainties and the uncertainty of the particle diameter, there are also additional uncertainties that have to be taken into account, due to the fitting procedures.

The different parameters that contribute to the uncertainties from the measurement setup are:

- the uncertainty of photon energy $u(E)$
- the uncertainty of the pixel size $u(s)$, which is needed to calculate the radius r of the intensity rings in the recorded scattering images with $r = s \cdot n_p$, where n_p is the ring radius in the scattering pattern image, in pixels.
- the uncertainty of the distance between sample and detector $u(L)$

The equation of the momentum transfer q given above,

$$q = \frac{4\pi E}{hc} \cdot \sin\left(\frac{1}{2} \arctan\left(\frac{s \cdot n_p}{L}\right)\right) \quad (\text{Eq. 24})$$

can be simplified for the uncertainty analysis due to the small angles of ($< 5^\circ$) used for SAXS to

$$q = \frac{2\pi E}{hc L} \cdot s \cdot n_p \quad (\text{Eq. 25})$$

With this, the uncertainty $u(q)$ for the momentum transfer becomes:

$$u(q) = \frac{2\pi}{hcL} n_p \sqrt{s^2 u^2(E) + E^2 u^2(s) + \left(\frac{E s}{L}\right)^2 u^2(L)} \quad (\text{Eq. 26})$$

Photon energy: Traceable determination of the photon energy is necessary especially for tunable X-ray sources. Here the photon energy can be determined e.g. by back scattering at a Silicon crystal. For lab setups using an X-ray tube as source operating at characteristic K-alpha emission lines the literature values can be used.

8

IN THIS CHAPTER

- Need to always apply the principles of metrology when measuring nanoparticles, no matter the technique used.
- Ensuring traceability to allow comparison of measurement results
- Following GUM approach to estimate uncertainty contributions and minimize the final one.

The overall aim of this guide was to present to a non-expert reader the principles of metrology and how to apply these concepts to nanoparticle measurements. It is important that people try to obtain traceability, the only meaningful way to compare measurement results. In order to do this, the key requirement is to estimate the uncertainty contributions and minimise the final associated uncertainties.

In this guide, this approach has been applied to two common single particle and two ensemble nanoparticle measurement techniques.

From this guide the reader should have been made aware that there are key parameters that need to be controlled in order to ensure low uncertainties. For single particle techniques these are:

- The number of particles measured. This is defined by the uncertainty level required and the size distribution of the sample. It has been demonstrated that for a particular technique / sample combination there is an uncertainty floor which cannot be realistically lowered.
- Effective calibration standards. Currently there are few transferable calibration standards in the 1 nm to 50 nm range that can be used to calibrate multiple instruments quickly and effectively. One must ensure that the calibration status does not change after calibration or during measurement. This is especially true for transmission electron microscopes.

For ensemble techniques, many thousands of particles are measured simultaneously, hence the particle count is not an issue. In addition, it is often difficult to calibrate the instruments in the traditional sense. In this case, the following need to be taken into account to ensure effective measurements:

- Precise control of the sample preparation to ensure that neither agglomeration (which can occur when altering the suspension fluid) nor particle break up (using excessive ultrasound or milling to dissipate agglomerates) occurs.
- Ensuring that no impurities are introduced.
- Tight control of environment during measurement, in particular temperature.

In all case and if possible, we would recommend that a full analysis should be done in accordance with the *Guide to the Expression of Uncertainty in Measurement* [100].

9

IN THIS CHAPTER

- List of current standards pertaining to nanoparticles.
- List of other standards belonging to close fields and using common instrumentation.

ISO TC24 Particle characterization including sieving

Published Standards

Number	Title	Published
ISO 9276-1	Representation of results of particle size analysis – Part 1: Graphical representation, ISO 9276-1:1998/Cor 1:2004	1998
ISO 9276-2	Representation ... -- Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions	2001
ISO 9276-3	Representation ... -- Part 3: Adjustment of an experimental curve to a reference model	2008
ISO 9276-4	Representation ... -- Part 4: Characterization of a classification process	2001
ISO 9276-5	Representation ... -- Part 5: Methods of calculation relating to particle size analyses using logarithmic normal probability distribution	2005
ISO 9276-6	Representation ... -- Part 6: Descriptive and quantitative representation of particle shape and morphology	2008
ISO 9277	Determination of the specific surface area of solids by gas adsorption-- BET method	2010
ISO 13318-1	Determination of particle size distribution by centrifugal liquid sedimentation methods -- Part 1: General principles and guidelines	2001
ISO 13318-2	Determination ... -- Part 2: Photocentrifuge method	2007
ISO 13318-3	Determination ... -- Part 3: Centrifugal X-ray method	2004
ISO 13320	Particle size analysis -- Laser diffraction methods	2009
ISO 13321	Particle size analysis -- Photon correlation spectroscopy	1996
ISO 13322-1	Particle size analysis -- Image analysis methods -- Part 1: Static image analysis methods	2004
ISO 13322-2	Particle size analysis -- Image analysis methods -- Part 2: Dynamic image analysis methods	2006
ISO/TS 13762	Particle size analysis -- Small angle X-ray scattering method	2001
ISO 14887	Sample preparation -- Dispersing procedures for powders in liquids	2000
ISO 14888	Particulate materials -- Sampling and sample splitting for the determination of particulate properties	2007
ISO 15900	Determination of particle size distribution -- Differential electrical mobility analysis for aerosol particles	2009
ISO 15901-1	Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption -- Part 1: Mercury porosimetry	2005
ISO 15901-2	Pore ... -- Part 2: Analysis of mesopores and macropores by gas adsorption	2006
ISO 15901-3	Pore ... -- Part 3: Analysis of micropores by gas adsorption	2007
ISO 20998-1	Measurement and characterization of particles by acoustic methods – Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy	2006
ISO 21501-1	Determination of particle size distribution -- Single particle light interaction methods -- Part 1: Light scattering aerosol spectrometer	2009
ISO 21501-2	Determination of particle size distribution – Single particle light interaction methods -- Part 2: Light scattering liquid-borne particle	2007

	counter	
ISO 21501-3	Determination of particle size distribution – Single particle light interaction methods -- Part 3: Light extinction liquid-borne particle counter	2007
ISO 21501-4	Determination of particle size distribution – Single particle light interaction methods -- Part 4: Light scattering airborne particle	2007
ISO 22412	Particle size analysis – Dynamic light scattering (DLS)	2008

Standards under Development

ISO/DIS 13099-1	Methods for zeta potential determination -- Part 1: Introduction	2011
ISO/DIS 13099-2	Methods for zeta potential determination -- Part 2: Optical methods	2011
ISO/NP 13099-3	Methods for zeta potential determination -- Part 3: Acoustic methods	2013
ISO/CD 26824	Particle characterization of particulate systems -- Vocabulary	2012
ISO/CD 20998-2	Measurement and characterization of particles by acoustic methods – Part 2: Guidelines for linear theory	2012
ISO/NP 27891	Calibration of aerosol particle number concentration measuring instruments	2013

New Proposals

ISO/NP 12154	Determination of density by volumetric displacement -- Skeleton density by gas pycnometry	
ISO/PWI 12177	Particle size analysis -- Image analysis method -- Determination of particle size and shape from discretised images	
ISO/PWI 12187	Particle size analysis – Dispersed stability characterization in liquids	
ISO/PWI TS 12918	Measurement of water sorption and other vapours in solids	
ISO/PWI 13099-4	Methods for zeta potential determination -- Part 4: Streaming potential method	
ISO/PWI 13099-5	-- Part 5: Other methods	
ISO/PWI 13318-1	Determination of particle size distribution by centrifugal liquid sedimentation methods -- Part 1: General principles and guidelines	
ISO/PWI 13762	Particle size analysis -- Small angle X-ray scattering method	
ISO/PWI 14411	Measurement protocols for the determination of certified values of reference materials for laser diffraction	
ISO/PWI 15901-1	Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption -- Part 1: Mercury porosimetry	
ISO/PWI 20998-3	Measurement and characterization of particles by acoustic methods -- Part 3: Guidelines for non-linear theory	

Other relevant ISO Committees

- TC201 Surface chemical analysis
- TC202 Microbeam analysis
- TC209 Cleanrooms and associated controlled environments
- TC229 Nanotechnologies
- TC146 Air Quality

Many of the written standards produced by the above Technical Committees may be not directly related to nanoparticles but to close fields where common terminology and instrumentation (XPS, Auger, AFM, TEM, SEM) is used. A wider and better knowledge on related matters and the instrumentation used conducts to more accurate results; hence the why to include here the following lists of standards:

ISO TC201 Surface chemical analysis –

Published Standards

ISO 10810:2010	Surface chemical analysis -- X-ray photoelectron spectroscopy -- Guidelines for analysis
ISO 14707:2000	Surface chemical analysis -- Glow discharge optical emission spectrometry (GD-OES) -- Introduction to use
ISO/TS 15338:2009	Surface chemical analysis -- Glow discharge mass spectrometry (GD-MS) -- Introduction to use
ISO 15470:2004	Surface chemical analysis -- X-ray photoelectron spectroscopy -- Description of selected instrumental performance parameters
ISO 15471:2004	Surface chemical analysis -- Auger electron spectroscopy -- Description of selected instrumental performance parameters
ISO 15472:2010	Surface chemical analysis -- X-ray photoelectron spectrometers -- Calibration of energy scales

Standards under Development

ISO/DIS 11039	Surface chemical analysis -- Scanning-probe microscopy -- Measurement of drift rate
ISO/WD 11775	Surface chemical analysis -- Scanning-probe microscopy -- Determination of cantilever normal spring constants
ISO/AWI 11939	Standards on the measurement of angle between an AFM tip and surface and its certified reference material
ISO/CD 11952	Guidelines for the determination of geometrical quantities using scanning-probe microscopes -- Calibration of measuring systems
ISO/AWI 13083	Definition and calibration of the spatial resolution of electrical scanning-probe microscopes (ESPMs) such as SSRM and SCM for 2D-dopant imaging and other purposes
ISO/PRF 13084	Surface chemical analysis -- Secondary-ion mass spectrometry -- Calibration of the mass scale for a time-of-flight secondary-ion mass spectrometer
ISO/AWI 13095	Surface chemical analysis -- Procedure for in situ characterization of AFM probes used for nanostructure measurement

ISO/AWI TR 13096	Surface chemical analysis -- Scanning-probe microscopy -- Guidelines for the description of AFM probe properties
ISO/CD 13424	Surface chemical analysis -- X-ray photoelectron spectroscopy -- Reporting of results for thin film analysis
ISO/DTR 14187	Surface chemical analysis -- Characterization of nanostructured materials
ISO/DIS 14701	Surface chemical analysis -- X-ray photoelectron spectroscopy -- Measurement of silicon oxide thickness
ISO/CD 16129	Surface chemical analysis -- X-ray photoelectron spectroscopy -- Procedures for assessing the day-to-day performance of an X-ray photoelectron spectrometer
ISO/DIS 16242	Surface chemical analysis -- Recording and reporting data in Auger electron spectroscopy (AES)
ISO/DIS 16243	Surface chemical analysis -- Recording and reporting data in X-ray photoelectron spectroscopy (XPS)
ISO/CD 16413	Evaluation of thickness, density and interface width of thin films by X-ray reflectometry -- Instrumental requirements, alignment and positioning, data collection, analysis and reporting
ISO/WD 16531	Surface Chemical Analysis -- Depth Profiling -- Guideline of ion beam alignment for depth profiling in Auger electron spectroscopy and X-ray photoelectron spectroscopy
ISO/DTR 19319	Surface chemical analysis -- Determination of lateral resolution in beam-based methods
ISO/DIS 20903	Surface chemical analysis -- Auger electron spectroscopy and X-ray photoelectron spectroscopy -- Methods used to determine peak intensities and information required when reporting results
ISO/FDIS 27911	Surface chemical analysis -- Scanning-probe microscopy -- Definition and calibration of the lateral resolution of a near-field optical microscope
ISO/FDIS 28600	Surface chemical analysis -- Data transfer format for scanning probe microscopy

ISO TC202 Microbeam Analysis

Published Standards

ISO 14594:2003	Microbeam analysis -- Electron probe microanalysis – Guidelines for the determination of experimental parameters for wavelength dispersive spectroscopy
ISO 14594:2003/Cor 1:2009 ISO 14595:2003	Microbeam analysis -- Electron probe microanalysis – Guidelines for the specification of certified reference materials (CRMs)
ISO 14595:2003/Cor 1:2005 ISO 15632:2002	Microbeam analysis -- Instrumental specification for energy dispersive X-ray spectrometers with semiconductor detectors
ISO 16700:2004	Microbeam analysis -- Scanning electron microscopy – Guidelines for calibrating image magnification
ISO 17470:2004	Microbeam analysis -- Electron probe microanalysis – Guidelines for qualitative point analysis by wavelength dispersive X-ray spectrometry
ISO 22309:2006	Microbeam analysis -- Quantitative analysis using energy dispersive spectrometry (EDS)

ISO 22489:2006	Microbeam analysis -- Electron probe microanalysis – Quantitative point analysis for bulk specimens using wavelength-dispersive X-ray spectroscopy
ISO 22493:2008	Microbeam analysis -- Scanning electron microscopy -- Vocabulary
ISO 22493:2008	Microbeam analysis -- Scanning electron microscopy -- Vocabulary
ISO 23833:2006	Microbeam analysis -- Electron probe microanalysis (EPMA) -- Vocabulary
ISO 24173:2009	Microbeam analysis -- Guidelines for orientation measurement using electron backscatter diffraction
ISO 25498:2010	Microbeam analysis -- Analytical electron microscopy – Selected area electron diffraction analysis using a transmission electron microscope
ISO 29301:2010	Microbeam analysis -- Analytical transmission electron microscopy -- Methods for calibrating image magnification by using reference materials having periodic structures

Standards under Development

ISO/DIS 11938	Microbeam analysis -- Electron probe microanalysis -- Methods of elemental area analysis using wavelength-dispersive spectroscopy
ISO/DIS 13067	Microbeam analysis -- Electron backscatter diffraction -- Measurement of average grain size
ISO/CD 15632	Microbeam analysis -- Instrumental performance parameters for the specification and checking of energy-dispersive X-ray spectrometers for use in electron probe microanalysis
ISO/CD 22029	Standard file format for spectral data exchange
ISO/FDIS 22309	Microbeam analysis -- Quantitative analysis using energy dispersive spectrometry (EDS) for elements with an atomic number of 11 (Na) or above
ISO/PRF TS 24597	Microbeam analysis -- Scanning electron microscopy -- Methods of evaluating image sharpness

ISO TC209 Cleanrooms and associated controlled environments

Published Standards

ISO 14644-1:1999	Cleanrooms and associated controlled environments -- Part 1: Classification of air cleanliness
ISO 14644-2:2000	Cleanrooms and associated controlled environments -- Part 2: Specifications for testing and monitoring to prove continued compliance with ISO 14644-1
ISO 14644-3:2005	Cleanrooms and associated controlled environments -- Part 3: Test methods
ISO 14644-6:2007	Cleanrooms and associated controlled environments -- Part 6: Vocabulary
ISO 14644-8:2006	Cleanrooms and associated controlled environments -- Part 8: Classification of airborne molecular contamination

Standards under Development.

ISO/DIS 14644-1	Cleanrooms and associated controlled environments -- Part 1: Classification of air cleanliness by particle concentration
ISO/DIS 14644-2	Cleanrooms and associated controlled environments -- Part 2: Specifications for monitoring and periodic testing to prove continued compliance with ISO 14644-1
ISO/FDIS 14644-9	Cleanrooms and associated controlled environments -- Part 9: Classification of surface cleanliness by particle concentration
ISO/CD 14644-10	Cleanrooms and associated controlled environments -- Part 10: Classification of surface chemical cleanliness

ISO TC229 Nanotechnologies

Published Standards

ISO 10801:2010	Nanotechnologies -- Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method
ISO 10808:2010	Nanotechnologies -- Characterization of nanoparticles in inhalation exposure chambers for inhalation toxicity testing
ISO/TS 10867:2010	Nanotechnologies -- Characterization of single-wall carbon nanotubes using near infrared photoluminescence spectroscopy
ISO/TS 11251:2010	Nanotechnologies -- Characterization of volatile components in single-wall carbon nanotube samples using evolved gas analysis/gas chromatograph-mass spectrometry
ISO/TR 11360:2010	Nanotechnologies -- Methodology for the classification and categorization of nanomaterials
ISO/TR 12802:2010	Nanotechnologies -- Model taxonomic framework for use in developing vocabularies -- Core concepts
ISO/TR 12885:2008	Nanotechnologies -- Health and safety practices in occupational settings relevant to nanotechnologies
ISO/TS 27687:2008	Nanotechnologies -- Terminology and definitions for nano-objects -- Nanoparticle, nanofibre and nanoplate
ISO 29701:2010	Nanotechnologies -- Endotoxin test on nanomaterial samples for in vitro systems -- Limulus amoebocyte lysate (LAL) test
ISO/TS 80004-1:2010	Nanotechnologies -- Vocabulary -- Part 1: Core terms
ISO/TS 80004-3:2010	Nanotechnologies -- Vocabulary -- Part 3: Carbon nano-objects
ISO/DTS 10797	Nanotubes -- Use of transmission electron microscopy (TEM) in walled carbon nanotubes (SWCNTs)
ISO/PRF TS 10798	Nanotechnologies -- Characterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis
ISO/AWI TS 10812	Nanotechnologies -- Use of Raman spectroscopy in the characterization of single-walled carbon nanotubes (SWCNTs)
ISO/DTS 10868	Nanotechnologies -- Characterization of single-wall carbon nanotubes using ultraviolet-visible-near infrared (UV-Vis-NIR) absorption spectroscopy
ISO/DTR 10929	Measurement methods for the characterization of multi-walled carbon nanotubes (MWCNTs)
ISO/DTS 11308	Nanotechnologies -- Use of thermo-gravimetric analysis (TGA) in the purity evaluation of single-walled carbon nanotubes (SWCNT)
ISO/TR 11811	Nanotechnologies -- Guidance on methods for nanotribology measurements
ISO/DTS 11888	Determination of mesoscopic shape factors of multiwalled carbon nanotubes (MWCNTs)
ISO/DIS 12025	Nanomaterials -- Quantification of nano-object release from powders by generation of aerosols
ISO/DTS 12805	Nanomaterials - Guidance on specifying nanomaterials
ISO/NP TS 14101	Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening: FT-IR method
ISO/AWI TR	Nanotechnologies -- Framework for nomenclature models for nano-objects

14786	
ISO/AWI TS 16195	Nanotechnologies - Generic requirements for reference materials for development of methods for characteristic testing, performance testing and safety testing of nano-particle and nano-fiber powders
ISO/NP TR 16196	Nanotechnologies - Guidance on sample preparation methods and dosimetry considerations for manufactured nanomaterials
ISO/NP TR 16197	Nanotechnologies - Guidance on toxicological screening methods for manufactured nanomaterials
ISO/NP 16550	Nanoparticles - Determination of muramic acid as a biomarker for silver nanoparticles activity
IEC/NP TS 62622	Artificial gratings used in nanotechnology -- Description and measurement of dimensional quality parameters
ISO/DTS 80004-4	Nanotechnologies -- Vocabulary -- Part 4: Nanostructured materials
ISO/DTS 80004-5	Nanotechnologies -- Vocabulary -- Part 5: Nano/bio interface
ISO/AWI TS 80004-6	Nanotechnologies -- Vocabulary -- Part 6: Nanoscale measurement and instrumentation
ISO/DTS 80004-7	Nanotechnologies -- Vocabulary -- Part 7: Diagnostics and therapeutics for healthcare
ISO/NP TS 80004-8	Nanotechnologies -- Vocabulary -- Part 8: Nanomanufacturing processes

ISO TC146 Air Quality

Published Standards

ISO 4225:1994	Air quality -- General aspects -- Vocabulary
ISO 4226:2007	Air quality -- General aspects -- Units of measurement
ISO 7708:1995	Air quality -- Particle size fraction definitions for health-related sampling
ISO 8672:1993	Air quality -- Determination of the number concentration of airborne inorganic fibres by phase contrast optical microscopy – Membrane filter method
ISO 9096:2003	Stationary source emissions -- Manual determination of mass concentration of particulate matter
ISO 9169:2006	Air quality -- Definition and determination of performance characteristics of an automatic measuring system
ISO 9855:1993	Ambient air -- Determination of the particulate lead content of aerosols collected on filters -- Atomic absorption spectrometric method
ISO 10155:1995	Stationary source emissions -- Automated monitoring of mass concentrations of particles -- Performance characteristics, test methods and specifications
ISO 10312:1995	Ambient air -- Determination of asbestos fibres -- Direct transfer transmission electron microscopy method
ISO 10473:2000	Ambient air -- Measurement of the mass of particulate matter on a filter medium -- Beta-ray absorption method
ISO 12141:2002	Stationary source emissions -- Determination of mass concentration of particulate matter (dust) at low concentrations – Manual gravimetric method
ISO 14956:2002	Air quality -- Evaluation of the suitability of a measurement procedure by comparison with a required measurement uncertainty
ISO 14966:2002	Ambient air -- Determination of numerical concentration of inorganic fibrous particles -- Scanning electron microscopy method
ISO 15202-1:2000	Workplace air -- Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry -- Part 1:

	Sampling
ISO 15202-2:2001	Workplace air -- Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry -- Part 2: Sample preparation
ISO 15202-3:2004	Workplace air -- Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry -- Part 3: Analysis
ISO 15767:2009	Workplace atmospheres -- Controlling and characterizing uncertainty in weighing collected aerosols
ISO 20988:2007	Air quality -- Guidelines for estimating measurement uncertainty
ISO 12141:2002	Stationary source emissions -- Determination of mass concentration of particulate matter (dust) at low concentrations – Manual gravimetric method
ISO 14956:2002	Air quality -- Evaluation of the suitability of a measurement procedure by comparison with a required measurement uncertainty
ISO 14966:2002	Ambient air -- Determination of numerical concentration of inorganic fibrous particles -- Scanning electron microscopy method
ISO 15202-1:2000	Workplace air -- Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry -- Part 1: Sampling
ISO 15202-2:2001	Workplace air -- Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry -- Part 2: Sample preparation
ISO 15202-3:2004	Workplace air -- Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry -- Part 3: Analysis
ISO 15767:2009	Workplace atmospheres -- Controlling and characterizing uncertainty in weighing collected aerosols
ISO 20988:2007	Air quality -- Guidelines for estimating measurement uncertainty

Standards under development

ISO/NP 8672	Air quality -- Determination of the number concentration of airborne
ISO/DIS 13138	Air quality -- Sampling conventions for airborne particle deposition
ISO/DIS 13271	Stationary source emissions -- Determination of PM ₁₀ /PM _{2,5} mass concentration in flue gas -- Measurement at higher concentrations by use of virtual impactors
ISO/WD 25597	Stationary source emissions -- Test method for determining PM 2.5

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