



International Standard

ISO 19430

Determination of particle size distribution and number concentration by particle tracking analysis (PTA)

*Détermination de la distribution granulométrique et de la
concentration en nombre par l'analyse de suivi de particule (PTA)*

**Second edition
2024-08**

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Foreword

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

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

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

This second edition cancels and replaces the first edition (ISO 19430:2016), which has been technically revised.

The main changes are as follows:

- Inclusion of particle counting and number concentration measurements.
- Inclusion of information on gravitational motion tracking.
- Inclusion of information on simultaneous multispectral detection.
- Inclusion of particle number concentration comparison to other methods.
- Inclusion of information on serial dilution for PTA.

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

Introduction

Regulatory, scientific and commercial requirements for nanomaterial characterization or characterization of particulate suspensions where particle sizing and counting is used provide a strong case for further development of techniques such as particle tracking analysis (PTA), also known as nanoparticle tracking analysis (NTA).^[1] Due to the fact that the term PTA covers a larger size range and is more generic,¹⁾ the term PTA is used throughout this document to refer to NTA and PTA.

PTA is based on measuring the diffusion movement of objects (particles, droplets or bubbles) in a dispersion, but can also be used to undertake gravitational migration tracking by means of laser illumination, imaging of scattered light, particle identification and localization, and individual particle tracking.²⁾ This document covers two tracking regimes.

— Brownian motion tracking for smaller particles.

— Gravitational fall tracking for larger particles.

In both cases, the suspension is an even dispersion of particles, gas bubbles or other liquid droplets. The hydrodynamic diameter of the individual particles, droplets or bubbles is related to Brownian motion parameters via the Einstein equation and via Stokes law for gravitational migration dynamics.

In recent years, the academic community working in fields such as liposomes and other drug delivery vehicles, nanotoxicology, viruses, exosomes, protein aggregation, inkjet inks, pigment particles, cosmetics, foodstuffs, fuel additives and ultrafine bubbles began using the PTA technology for characterization. ASTM E2834-12 was developed to give guidance to the measurement of particle size distribution by means of NTA. This document aims to broaden the scope of the specification and to introduce system tests for PTA operation as well as to extend the particle size range from nanoscale to microscale sizes. One way to do this is to combine Brownian motion tracking with gravitational migration tracking in the same device on the same sample.

For a number of years, the stakeholders working with nanomaterials safety, regulation, compliance and fundamental research into applications such as biomedicine, catalysis, fuel additives and others were looking for a method (or a combination of methods) for counting and sizing particles in a wide size range (larger than 1 nm to 100 nm). Particle size distributions are often used to evaluate nanomaterials for regulatory purposes (see Reference [41] on the definition of nanomaterial) or for material specification compliance. A number of techniques are available for such characterization, but samples need to be monodisperse. A bigger challenge is to provide an accurate particle count. Techniques such as PTA, electron microscopy, spICP-MS or electrical sensing zone (see ISO 13319-1) allow particle count but have method-specific issues.

One of the key aspects of PTA is the interpretation of data. The key measurand obtained from PTA measurement is the number-based particle size distribution where the size is taken to mean the hydrodynamic diameter of the particles in the sample. The hydrodynamic particle diameters measured with PTA can be different from equivalent particle diameters obtained with different techniques^[3] such as dynamic light scattering (DLS) (see ISO 22412:2017) or electron microscopy (see ISO 21363 and ISO 19749).

1) NTA is the most recognised abbreviation for the technique described in this document. However, PTA includes NTA in its size range of measurements.

2) For the purpose of this document, “tracking” is intended to mean “following in terms of particle’s x and y position”; “track” is defined in 3.32.

Determination of particle size distribution and number concentration by particle tracking analysis (PTA)

1 Scope

This document specifies the particle tracking analysis (PTA) method under static (no flow) conditions for the determination of the number-based particle size distribution and the number concentration in liquid dispersions (solid particles, liquid droplets or bubbles suspended in liquids).

This document covers two tracking regimes.

- Brownian motion tracking for smaller particles.
- Gravitational fall tracking for larger particles.

This document outlines the theory and basic principles of the PTA method along with its limitations and advantages for both size evaluation and number concentration measurements. It also describes commonly used instrument configurations and measurement procedures as well as system qualifications and data reporting.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 13322-1:2014, *Particle size analysis — Image analysis methods — Part 1: Static image analysis methods*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

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

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

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

3.1 nanoscale

length range approximately from 1 nm to 100 nm

[SOURCE: ISO 80004-1:2023, 2.1]

3.2 nanoparticle

discrete piece of material with all external dimensions in the *nanoscale* (3.1)

Note 1 to entry: If the dimensions differ significantly (typically by more than three times), terms such as "nanofibre" or "nanoplate" are preferred to the term nanoparticle.

[SOURCE: ISO 80004-1:2023, 3.3.4]

3.3

particle

minute piece of matter with defined physical boundaries

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

Note 2 to entry: This general particle definition applies to nano-objects.

[SOURCE: ISO 80004-1:2023, 3.2.1]

3.4

particle size

linear dimension of a *particle* (3.3) determined by a specified measurement method and under specified measurement conditions

Note 1 to entry: Different methods of analysis are based on the measurement of different physical properties. Independent of the particle property actually measured, the particle size can be reported as a linear dimension, e.g. as an equivalent spherical diameter.

[SOURCE: ISO/TS 80004-6:2021, 3.1.1]

3.5

particle size distribution

distribution of the quantity of *particles* (3.3) as a function of *particle size* (3.4)

Note 1 to entry: Particle size distribution may be expressed as cumulative distribution or a distribution density (distribution of the fraction of material in a size class, divided by the width of that class).

Note 2 to entry: The quantity can be, for example, number, mass or volume based.

[SOURCE: ISO/TS 80004-6:2021, 4.1.2]

3.6

equivalent diameter

diameter of a sphere that produces a response by a given *particle size* (3.4) measurement method that is equivalent to the response produced by the *particle* (3.3) being measured

Note 1 to entry: The physical property to which the equivalent diameter refers is indicated using a suitable subscript (see ISO 9276-1:1998).

Note 2 to entry: For discrete-particle counting, light-scattering instruments, an equivalent optical diameter is used.

Note 3 to entry: Other material constants like density of the particle are used for the calculation of the equivalent diameter like *Stokes diameter* (3.22) or sedimentation equivalent diameter. The material constants, used for the calculation, should be reported additionally.

Note 4 to entry: For inertial instruments, the aerodynamic diameter is used. Aerodynamic diameter is the diameter of a sphere of density $1\,000\text{ kg m}^{-3}$ that has the same settling velocity as the irregular particle.

[SOURCE: ISO/TS 80004-6:2021, 4.1.5, modified — Note 1 to entry and Note 3 to entry changed.]

3.7

light scattering

change in propagation of light at the interface of two media having different optical properties

[SOURCE: ISO/TS 80004-6:2021, 4.2.5]

3.8

hydrodynamic diameter

equivalent diameter (3.6) of a *particle* (3.3) in a liquid having the same diffusion coefficient as a spherical particle with no boundary layer in that liquid

Note 1 to entry: In practice, *nanoparticles* (3.2) in solution can be non-spherical, dynamic and solvated.

Note 2 to entry: A particle in a liquid will have a boundary layer. This is a thin layer of fluid or adsorbates close to the solid surface, within which shear stresses significantly influence the fluid velocity distribution. The fluid velocity varies from zero at the solid surface to the velocity of free stream flow at a certain distance away from the solid surface.

[SOURCE: ISO/TS 80004-6:2021, 4.2.6]

3.9

particle tracking analysis

PTA

method where *particles* (3.3) undergoing Brownian and/or gravitational motion in a liquid *suspension* (3.14) are illuminated by a laser and the change in position of individual particles is used to determine their *equivalent diameters* (3.6)

Note 1 to entry: Analysis of the time-dependent particle position yields the translational diffusion coefficient and hence the *hydrodynamic diameter* (3.8) using the Einstein relationship.

Note 2 to entry: Nanoparticle tracking analysis (NTA) is often used to describe PTA. NTA is a subset of PTA, since PTA covers a length range that exceeds the *nanoscale* (3.1).

3.10

nanomaterial

material with any external dimension in the *nanoscale* (3.1) or having internal structure or surface structure in the nanoscale

Note 1 to entry: See “engineered nanomaterial”, “manufactured nanomaterial” and “incidental nanomaterial” in ISO 80004-1 for definitions of certain types of nanomaterial.

Note 2 to entry: The nanoform of a material is a nanomaterial.

[SOURCE: ISO 80004-1:2023, 3.1.4, modified — Reference to ISO 80004-1 added to Note 1 to entry.]

3.11

diluent

non-volatile homogeneous liquid which is used to decrease the concentration of *particles* (3.3) in a *suspension* (3.14) without any deleterious effects such as changing particle total number, state of aggregation, *particle size* (3.4) or surface chemistry

3.12

viscosity

η

ratio between the applied shear stress and rate of shear of a liquid

Note 1 to entry: It is a measure of the resistance to flow or deformation of a liquid.

Note 2 to entry: The term “dynamic viscosity” is also used in a different context to denote a frequency-dependent quantity in which shear stress and shear rate have a sinusoidal time dependence.

[SOURCE: ISO 3104:2020, 3.2, modified — Preferred terms and Note 3 to entry have been deleted.]

3.13

dispersion

multi-phase system in which discontinuities of any state (solid, liquid or gas: discontinuous phase) are distributed in a continuous phase of a different composition or state

Note 1 to entry: This term also refers to the act or process of producing a dispersion; in this context, the term “dispersion process” should be used.

Note 2 to entry: If solid *particles* (3.3) are distributed in a liquid, the dispersion is referred to as a *suspension* (3.14). If the dispersion consists of two or more immiscible liquid phases, it is termed an “emulsion”. A suspoemulsion consists of both solid and liquid phases distributed in a continuous liquid phase.

[SOURCE: ISO/TS 80004-6:2021, 3.14]

3.14

suspension

heterogeneous mixture of materials comprising a liquid and a finely dispersed solid material

[SOURCE: ISO/TS 80004-6:2021, 3.13]

3.15

simultaneous multispectral detection

SMD

method where optically scattering objects [such as *particles* (3.3) or bubbles] are detected, counted and tracked by means of *particle tracking analysis* (3.9), using light sources of different wavelengths and different powers.

Note 1 to entry: Detection, counting and tracking of objects is performed independently in each spectral regime.

3.16

total particle count method

particle (3.3) counting method in which the total number of particles in a certain sample volume is determined without classification according to size

[SOURCE: ISO 29463-4:2011, 3.2]

3.17

particle counting and sizing method

particle (3.4) counting method which allows both the determination of the number of particles and also the classification of the particles according to size

[SOURCE: ISO 29463-4:2011, 3.3]

3.18

particle number concentration

number of *particles* (3.3) per unit of volume of suspension (3.14)

3.19

number concentration distribution density

distribution density (frequency) of the *particle number concentration* (3.18) represented as a function of the *particle size* (3.4)

[SOURCE: ISO 26824:2022, 3.9.5]

3.20

limit of quantification

quantification limit

LOQ

lowest amount of an analyte that is quantifiable with a given confidence level

Note 1 to entry: The confidence level can be calculated as ten times the standard deviation of blank measurement results. This concept applies to concentration measurements only.

Note 2 to entry: The value LOQ can be used as a threshold value to assure quantitative measurement of an analyte accurately.

[SOURCE: EN 1540:2021, 5.3.5, modified — Note 2 to entry has been modified and Note 3 to entry has been deleted.]

3.21

limit of detection

LOD

lowest amount of an analyte that is detectable with a given confidence level

Note 1 to entry: The limit of detection can be calculated as three times the standard deviation of blank measurement results. This represents a probability of 50 % that the analyte will not be detected when it is present at the concentration of the LOD.

Note 2 to entry: The LOD can be used as a threshold value to assert the presence of a substance with a known confidence.

Note 3 to entry: The LOD only refers to concentration measurements and not to particle sizing.

[SOURCE: EN 1540:2021, 5.3.4, modified — Note 3 added]

3.22

Stokes diameter

equivalent diameter (3.6) of a sphere that has the same buoyant density and terminal sedimentation velocity as the real *particle* (3.3) in the same liquid under creeping flow conditions

[SOURCE: ISO 26824:2022, 3.4.4]

3.23

migration velocity

absolute value of sedimentation or creaming and flotation terminal velocity

Note 1 to entry: Velocity of creaming and flotation is indicated by a negative sign.

[SOURCE: ISO 18747-1:2018, 3.3]

3.24

migration

directed *particle* (3.3) movement (sedimentation or creaming and flotation) due to acting gravitational or centrifugal fields

Note 1 to entry: Sedimentation occurs when the density of droplets or particles is larger than that of the liquid. Creaming and flotation occur when the density of droplets or particles is smaller than that of the liquid. In these two processes, particles move in opposite directions.

[SOURCE: ISO 18747-2:2019, 3.3]

3.25

analyte

element or constituent to be determined

[SOURCE: ISO 10136-2:1993, 3.3]

3.26

track

path of an object through space

3.27

frame

single static image obtained by a camera in a video recording process

Note 1 to entry: For the purpose of this document, the term "frame" does not include the edge of the *field of view* (3.31).

3.28

transparent medium

medium which has a high transmittance of light in a given spectral range

3.29

aspect ratio

ratio of length of a *particle* (3.3) to its width

[SOURCE: ISO 14966:2019, 3.7]

3.30

tracking

process of obtaining a *track* (3.26) in *x* and *y* coordinates

3.31

field of view

area viewed by the imaging probing system

[SOURCE: ISO 10360-7:2011, 3.3]

4 Symbols and abbreviated terms

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

CCD	charge coupled device	
CMOS	complementary metal oxide semiconductor	
CRMs	certified reference materials	
DLS	dynamic light scattering	
MSD	mean square distance	
d_h	hydrodynamic diameter	m
d_s	Stokes diameter	m
v	terminal velocity	$\text{m}\cdot\text{s}^{-1}$
g	gravitational acceleration	$\sim 9,8 \text{ m}\cdot\text{s}^{-2}$
ρ	apparent density of the particle	$\text{kg}\cdot\text{m}^{-3}$
ρ_0	density of the liquid	$\text{kg}\cdot\text{m}^{-3}$
D_x	translational diffusion coefficient in 1 dimension	m^2s^{-1}
D_{xy}	translational diffusion coefficient in 2 dimensions	m^2s^{-1}
D_{xyz}	translational diffusion coefficient in 3 dimensions	m^2s^{-1}
η	viscosity of the suspension medium	$\text{N}\cdot\text{s}\cdot\text{m}^{-2}$
k_B	Boltzmann's constant	$\text{N}\cdot\text{m}\cdot\text{K}^{-1}$
T	absolute temperature	K
t	time	s
$\overline{(x)^2}$	mean square displacement in 1 dimension	m^2
$\overline{(x,y)^2}$	mean square displacement in 2 dimensions	m^2
$\overline{(x,y,z)^2}$	mean square displacement in 3 dimensions	m^2
C	total particle number concentration	m^{-3}
N	total particle number in a sampling volume	
V_s	sensing volume	m^3
N_o	array of values containing original total number of particles in size-bins before sample dilution	

c_s	array of values containing PTA results as number of particles per unit volume in size-bins	m^{-3}
V_d	volume of diluent used	m^3
V_o	original volume of the dispersion before dilution	m^3
c_d	array of values containing diluent number of particles per unit volume in size-bins	m^{-3}
c_o	array of values containing original number of particles per unit volume in size-bins before sample dilution	m^{-3}
Re	Reynolds number	

5 Principles

5.1 General

In general, PTA can be used to detect, size and count individual particles, droplets or bubbles through Brownian motion or gravitational fall particle dynamics. Historically, only Brownian motion was used for particle size determination and construction of number-based particle size distribution. However, this document covers all known particle tracking methods, thus including gravitational fall. This document is focused on particle sizing, particle counting, particle number concentration and constructing number-based particle size distribution.

NOTE This document is applicable to particles, droplets and bubbles in liquid dispersions which are referred to in the text as particles.

5.2 Measurement types

5.2.1 General

Determination of particle size distribution by PTA commonly makes use of:

- Brownian motion of particles, or
- the gravitational fall or floating of particles, or
- both of the above

combined with the light scattering properties of particles suspended in liquids.

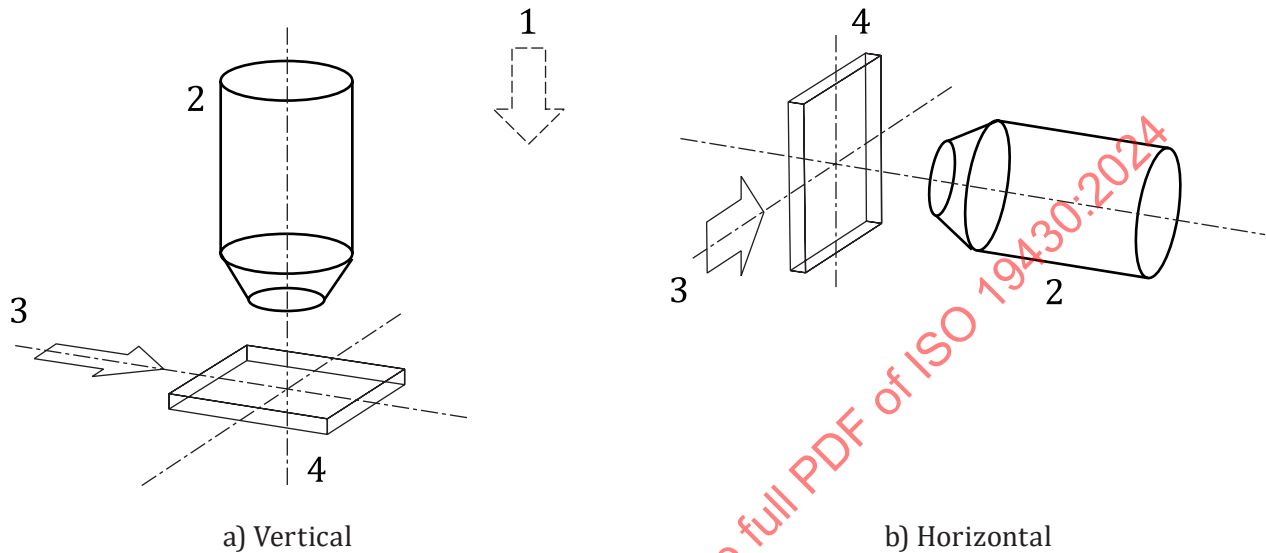
Irradiation of the sample (typically by means of one or several laser beam(s) of wavelength(s) in the visible region) leads to light scattering by objects with a refractive index that is different from that of the surrounding medium. Light scattered from each particle is collected by magnifying optics and visualized by way of a suitable camera, equipped with a charge coupled device (CCD) or complementary metal oxide semiconductor (CMOS) sensor. By recording a series of sequential images, the instrument's software tracks positions of particles as a function of time, allowing analysis of their movement.

By tracking individual particles undergoing Brownian motion^{[3],[4]} or gravitational migration (see ISO 13317-1) from frame to frame, the average spatial displacement of the particles per unit time can be calculated. This displacement can be related to the hydrodynamic diameter of the particles through the Einstein equation^[5] or through Stokes law for gravitational migration of particles.

NOTE In practice, the particles are either tracked in Brownian motion regime or in gravitational migration regime.

PTA instruments use a dark field imaging configuration, with the optical capture axis (Figure 1) commonly oriented vertically (a) or horizontally (b).

A broad range of particle sizes in a dispersion must be detected, sized and counted for the context of this document. Different methods are used to effectively extend the size range of the PTA method. The size working range of the method is often determined by the geometry of the instrument as well as data processing steps, properties of the optical system, the size and optical properties of the particles. There are a number of publications outlining optimum data processing methods used for track analysis (see ASTM E2834-12 and Reference [6]) but their use varies from one manufacturer to another.



Key

- 1 direction of gravity
- 2 is optical capture
- 3 illumination
- 4 sample volume

Figure 1 — PTA configurations

The illumination may be provided by a single wavelength laser or a number of lasers with different wavelengths and power settings, allowing for wider particle size distribution and polydispersity in the sample. Detailed requirements for PTA instruments are outlined in Clause 6. Table 1 summarises the availability of measurements in various instrument geometries.

Table 1 — Summary of instrument configurations and capabilities

	Vertical [Figure 1 a)]	Horizontal [Figure 1 b)]
Single laser	Available	Available
Multiple laser (SMD)	Not known to exist	Available
Gravitational tracking	Not possible	Available

Both horizontal and vertical system geometries are available with a single laser, while no vertically orientated instruments are known to exist for multiple laser illumination. Gravitational fall tracking can only be implemented in horizontal geometries [Figure 1 b)].

5.2.2 Particle detection

The scattering from particles in a dispersion are detected by imaging them in a dark-field mode under a microscope which provides a quantifiable magnification. Once a microscope is combined with a camera, this magnification shall be calibrated. This is important for evaluation of several length measurements such as

the track steps lengths used to calculate the diffusion coefficient as well as the effective field of view for the device as this affects the effective sensing volume used for particle number concentration evaluation.

5.2.3 Brownian motion tracking

When a particle is detected on microscope images, its position is recorded as a function of time in a series of steps making up tracks of individual particles. The underlying physics were described by Einstein.^[5] The Navier-Stokes equation with small Reynolds number limit is solved in [Formula \(1\)](#), giving [Formula \(2\)](#):

$$m \frac{d\vec{v}}{dt} = -3\pi\eta d_h \vec{v} + \vec{F}(t) \quad (1)$$

For $Re = \frac{v\rho d_h}{\eta} \ll 1$, and because $m\langle v^2 \rangle = k_B T$ and $\langle xF(t) \rangle = \langle x \rangle \langle F(t) \rangle = 0$, the solution is:

$$\langle r^2 \rangle = \frac{4k_B T}{3\pi\eta d_h} \Delta t \quad (2)$$

where the averaging is over the assembly of particles observed. Analysis of the track is done by evaluating the mean square distance (MSD) that single particle has travelled in 2-dimensions (2D) over N frames (with the 3rd dimension parallel to the microscope axis). It has been shown that in order to replace time average with assembly average (the so-called ergodicity), a delay (lag) of n frames should be introduced^[7]:

$$MSD(n) = \frac{1}{N-n} \sum_{i=1}^{N-n} (x_{i+n} - x_i)^2 + (y_{i+n} - y_i)^2 \quad (3)$$

The diffusion coefficient D obtained by the optimised least-square fit of $MSD(n)$ as a function of n is given by $(4\Delta t D)n$ (see [Annex A](#)). Hence the resulting PTA hydrodynamic diameter d_h is given by:

$$d_h = \frac{k_B T}{3\pi\eta D} \quad (4)$$

Due to the fact that the unrestricted diffusion process in 3-dimensions (3D) is separable (see [Annex A](#)) into x , y and z independent components, measuring $\langle x \rangle^2$ in the x direction is sufficient for particle hydrodynamic diameter (d_h) evaluation.

5.2.4 Gravitational motion tracking

Brownian motion PTA has a limit in terms of the largest particle size it can measure due to slow movement of large particles. This limit is reached for particles of approximately a few micrometres in diameter, which are relatively immobile. The large particles require better image processing to find particle centres and long tracking times for appropriate measurement statistics. Particles of such a size also scatter a lot of light and can prevent the detection and tracking of much smaller particles in the same dispersion.

In addition to the Brownian motion tracking method, it is possible to use the particle tracking data to calculate the gravitational fall or floating dynamics of larger particles in the dispersion. This method can only be applied in the instrument configuration in [Figure 1 b](#)), i.e. the horizontal configuration. This method extends the range of sizes of particles that can be sized and counted by PTA to large particle fractions.

For particles exhibiting gravity-induced motion, equivalent diameters are determined from the Stokes constant-speed falling sphere approximation (migration velocity); Stokes diameter (d_s) of a tracked particle is then:

$$d_s = \sqrt{\frac{18\eta}{g(\rho - \rho_0)}} \quad (5)$$

The sensitivity of gravitational motion tracking is limited to particles with densities that are different from the dispersion medium. The sizing process is also very sensitive to the changes in liquid viscosity and

temperature. However, these parameters affect size measurements but do not directly affect the ability to count particles in the number concentration measurement.

PTA instruments offering a gravitational sedimentation option for large particle sizing operate in a “homogeneous mode” where the sample is homogenised at the start of the measurement. The terminal velocity (v) of the particle in [Formula \(5\)](#) is obtained by measuring the distance that a tracked particle has travelled in vertical direction in a given time. Such vertical motion can be virtually zero for particles with either equivalent diameters below 1 micrometre or apparent densities close to the density of the continuous phase, or both. For larger particle sizes (typically over 1 μm diameter), the Brownian motion is negligible compared to the gravitational migration, so they display gravitational fall with terminal velocity (v). The terminal velocity is often reached very quickly compared to the video acquisition time. For particles between the purely Brownian and purely gravitational regimes, there exists a range of sizes where neither effect dominates and neither effect can be neglected, leading to a very high uncertainty in sizing.

NOTE The particle gravitational sizing process also shows a large uncertainty for samples where continuous medium and particles have similar densities. The effect is a very slow sedimentation because of the near-zero value of $|(\rho - \rho_0)|$.

PTA sedimentation tracking offers an individual particle sizing method rather than the ensemble sedimentation methods that are described in the ISO 13317 series. An example of such data sets is given in [Annex C](#).

5.3 Key physical parameters

[Formula \(4\)](#) shows that the diffusion coefficient is calculated from particle positions. Sample temperature shall be measured. The viscosity of the sample shall be known, in order to calculate the hydrodynamic diameter of individual particles, droplets or bubbles in a dispersion.

In order to evaluate particle number concentration, the total particle number and the sampling volume shall be known or measured.

5.4 Limits of detection

5.4.1 General

Like any measurement technique, PTA has limits of detection in terms of the particle size and the particle number concentration. These limits mainly depend on the physical properties (e.g. equivalent diameter, apparent density, refractive index) of the particles and the polydispersity of the sample. The refractive index of the medium (diluent) and that of particles have an effect on the tracking ability and therefore on limits of detection. Particles with same or similar refractive indexes as the diluent are possibly not readily detectable as they scatter light very poorly.

Detection and tracking of particles are therefore two inseparable processes that should be performed in order to obtain particle tracking data. Tracking in PTA requires isolated particles to be tracked for long enough and to satisfy the track rejection criteria outlined in [Figure 6](#). For this reason, some tracks are rejected. This is normal and forms the basis of PTA. The tracking data can be immediately converted into particle sizes. In other words, counting particles in a sensing volume is not possible without tracking them for PTA data output. After this process it is also possible to obtain the total number of detected (tracked) particles by adding them all together irrespective of their sizes. The resulting number is the total particle number in a given sensing volume which gives a particle number concentration.

The lower and upper limits for particle detection are often determined by the intensity of light scattered by the particles, the strength of illumination, and sensitivity of the detector. Depending on the physical properties of the particles and details of PTA instrument optics, the typical working range of the PTA method is from approximately 10 nm to about 2 μm in diameter and from 10^6 to 10^9 particles/mL. The lower limit largely depends on the instrument setup, the duration of recording and number of videos. It can be lower than 10^6 particles/mL in some cases with very long measurement times.

While PTA does not have a fundamental limit on the aspect ratio of the particles in the sample, PTA should be applied to samples of aspect ratio 0,5 to 1. Rod-like or disk-like particles display complex behaviour and

anisotropic diffusion coefficient. The scattered light intensity can fluctuate due to particle rotation. These concepts are beyond the scope of this document.

5.4.2 Lower size limit of detection

The lower limit of detection in terms of the particle hydrodynamic diameter is determined (apart from sensitivity and dynamic range of the camera) by the light scattering from the particles. The difference in refractive index between the particles and the surrounding continuous liquid phase determines how easily particles are detected. A large difference in refractive indexes results in higher scattering and therefore lower limits of detection for all other parameters being the same. [Table 2](#) shows the limits of detection for commonly used dispersions (particle-diluent combinations). In this case, the diluent used was water.

Table 2 — Typical lower size limits of detection for monodisperse suspensions of nanoparticles

Particle material	Approximate lower limit of detection – smallest size detected (Hydrodynamic diameter in nm)
Gold	10
Silver	10
Other metals or metal oxides	25
Polystyrene ^[8]	40
Silica ^[8]	40
Vesicles, bacteria and viruses	65
Ultrafine bubbles (ISO 20480-1)	40

All the values in [Table 2](#) are given for monodisperse samples.

General effects of samples and measurement parameters on the limits of detection are described in [5.4.3](#) to [5.4.5](#). The typical values quoted for room temperature water dispersion are provided in [Table 2](#). These values are approximate and can vary (as much as 30 %) depending on factors such as porosity of silica or the type of biological material.

Generally, small particles scatter so little light that their presence will possibly not be detected. Additionally, very small particles move very fast and require short camera exposure time which reduces the number of photons they get to form images. Fast moving objects also leave non-circular images on the captured frames due to movement during exposure, thus leading to localization (or positioning) errors as well as to the difficulty to detect and track such particles.

The ability to detect (to image) very faint particles is further reduced in the presence of larger particles in the same dispersion. This issue of polydispersity is important for particle sizing and counting as the presence of larger particles can significantly reduce the ability to detect and track smaller size fractions.

Size-dependent fluctuations in the Mie scattering cross section are often the greatest when the diameter is comparable to the illumination light wavelength. Using more than one laser with different wavelengths appropriately spread from red to blue can allow detection of more particles in a poly-dispersed sample. The particles are then tracked in all colours and the software algorithm assures no double counting so under counting takes place. It should be noted that single laser instruments are able to count particles as described in this document but with different uncertainties.

5.4.3 Upper size limit of detection

The upper particle size (equivalent diameter) limit is due to slowing Brownian motion at larger particle sizes and long observation periods can be necessary. Another issue is that their size can prevent obtaining good sizing and count statistics in a limited field of view of a PTA instrument. For this reason, a lower magnification possibly needs to be used, reducing the sensitivity to the already slow dynamics of very large particles. Very large particles can occupy a relatively large part of the field of view of an instrument and can overlap by being on top of each other. This can lead to an undercount and lower reported number concentration.

Brownian motion tracking described in 5.2.2 can be complemented by another independent tracking method such as gravitational tracking (5.2.3) to offer larger size working range in one instrument. In horizontal experimental geometry (Figure 1 b), it is possible to observe and measure the Stokes velocity of larger particles. This can be used in turn to evaluate particle size via Formula (5). This is done by evaluating a net mean particle movement in vertical direction (5.2.3). This provides a measure of the particle's Stokes velocity and gives Stokes diameter d_s . The upper sizing limit for the gravitational motion sizing depends heavily on the particle density (the difference of particle density and the density of the continuous medium used as diluent). Most metallic particles with sizes over 10 μm sediment too fast to obtain good tracking statistics.

NOTE Hydrodynamic diameter derived from Brownian motion diffusion equation d_h is not the same as the Stoke particle diameter. Hydrodynamic diameter is often known to be slightly larger than Stoke's diameter for the same particle.

5.4.4 Particle number concentration measurement limits

Number concentrations are derived from tracking and counting all individual (tracked) particles in a known volume. By nature, there will be a limit of quantification for the number concentration related to the counts needed for statistically meaningful results and an upper number concentration limit to minimize the effects of overlapping particle tracks which can result in tracks being rejected and particles not being counted.

When accurate concentration measurements are important, the users should determine particle concentration limits for their situation from a concentration linearity analysis whereby expected concentrations are plotted vs. measured concentrations. Samples for this analysis should be prepared by serial dilutions (Annex D) of reference materials with certified particle number concentrations and hydrodynamic diameters similar to the diameters of the particles in the sample. The linear range from this analysis defines the range of accurate particle number concentration for that specific situation. This method is described in more detail in Annex D.

NOTE 1 As described in 5.4, particle count and number concentration measurements without tracking (also known as "total particle count method") are outside the scope of this document and are not viewed as PTA experiments. Comparison of such counting results with PTA data is inappropriate due to significant differences of conditions in which data are acquired and processed. As described above, tracking algorithms can reject some tracks due to track crossing or inadequate track lengths which can lead to undercount. As a result, at high number concentrations it is not possible to compare PTA and the total particle method results.

NOTE 2 For a PTA SMD system with a 20x magnification and a nominal sensing volume of 2,5 nL and by recording 7-second videos (each of 300 frames) of 25 fresh aliquots of a sample, it has been found that $5 \cdot 10^6$ to 10^8 particles/mL are reasonable lower and upper particle concentration limits. These limits were established using monodisperse polystyrene latex spheres of sizes 100 nm to 400 nm (each measured individually).

The lower concentration limits of quantification can vary depending on the components in the optical path of the detector which will define the field of view and depth of focus and thus directly affect the number of particles captured in the videos for a sample of a given concentration. Another variable, particularly for the lower number concentration limit, is the time allotted for testing and desired level of statistical significance. For very low concentration samples, i.e. with single digit counts per video, better statistics can be obtained by introducing a larger number of fresh aliquots from the same sample into the sensing volume and recording videos from each aliquot (which will extend the time of the test). Other factors, including the shape of the sensing volume, or risk of contamination from external sources like air in laboratory, liquid viscosity and particle size can have effects on the number concentration limits.

NOTE 3 LOD is often calculated as three times the standard deviation of the result obtained in the blank test (as pure diluent as possible). Hence LOD is often estimated for 10 to 20 particles per field of view per video recorded.

Samples with very low particle concentrations can be affected by a systematic effect of particle content in an pure liquid that is used to make the dispersions for testing. Even high purity liquids used in the laboratory can contain contaminating particles. This effect can be as large as 10 % to 15 % by number. The method for eliminating this systematic effect is described in 7.8.3.

At the upper end of the working range of concentration, the crossing and overlapping of tracks leads to track rejections and can lead to particle undercounting.

NOTE 4 Upper limit of the working range of concentration can be defined as the concentration at which the deviation from liner behaviour in series dilution experiment ([Annex D](#)) exceeds ten standard deviations of a blank sample. In this case, the upper limit coincides with the upper LOQ.

One limiting factor is the overlapping of the particle images as seen by the image capturing system or crossing particle tracks. Overlapping particle images can be erroneously counted as one particle or be rejected by some more advanced static image analysis algorithms (ISO 13317-1).

In addition, the highly concentrated samples where particle to particle interaction (limited Brownian motion) can lead to agglomeration or other forms of instabilities, are susceptible to changes in temperature or presence of a dispersant. For this reason, the measurements on highly concentrated samples require stricter control over the sample preparation and experimental parameters such as temperature.

5.4.5 Sample and sampling volume

Sample and sampling volume are critical for all concentration measurements. Typically, instruments discussed in this document require approximately 1 mL of sample to be used for measurement.

The subsampling methods can vary between manufacturers, yet the sampling volume of liquid that is being investigated within the PTA microscope field of view is often limited to a range of approximately 0,1 nL to 1 nL volume. The sampling volume, for the PTA measurement is limited laterally by the optical field of view of the system to (typically of the order of) 100 μm by 100 μm area. The particles in that area are tracked using imaging power of the optics with an approximate focus depth of (the order of) 10 μm which is taken as the sampling volume depth. This results in a sampling volume of 0,1 nL. Larger sampling volumes can be obtained for optical systems with larger field of view or lower magnification and higher focus depth.

In order to obtain a representative measurement of a sample (especially for low particle number concentrations), the sampling volume should be increased. This is often achieved by sampling multiple parts of the sample and performing a new measurement as described in [Clause 7](#).

5.5 Measurement precision and uncertainties

5.5.1 Size measurement uncertainty

The number of tracks of different particles determines the level of representative sampling. Enough particles for an appropriate statistical representation of the sample must be tracked. Ultimately the longer the track length, the better the estimate of D is and thus the lower the measured particle size uncertainty.^[8] However, in practice, the lengths of tracks are finite which allows only a finite number of tracks to be accepted. This leads to higher uncertainty in measured particle sizes. In addition to this due to finite sensitivity of the camera and finite shutter speed, the image of a particle can be smeared or blurred, leading to a yet greater uncertainty in the particle localization. Some manufacturers set a constant minimum track length for all particles. Statistically, the longer the minimum track length, the more accurate each particle diameter but there are less of such tracked particles. Therefore, the experiment should run longer to accumulate statistically significant samples as shown in [Table 3](#). An example of an approximate relationship between the number of tracks and the PTA measurement precision expressed in CV of modal particle size for a monodisperse 100 nm sample is reported in [Table 3](#).

Table 3 — Approximate values showing dependence of PTA measurement on the number of tracked particles

Number of tracks	CV of particle size %
400	< 10
700	< 8
1 000	< 5
2 000	< 3
NOTE: Data were obtained for a 100 nm monodisperse sample of polystyrene spheres.	

[Table 3](#) should be used as guidance in planning experiments. Some manufacturers use the optimization routine to take into account particle motion blur, particle localization uncertainty and finite track length to minimize individual particle sizing uncertainty.^[9]

Other size bins may be used in other measurements. Larger size bins allow better count statistics but compromise size resolution.

For a given bin size, the number of tracks recorded can be used as an indication of the data precision. For such a Poisson distribution, the square root of the number of tracks in a size bin represents the precision estimate. An assessment of precision should be implemented by sampling the same sample three or more times and obtaining a CV for each size bin.

The precision of PTA measurement is affected by polydispersity. Thus, the values in [Table 3](#) represent a relatively ideal case. This means that, to achieve a desired precision, a higher number of tracks should be counted for more polydisperse samples. The methodology described in ISO 13322-1:2014, Annex A for ab-initio calculation of the number of tracks is required for a given precision. [Table 3](#) indicates the minimum number tracks to achieve a given CV of modal particle size. Number of tracks, number of frames and video length follow an approximate linear relationship (within the experimental fluctuations of [Table 3](#) data).

Based on [Formula \(4\)](#) in [Clause 5](#), the particle diameter (hydrodynamic diameter, d_h) depends on uncertainty in the temperature of the sample, in the used value of viscosity (which is itself temperature-dependent) and on the error involved in measuring the particle mean displacement while tracking.

The temperature of the sample shall be measured within to $\pm 0,5$ K. It is also required to bring the sample and instrument into thermal equilibrium before starting any measurement. For any video acquisition time longer than 30 seconds, a measurement of starting and finishing temperature is required.

Values for viscosity will often be taken from tabulated or extrapolated values for a given diluent. Viscosity varies with temperature.

Any vibration or movement that is not a result of particle Brownian motion or gravitational migration will lead to a decrease in the reported size of particles, increase in particle sizing uncertainty and as such shall be avoided. Therefore, steps shall be taken to prevent vibration affecting the measurement. This should be achieved by placing the instrument on vibration isolation table or conducting experiments in a basement room with steady floor.

Detection and tracking of particles are enhanced by using higher power laser, however it can have a heating effect on particles which acquire a drift velocity due to laser heating. This is a significant effect to be avoided since this drift velocity is not possible to compensate or remove.

5.5.2 Counting efficiency

This document focuses on the PTA particle counting and sizing method which means that the overall particle counting efficiency is determined by the detection efficiency, tracking efficiency and data processing

algorithms (for example track rejection rules discussed in [7.7](#)). Particle counting efficiency is often defined as the ratio of the reported number of particles in a given size range to the actual number of such particles.

NOTE PTA counting efficiency is distinct from intrinsic issues with counting particles in dispersion. In certain samples, the particles adhere to the vessel or the sample cell leading to lower effective concentration. In other samples, aggregation can reduce the number of particles over time or dissolution reduces their size. Both issues are known to affect samples over time and can affect the storage of such samples.

5.5.3 Size resolution

Size resolution refers to the ability to distinguish two closely size-related particle populations (each monodisperse). This parameter is determined by the uncertainty in measurement of a single particle, the reproducibility of the data in each size bin as well as the counting efficiency (see [5.5.2](#)) in each size class of the particle distribution.

5.5.4 Polydispersity

Real world samples are often polydisperse. It is therefore a great advantage for an analytical technique to be able to characterise such samples. Larger particles scatter disproportionately more light into optical systems than smaller ones. This issue adds a sample-dependent systematic undercounting or no counting at all error of smaller size fractions in a poly-dispersed samples which can also affect the number-based particle size distribution.

NOTE The dynamic range of scattering intensity as a function of particle size can be increased by the PTA SMD method, which illuminates the sample illumination with multiple light beams of different power and colour, coupled with the capability to capture and analyse scattered light images in each colour from each particle separately. By using multiple lasers, with individually adjustable power levels, it is possible to divide the dynamic range of scattered light intensity from sub-micrometre and micrometre sized particles into manageable segments and therefore visualize as well as measure the size and concentration of particles in samples containing a wide range of particle sizes.

5.5.5 Sensing volume

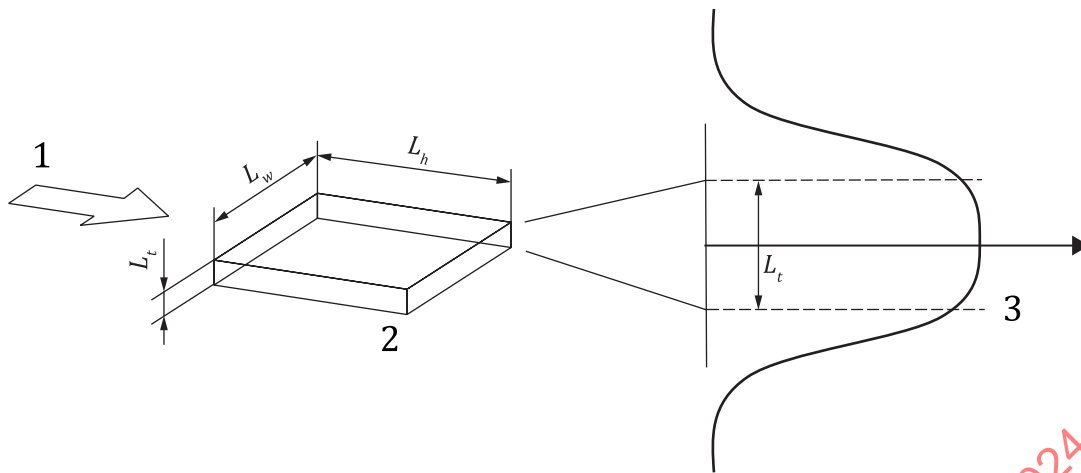
Although details of the sensing volume and its geometry are not critical for PTA sizing measurements, they are critical for particle counting and evaluation of number concentration. Because of the discussions in [5.2.2](#), the sensing volume cannot be restricted by hard surfaces and is often taken for a shape of the light sheet without restricting walls. The sensing volume refers to the volume in which particles are optically detected and tracked. The sensing volume, its geometry and uncertainties in its dimensions are very important for the evaluation of number concentration by means of PTA.

- a) Ideally, particles of all sizes must be equally illuminated in the sampling volume in order to have a uniform ability to track particles of similar sizes in every part of the sampling volume.
- b) Ideally, the imaging quality (e.g., focusing) should not vary across the sampling volume or its depth. Uneven imaging quality can lead to weaker detection and lower ability to track smaller particles. In turn, this will lead to particle undercount.

A major systematic counting error can be introduced if the limited depth of focus leads to detecting and tracking of only larger particles where the focus is not sharp. Large particles can still be tracked and counted even if they are not in focus.

In reality, the effective sensing volume of a PTA instrument is not a perfect rectangle. Its effective shape and size are affected by the illumination profile, polydispersity and refractive indices of particles tracked.

Common illumination of the sensing volume is done using a focussed laser(s). It is the property of every optical system to have a slightly decreased intensity of light at the edge of the illuminating zone as shown in [Figure 2](#).

**Key**

- 1 incident laser
- 2 (effective) sample volume
- 3 light intensity

Figure 2 — Light intensity variation across the sensing volume

The intensity of illuminating light is often not uniform across the thickness of the sensing volume. This introduces an uncertainty in the depth of that volume (L_t). In addition, the tracking algorithms make the edges of the viewing area less likely to collect long enough tracks for sizing and counting.

Yet another complication is that both deviations of the shape of the sensing volume from a rectangular shape also depends on the wavelength. It is therefore required to use similar size and material of particles to experimentally evaluate the effective sampling volume of a PTA instrument. Different manufacturers have their own methods of evaluating and calibrating the effective sample volume. A typical process flow is shown in [Figure 3](#).

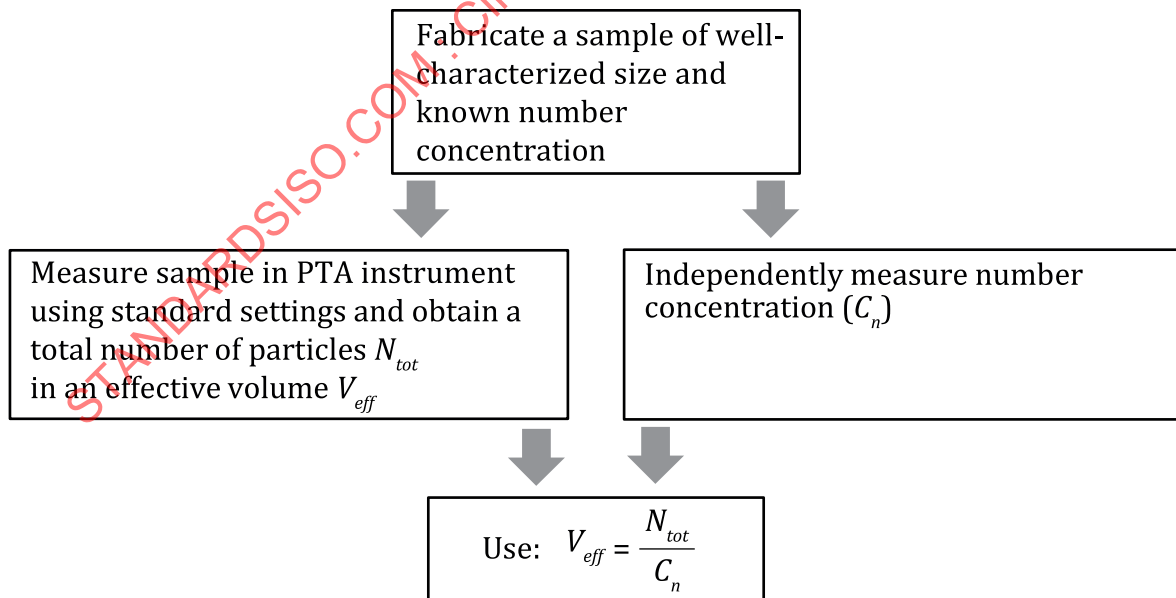


Figure 3 — Schematic process flow for estimation of effective sampling volume

Different manufacturers can employ different calibration regimes for this procedure. The method relies on the availability of a reference sample of known size, known refractive index and known number concentration. The size of particles and their refractive index are relevant since this affects the effective

sensing volume. Therefore, the test sample(s) should be made as close to the sample of interest as possible in number concentration, size and material. Alternatively, the intensity of scattered light should be comparable between standard and measured sample. Ultimately, the effective sensing volume is different for each size fraction and material type in polydisperse samples, reflecting the fact that different particles with different sizes can be detected and tracked by a given PTA optical system at different depth of liquid. This makes effective sensing volume a function of particle size $V_{eff}(d_B)$ and material refractive index. Some instruments can automatically correct effective measurement volume by comparing scattered intensity of light for each tracked particle with known standards for which effective volumes were predetermined and stored into lookup tables.

Independent evaluation of number concentration can carry its own uncertainty and bias but is not part of the scope of this document as it relates to independent methods. The user of this document should consult relevant specifications in order to assess the method applicability to their hardware.

6 Apparatus

6.1 General

PTA equipment will generally comprise a common collection of basic components, with the possibility of additional peripherals that can be desirable or required for specific experiment types. A number of equipment configurations are described in 5.1. These are all composed of a sample cell (6.2), illumination system (6.3), image capturing (6.4) and image processing (6.5) parts.

6.2 Sample cell (with sample in dispersion)

The sample to be analysed is held in the sample cell. This cell shall be inert to the sample. It shall be able to hold the sample at a stable thermal equilibrium. There are general requirements for the sample cell specifically for the particle counting and particle number concentration evaluation. The following are required for particle sizing and counting:

- Sample cell shall have clearly stated parameters for sensing volume as outlined in 5.5.5 with specified size uncertainties that are used to evaluate number concentration uncertainty according to Formula 4.
- Sample cell shall allow resampling by automatically or manually introducing fresh volume of dispersion in the sensing volume. The fresh volume of the dispersion shall be allowed to stop before a new round of data sampling.

NOTE This document deals with measurements on static liquid where no de-drifting or mathematical subtracting of the ensemble average displacement at each time step^[10] is used. Any such technique is outside the scope of this document.

- Sample cell temperature and its stability shall be within $\pm 0,5$ K for sizing and particle counting using PTA.
- Sample cell shall be easy to clean.

6.3 Illumination

The laser source shall be such that the intensity and wavelength provide appropriate scattering from particles without bleaching, destroying or modifying them in any way. The wavelength and intensity shall also be appropriate for image of scattered light collection by the digital camera. The beam shall be focused to maximize illumination of in-focus particles, and to minimize optical noise generated by the illumination of out-of-focus particles (coordination of laser sheet thickness and microscope depth of focus).

NOTE Strong irradiation can give rise to minimal localized heating or photophoresis.

For certain experimental procedures, such as the visualization of fluorescently labelled particles against a non-fluorescent scattering background, the radiation shall be monochromatic and the wavelength matched to both the excitation wavelength of the fluorophore and the optical filters used in signal collection.

There are general recommendations for the sample illumination in the sample cell that are particularly relevant to particle counting and number concentration measurements.

- a) Illumination should be uniform throughout the sensing volume (see [5.5.5](#)).
- b) Illumination strength and wavelength should be selected to maximise the image capture quality (see [6.4](#)).

The illumination can be made by either or a combination of the following:

- one continuous light source (e.g. laser);
- several continuous light sources of different wavelengths;
- gated and modulated light source(s).

For the case of multiple light sources, the power output of each should be calibrated and controlled. Such an illumination system may include polarization of each wavelength, which optimizes the useful illumination intensity for a given laser power level. This optimization stems from the fact that the detection system is primarily observing scattered light at 90 degrees (typically ± 15 degrees, depending on numerical aperture of the used optical system) from the illumination light source. This observation-illumination path forms a 2D plane and light scattered in directions orthogonal to this plane is not detectable by the camera and essentially useless. With polarization that removes the useless components from the illumination sources, higher useful illumination energy levels can be achieved without inducing drift or other deleterious effects on particles for a given laser power level.

6.4 Optical image capturing

Scattered (or emitted) light is collected and delivered to the image capture apparatus through a series of lenses, filters and mirrors generally resembling a conventional optical microscope. It is worth noting that magnification sufficient to clearly image the particles is unnecessary, the only requirement being sufficient resolution to allow suitable measurement of the motion of the images of light scattered by particles being measured.

The images should be captured using a digital camera. The following requirements apply for the practical properties of the image capturing system apply, regardless of the system used:

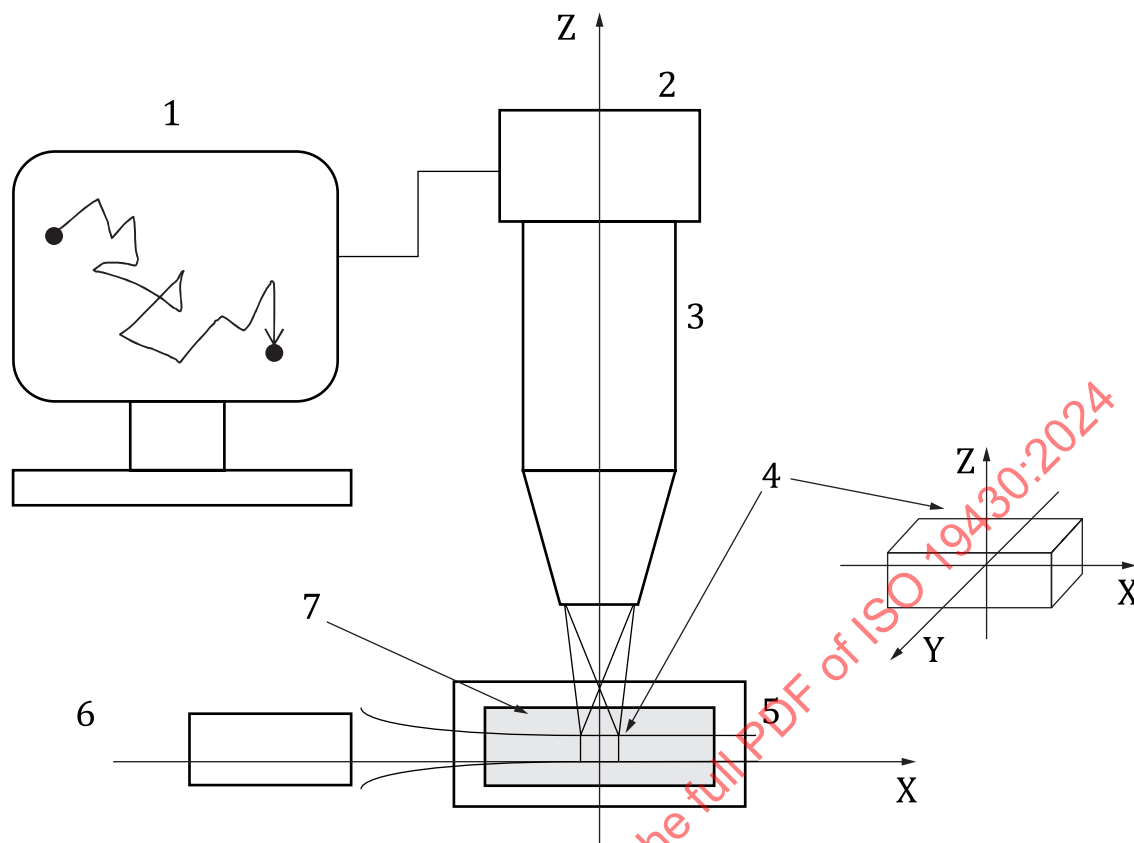
- a) The camera detector array shall be sensitive enough to image particles with lowest scattering cross section of interest with the illumination provided ([6.3](#)).
- b) The camera frame rate (typically 10 to 60 frames/s, but in special cases lower or higher frame rate can be needed, such as for elongated particles measurements) shall be such that sufficient displacement data are collected for each particle, allowing accurate particle tracking and determination of particle size. In the case of the multiple illumination sources, the detection system shall be able to capture images of scattered light from each particle in each of the relevant spectral bands (e.g. blue, green, and red). This can be achieved with three separate detectors if the scattered light is first separated into the individual spectral components, or with a single detector that is capable of individually resolving the blue, green and red images of scattered light from each particle for example a colour video recording device.

6.5 Image analysis, tracking and data processing computer.

A video of illuminated particles shall be captured. The video shall be analysed using particle tracking software. The software should be capable of:

- processing each of the video images;
- identifying, localizing, and counting individual particles;
- tracking discrete particles from frame to frame by recognizing the same particle in separate frames of the video;
- calculating the diffusion coefficient of each particle;

—integrating and outputting the gathered data.



Key

- | | | | |
|---|---------------------------------------|---|----------------------|
| 1 | tracking and data processing computer | 5 | sample |
| 2 | sensitive digital camera | 6 | laser(s) |
| 3 | optical magnification | 7 | sample in dispersion |
| 4 | sampling volume 5.4.5 | | |

Figure 4 — Schematic representation of the vertical PTA experimental

[Figure 4](#) illustrates a common geometry of the PTA experimental setup [for a vertical geometry, see [Figure 1 b](#)].

NOTE The right angles between the illumination laser and the optical detection are not a requirement as other angles, allowing dark field imaging of the sample, are also used.

Image analysis process should be able to provide the following data:

- Identify particle centres (even in cases of blurred or smeared particle images).
- Particle positions as they are tracked from frame to frame (connected positions).
- Total particle count in sensing volume.
- Total particle number concentration (taking into account the effective sensing volume).
- Particle size distribution.

Particle size distributions and particle size distribution density shall be reported as particle number concentrations and a function of particle sizes.

7 Measurement procedure

7.1 General

This clause describes procedural steps for the PTA measurement. Some manufacturers have specific recommendations for each stage, but the schematic in [Figure 5](#) aims to outline the common process flow.

The schematic in [Figure 5](#) describes the process flow of the PTA measurement. Solid lines indicate compulsory steps in the process while dotted lines indicate optional steps and procedures. Optional steps help improve data noise levels and repeatability.

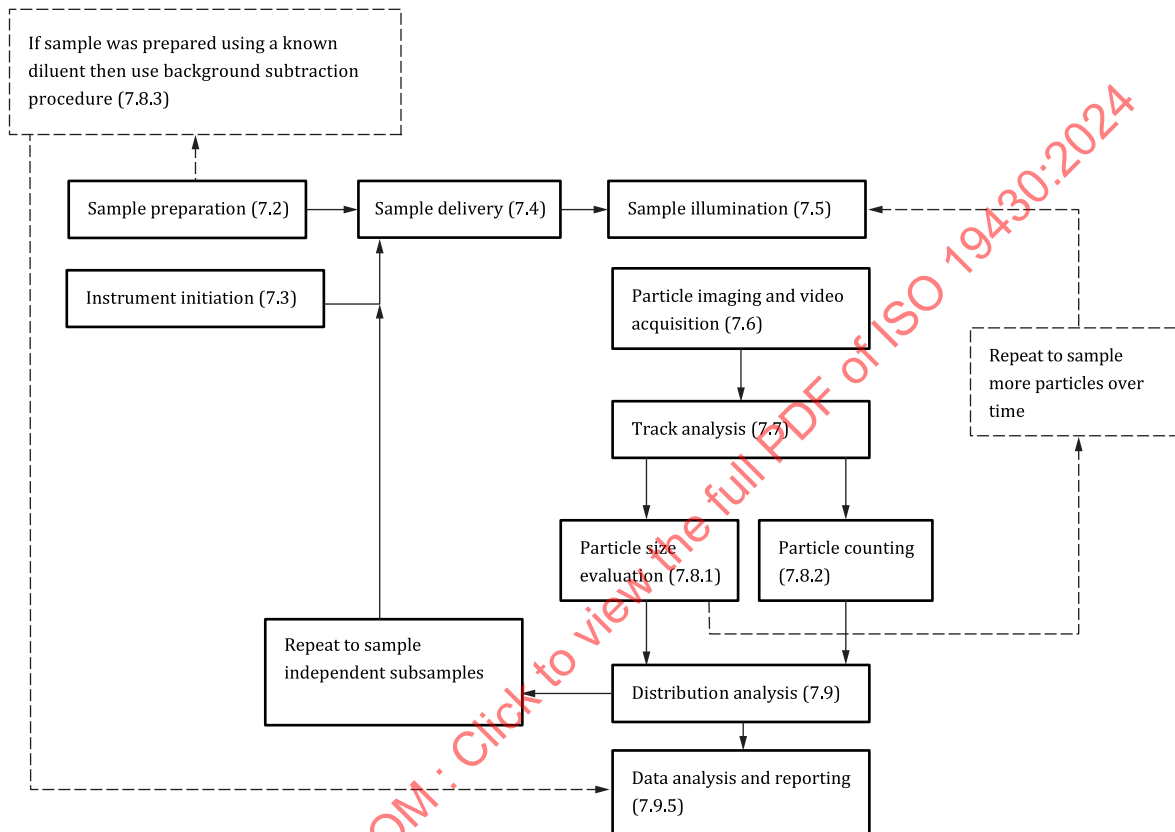


Figure 5 — Schematic of PTA measurement procedure

In some cases, when a known diluent is used to either prepare dispersed sample from dry powder or to dilute the existing dispersion to a recommended concentration for PTA analysis, then the diluent itself can need testing. The reason for this is that most diluents have some particle contaminants that are difficult to remove. This means that adding such a liquid to the sample can introduce new particles which can affect the result. Prior to dispersion of a sample, it shall be ensured that the diluent has the lowest possible count of background particles.

7.2 Sample preparation

The determination of particle size distribution and number concentration by PTA requires small volumes of sample (see [5.5.5](#)) to be dispersed in a suitable liquid (diluent). The sample analysed by the PTA instrument shall be representative of the population being investigated. ISO 14488 includes guidance on the problems of representative sampling. Powders shall be dispersed before analysis. Consultation of ISO 14887 is recommended in the case of powders. Appropriate sample preparation and sampling are critical for reproducible measurements.

Samples shall be homogeneously dispersed in a suitable liquid (diluent), such that particles and the diluent have different refractive indices. The diluent will be a transparent medium (see [3.12](#)). The diluent shall be

compatible with both the test sample, i.e. it shall not affect the physicochemical properties of the dispersed particles, and the PTA apparatus. The diluent shall form a stable suspension of particles and be of a suitable viscosity at the measurement temperature to allow a useful magnitude of particle displacement via Brownian motion or motion under gravity. The user shall ensure that the sample cell is free of contamination or other particles that can contribute to the measurement. The cleaning shall be performed according to manufacturer's specifications.

If dilution of the sample is required, a compatible diluent should be used. It should have similar refractive index, concentration of salts, surfactants, pH, etc., as the original sample's liquid, in order not to change the surface chemistry of the particles. For accurate particle number concentration measurements, dilution of the sample shall result in particle concentrations that are within the manufacturer's recommended upper and lower concentration limits as discussed in [5.4.4](#). Prior to dispersion of the sample, it shall be ensured that the diluent has the lowest possible count of background particles. Methods like syringe filters can be used to sufficiently reduce particle counts in most diluents if needed. Since completely particle-free liquids are not practical, the particle number concentration distribution density of the diluent should be measured prior to dilutions, in order to ensure that the background counts are reasonably low relative to the particles of interest in the sample.

7.3 Instrument set-up and initialisation

Before measurements are performed, it is necessary to initialise the instrument. Power shall be supplied to:

- hardware (controllers);
- image capture equipment;
- irradiation source;
- temperature control and readout;
- any motors, valves, or pumps.

Correct communication between the various components shall be verified. The system shall be allowed to reach thermal equilibrium in order to provide the most stable environment possible. The sample temperature shall be known to within ± 0.5 K or better. The sample temperature shall be stable over the measurement period. Stability of the sample and instrument are particularly important for the comparative measurements with background subtraction.

The first settings to be optimised should be the coordinates of particle observation. The observed position shall be such that illumination of visible particles is maximised, background interference is minimized, and as large a proportion of particles as possible can be seen in sharp focus. Settings shall be appropriate for a particular sample under investigation. For example, small or weakly scattering particles require relatively high laser power and high camera gain settings in order to allow sufficient signal to be detected for tracking purposes. Once the settings are optimized for a particular application, they should be saved in the system as a protocol for future work on same or similar samples.

Once the region of the sample is in focus, suitable image capture settings should be selected. These can include camera gain and shutter and analysis duration. Settings shall be appropriate for a particular sample under investigation. A fast frame rate may be appropriate for small, rapidly diffusing particles, but can be limited by the need for a suitable exposure time in each image. It should be noted that the duration of the experiment is a function of the degree of repeatability needed, with increased analysis duration leading to an increasingly representative sampling of the particle population.

7.4 Sample delivery

The sample can be introduced manually or automatically (with optional motorised dilution and delivery). The sample should remain stable in the sampling volume during image recording (see [5.5.5](#)). The sample shall avoid significant particle drift or particle immobilisation (e.g. sedimentation in case of large particles or creaming in case of bubbles), unless gravitational migration tracking is used.

7.5 Sample illumination

The sample is illuminated by one or several laser sources (6.3) (or another strongly directional light source) with wavelength(s) that is appropriate for an optimum contrast of the sample (dilution medium and particles). Typical wavelengths can range from 405 nm (violet) to 650 nm (red). Some systems offer multiple lasers with different wavelengths, which allows detection and tracking in one or all the available wavelengths.

The sample should be illuminated to give the best contrast for particle tracking. This means that particles should be clearly visible on the video capture system (6.4) while the background level should be minimized. Poor contrast in images can result in a lower number of smaller particles being successfully tracked. This, in turn, can therefore affect the resulting particle size distribution by reducing the number of smaller particles detected, sized and counted.

7.6 Particle imaging and video capture

Requirements for the optical imaging and capture have been specified in 6.4.

The image size calibration (nm/pixel) is usually performed by the manufacturer and does not typically require the attention of the operator. However, if the instrument has been modified or new optics (e.g. different microscope lens) have been installed, then such a calibration should be performed according to the manufacturer's instructions.

The contrast difference between the small bright spots representing the particles and the background shall be optimized by focusing the illuminating laser on the sample volume and by also focusing the imaging lens system for maximum particle clarity. The procedures can vary for different manufacturers. The manufacturer's specified settings and procedures shall be followed in order to improve the imaging contrast.

After thermal stabilization and drift settling of the sample and the selection of suitable method parameter settings, videos of the illuminated sample are recorded. Typically, the sample should be changed between videos (a new sample should be delivered to the measurement cell), in order to introduce an entirely new particle population. In line with other particle size analysis methods, it is necessary to have at least three such independent sub-samples. The number of aliquots that need to be analysed under repeatability conditions depend on the target measurement uncertainty to be achieved. See 5.5.1 for information regarding the length of the video and number of tracks required.

7.7 Track analysis

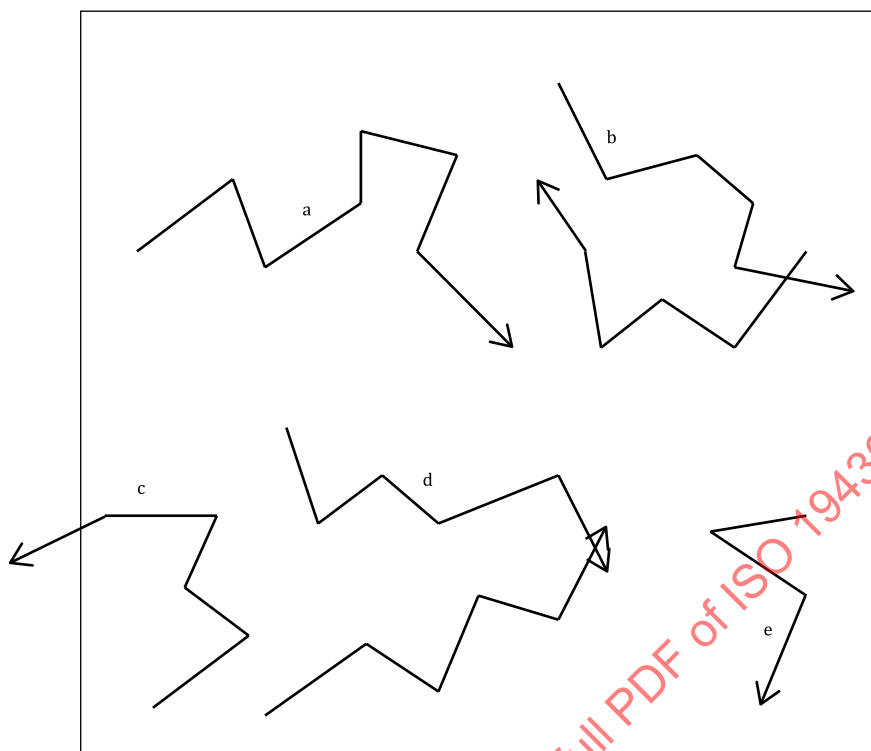
After selection of the appropriate analysis settings, the software locates the centre of each detected small bright spot in every frame of the video. For each such bright spot, the distance moved between every frame of video in which it is visible is measured. Over the course of a single video, hundreds or thousands of particles can be identified and tracked, and the mean squared displacement between frames is calculated for each particle centre individually. This mean squared displacement per time is used in conjunction with the known temperature of the sample and the viscosity to calculate the hydrodynamic diameter of the observed particles on a particle-by-particle basis. Finally, particle size data are combined to produce a particle size distribution.

Visual inspection of the particle movements on the screen can give a good estimate for the data quality obtained from these tracks. A constant drift, mechanical noise patterns (all tracks have similar shapes) or a one off (non-statistical jump) behaviour of a single particle can reduce the ability to track or even measure the particle size from such data. A visual inspection of tracks can help the user to establish the level of confidence in the sample under test.

Particle tracks are analysed for their completeness and some are rejected if the tracks are too short or ambiguous due to track crossing. These are two main track rejection criteria for the particle tracks during PTA measurements. A track is rejected:

- a) if two or more independent particles cross their paths in one of the steps during tracking (i.e. cross their paths between two consecutive frames) [see Figure 6 d)];

- b) if a particle escapes field of view (laterally or out of focus) before a set track length is reached. Such prematurely terminated tracks are shown in [Figure 6 c\) and e\)](#).



Key

- a normal full track,
- b two unambiguously crossing tracks
- c escaping track
- d ambiguously crossing tracks
- e too short track

Figure 6 — Particle track rejection scenarios

Several types of measurements are possible with the track data. The main two types are the particle size measurement and particle counting (particle number concentration) evaluation. Track rejection is one of the reasons for counting less particles than expected in higher concentration samples, due to ambiguous track crossing [see [Figure 6 d\)](#)] and premature track termination [see [Figure 6 c\)](#) and [e\)](#)] in higher concentration samples. This effect is demonstrated in [Annex D](#).

7.8 Measurements

The sample preparation and instrument initiation steps should be followed as described in [7.2](#) and [7.3](#) respectively. The process flow for the measurements shall follow the flowchart in [Figure 5](#).

7.8.1 Particle sizing and number-based size distribution

Track analysis ([7.7](#)) leads to the size evaluation of the individual particles according to [5.2.2](#) and [5.2.3](#) subject to rules of track acceptance and experimental limitations. PTA method is often referred to as “particle counting and sizing method”. The measurement procedure for this method should follow all the steps outlined in [Clause 7](#), including the flow diagram in [Figure 5](#). The tracked particles are sized and appropriately allocated to size bins that make up the particle number-size distribution.

7.8.2 Total particle number measurement

In some circumstances, it can be necessary to count particles in the sensing volume regardless of their size or luminosity. This can be necessary in order to correlate the sample preparation procedures which can use particle weighing and dispersing with the particle count in the PTA instrument. This can also be used to characterise the output of a particle or bubble generator where the size of objects is not critical.

As described in [5.4](#), the particle sizing and counting are not separable. This means that even though the total particle count is size-independent, the particles shall be tracked according to the PTA algorithm to be counted in the total particle count.

Sampling limitation can be highlighted by the fact that the sensing volume is only a small fraction of the entire sample. This means that changing the sample in the sensing volume between consecutive measurements is necessary to approach the representative sampling situation.

7.8.3 Particle background count

PTA is a single particle-based technique and small numbers of particles that can be present in the diluent, prior to adding the dispersed phase, can affect the overall result, particularly for very low concentration samples. The main issue is that diluents can contain contamination particles of different size or refractive index from the sample, making it difficult to interpret the final particle size distribution. There are a number of manufacturer-specific solutions for the particular case when the diluent is known and the amount of diluent added to (a) dry powder or a dispersion is known.

— By judicious preparation of the diluent, it is possible to reduce the number of diluent particles per frame to less than the particle number variation from frame to frame during the sample measurement, which avoids the issue of over-subtraction of the background level from the sample.

— The total number of particles in a blank sample can be subtracted from the total number of particles detected in the sample.

Subtraction of particle size distributions (sample PSD from diluent PSD) can lead to resulting PSD with negative size bins, which is a problem. It is therefore recommended to prioritise cleaning the diluents in order to reduce the effect of contamination.

7.8.4 Volume concentration

The volume concentration is derived from the particle number and sensing volume. In some areas, this is known as “particle volume concentration” which is the ratio of the particle volume to volume of diluent at the same pressure and temperature. In some areas of science, this is also known as volume fraction of a dispersion.

7.9 Results evaluation

7.9.1 General

The algorithms for data analysis may vary between manufacturers but should follow the key steps outlined in [7.1](#). Subclause [7.9](#) deals with common steps in data interpretation.

7.9.2 Particle size evaluation

Particle size is evaluated according to the derivation in [Annex A](#), summarized in [Formulae \(1\) to \(3\) in Clause 5](#). The size of the particle refers to the hydrodynamic diameter (see [3.8](#)) or Stokes diameter (see [3.22](#)).

7.9.3 Particle count results interpretation

The results of a PTA measurement and count of particles should be taken into account in context of the limitations of the method described in the [Clause 5](#). Experimental data comparing PTA number concentration measurement with other techniques such as spICPMS, UV-vis, DCS for gold particles is reported in [Annex B](#).

If there are intrinsic processes taking place in the sample such as sedimentation, agglomeration, flocculation or dissolution, the results of the PTA number count and sizing are time-dependent and will possibly have to be carefully examined. If changes to dispersion parameters are faster than the PTA measurement cycle, the results will possibly be difficult to interpret.

7.9.4 Distribution analysis

The results from each track are analysed and placed in the size bins to construct a particle size distribution. In order to achieve a required uncertainty, a certain minimum number of particle tracks should be used to fill the size-bins in the particle size distribution as described in [Clause 5](#). Data quality control shall therefore be in place to ensure that enough data points populate the size bins.

7.9.5 Data analysis and results display

Data reporting can vary between manufacturers; however, the key parameters needed for a complete data report on the PTA measurement should follow [Clause 10](#).

8 System qualification and quality control

8.1 General

The PTA system shall conform to the requirements outlined in this clause. These requirements are placed on system installation, maintenance, operation and qualification. These requirements should be considered as minimum requirements, since some manufacturers can impose their own system-specific maintenance requirements.

Periodic verification of instrument performance is necessary for quality control purposes. It should be carried out by the analysis of particle size reference material with metrologically traceable certified values.

8.2 System installation requirements

Installation requirements for the PTA system are commonly specified by the manufacturer. The manufacturer guidelines can include a more detailed list of technical steps and checks before the system is used. [8.2](#) sets minimum requirements for the operation of the PTA system.

The equipment shall be located in the area free from direct sun light and away from strong heat sources (such as radiators). The area should be well ventilated and void of excess humidity and dust, especially during sample insertion and injection into the measurement cell (dust can contaminate samples). Humidity should be less than approximately 45 % $\pm$ 10 % RH. Equipment storage requirements shall be taken from the manufacturer's manual.

Vibration can contribute to apparent larger particle movements and therefore systematically smaller sizes of particles will be detected. In this case, vibration isolation shall be implemented, e.g. as a vibration isolation table.

8.3 System maintenance

Maintenance procedures specified by the manufacturer shall be followed. [Subclause 8.3](#) specifies general maintenance steps that are common to all PTA systems.

The PTA system should be left clean and dry when the system is not in use. After each use, the system shall be flushed with diluent liquid to remove all traces of sample from the tubing and optical surfaces. If using diluent with a high concentration of dissolved solids (i.e. saline solution), clean water shall be flushed through the system after use. The controlling software should be shut down.

Optical components of the system (laser, lenses, etc.) shall be kept clear of the solvent or other contaminants at all times.

8.4 System operation

Before the operation of the PTA system, it is important to undertake some control steps to ensure there is no cross-contamination with previous runs and that the system is functioning within specifications. The following are general steps for PTA equipment operation:

- The system should be checked to determine it is clean and that the buffer or dispersing liquid used do not contain contaminant particles.
- The system should be checked to determine that the optical part of the system is optimized (clear sharp image of particles and an appropriate illumination level) as described in [7.5](#) and [7.6](#).
- If the illumination source is temperature controlled, then appropriate thermal equilibrium should be reached before the measurements. Also the sample temperature should be checked to determine that it is settled ($\pm 0,5$ K with minimal overshoot). Typical settling times can vary, but can be approximately a few minutes. Checking for external noise contributions is not always necessary, as most commercial systems have good mechanical and acoustic noise compensation routines.

8.5 Instrument qualification (for sizing)

The most appropriate system qualification is the ability of the PTA instrument to measure the known number-based particle size distribution of a reference material correctly. Ideally, this reference material should be of similar material, particle size and particle concentration to the real sample.

PTA results (for sizing) are method-defined; hence comparison of measurement results with certified values only makes sense if the certified values were also derived by PTA. If such CRMs are not available, values derived by DLS for highly monodispersed suspensions of spherical particles can be an alternative. Alternatively, results from other methods obtained on spherical particles can be used.

NOTE Non-spherical particles are not good for orthogonal method comparison.

Most reference materials are monodispersed while real samples are often not. Ideally, one uses a CRM consisting of polydisperse particles of the same material, certified for the number-size distribution obtained by PTA. The nearest objective test of the system can be performed on at least one certified reference material, such as a 100 nm or 150 nm sized spherical monodispersed particle sample. Polystyrene spheres dispersions with certified size make a good test sample. Sensitivity and measurement confidence of the system should therefore be assessed independently using such reference materials. Resulting system confidence should be reported with the results of the real sample measurement.

It is recommended to use certified materials with one or several peaks for instrument qualification. Their particle size distribution peaks (calculated as the steepest part of the cumulative distribution) should be measured against DLS, but not exclusively. The CRMs of 100 nm and 150 nm should be used. CRMs consisting of larger particles can be useful for systems with gravitational tracking capability.

CRMs with narrow size distribution for one or several of their peaks (each calculated as the steepest part of the cumulative distribution) such as 5 % CV (equal to standard deviation divided by average diameter of the peak) with average particle diameter (as measured by another standardized technique such as DLS) of 100 nm or 150 nm can be used. For such dispersions, the peak position for particle size as measured by PTA shall be within ± 6 % of the stated mean size. See [Table 4](#).

Table 4 — Accuracy requirements summary

	Deviation from the mean certified values nm	CV of the measured average values %	Recommended number of analysed tracks
100 nm sized particle	6	6	1 000
150 nm sized particle	9	6	1 000

In addition to the tests for 100 nm or 150 nm sized particles, an internal reference sample should be chosen that is similar to the sample in shape, size and material type to the samples to be investigated. At least one size qualification measurement should be performed.

NOTE A larger (i.e. more than one) number of size qualification measurements increases data confidence. Further guidance for the choice of RMs for particle size determination is given in ISO/TS 4807.

For instruments that use sedimentation, one size calibration for sedimentation regime (approximately 1 micrometre and above) is necessary to qualify the instrument. The same ± 6 % range should apply for size measurement qualification.

DLS is used as a comparative technique as it can give the closest reference to the hydrodynamic diameter of the reference material. Scanning electron microscopy is often used as orthogonal technique for size evaluation. The particle diameter obtained is slightly different from the equivalent hydrodynamic diameter obtained by PTA.

The number of tracked particles has a direct influence on the statistics of the PTA measurement. The number concentration of reference material samples shall be matched where possible to the real sample and be guided by [7.2](#) and the linearity tests described in [Annex D](#).

In a PTA measurement, a number of particles are tracked to obtain a statistical ensemble of the sample. It is understood that tracking more particles leads to increased confidence in the measurement. An exact calculation of the number of particles needed for a given level of confidence can be found in ISO 13322-1.

8.6 Instrument qualification (for number concentration)

The number concentration measurements described in [Clauses 5 to 7](#) have clear limitations and difficulties. Unlike sizing, the particle number concentration measurement requires knowledge of the sensing volume (see [5.5.5](#) and [Figure 2](#)) in which particles are counted and tracked. The sensing depth in which each size fraction is detected and tracked is different and is a function of the size d_p and type of a material. For this reason, the choice of the instrument qualification reference sample shall have a known concentration as well as size. A CRM with both size and number concentration is required. In the absence of such CRM, an in-house reference material can be obtained by making a dispersion of known mass of monodisperse particles (e.g. polystyrene spheres) in a known quantity of diluent.

For the case of polystyrene, it is possible to calibrate the newly prepared in-house reference sample by measuring the total carbon content in a given volume. Pure carbon-free diluent (such as pure water) should be used to avoid carbon count from the impurities in the diluent. If this cannot be ensured, the total carbon count method does not apply.

It is recommended to adjust the test sample concentration to be in the linear region of the dilution series (or serial dilutions) experiment outlined in [Annex D](#). The linear region is characterised by optimal counting of particles and therefore leads to the most reproducible counting.

The sensing volume (or effective sensing volume depending on manufacturer's assumptions) should be evaluated as described in [5.5.5](#).

Sample should be treated and handled according to [7.2](#). The measurement should follow the outline of [7.1](#).

The validation procedure requires that the measured number concentration matches the assigned reference material number concentration value within ± 10 %. A single point qualification is required at a minimum.

A single point qualification can lead to higher uncertainties. For this reason, varied concentrations should be tested. In case CRMs are used, the method of comparing CRM values with values obtained with a measurement method are clearly described in Reference [\[11\]](#).

9 Data recording

This clause deals with the data that needs to be recorded by the instrument software in order to evaluate data and report it in the format prescribed in [Clause 10](#). There can be some variation on the internal data

handling, but for maximum traceability, the equipment shall record the calculated size of each particle successfully tracked, along with the length of time (in terms of number of frames) for which it was tracked.

The following shall be recorded:

- a) videos of all aliquots analysed;
- b) PTA equipment model reference or serial number;
- c) camera-objective calibration constant (nm per pixel);
- d) camera gain (dB) laser(s) wavelength (nm);
- e) laser(s) power (mW);
- f) frame rate (frames/s);
- g) measured sample cell temperature (K);
- h) dynamic viscosity ($\text{N}\cdot\text{s}\cdot\text{m}^{-2}$);
- i) total number of frames recorded;
- j) processed number of frames;
- k) accepted number of tracks;
- l) measurement uncertainty level — this parameter is obtained independently from measuring a reference sample as described in [8.4](#).

For maximum reproducibility of the results, all data required for the test report ([Clause 10](#)) shall be recorded.

10 Test report

In addition to the requirements specified in ISO/IEC 17025, the test report shall contain at least the following information:

- a) a title (e.g. “Test report”);
- b) unique identification of the test report (such as the serial number), on each page to ensure that the page is recognized as a part of the test report, and a clear identification of the end of the test report;
- c) date and time of experiment;
- d) name of operator;
- e) the name and address of the laboratory, and the location where the tests were carried out, if different from the address of the laboratory;
- f) PTA equipment model reference or serial number and software version;
- g) a description of, the condition of, and unambiguous identification of the sample tested including details of particle shape and homogeneity (if known);
- h) camera-objective calibration constant (nm per pixel);
- i) the suspension conditions:
 - 1) diluent with its filtering procedure and dispersing agents and their concentration (if applicable);
 - 2) particle size distribution density in the diluent;
 - 3) dispersing procedure, including sonication conditions: time, frequency and applied power (if applicable);

- 4) viscosity of the suspension ($\text{N}\cdot\text{s}\cdot\text{m}^{-2}$);
- 5) density of diluent and particle ($\text{kg}\cdot\text{m}^{-3}$) (only needed for non-Brownian motion-based results, typically particles over 1 micrometre diameter);
- 6) temperature of the sample (K);
- j) the measurement conditions and inputs:
 - 1) laser(s) wavelength (nm);
 - 2) laser(s) power (mW);
 - 3) camera type;
 - 4) camera shutter (ms);
 - 5) camera gain (dB);
 - 6) frame rate (frames/s);
 - 7) number of processed frames;
 - 8) number of valid tracks;
- k) results:
 - 1) graphical particle size distribution in either histogram with variable bin widths or cumulative S-curve format;
 - 2) image/frame from one video;
 - 3) D10 (nm) - 10 % of cumulative distribution by number;
 - 4) D50 (nm) - 50 % of cumulative distribution by number;
 - 5) D90 (nm) - 90 % of cumulative distribution by number;
 - 6) average particle size with SD and CV;
 - 7) measured particle number concentrations investigated;
 - 8) particle number concentration from user defined integrated range of sizes with and without the diluent particle number concentration being subtracted;
 - 9) kinetic process rate information if measured;
 - 10) measurement uncertainty;
- l) all operating details not specified in this document, or regarded as optional, together with details of any incident that can have influenced the result(s);
- m) the name(s), function(s) and signature(s) or equivalent identification of person(s) authorizing the test report.

Operators should record all other relevant evaluation and measurement parameters. These, however, do not have to be added to the test report

Annex A (informative)

Theory

This annex gives the derivation of the n -dimensional diffusion coefficient that is used^[13] to evaluate the size of tracked particles. By definition, a particle flux through point, length or an area per unit time is given by $J(x_n, t)$.

$$J(x_n, t) = c(x_n, t)v(x_n, t) \quad (\text{A.1})$$

where x_n is the set of coordinates in n dimensions ($n=2$ for diffusion in the plane of 2D and $n=3$ for movement in x , y and z directions). Statistical mechanics gives the chemical potential which is related to local probability density (or local concentration) as:

$$\mu(x_n, t) = \mu_0 + k_B T \ln \left(\frac{c(x_n, t)}{c_0} \right) \quad (\text{A.2})$$

where μ_0 and c_0 are constants. The variation of chemical potential in space creates a force on the particles given by general differential Navier-Stokes equation:

$$F = -\nabla \mu(x_n, t) = -\frac{k_B T}{c(x_n, t)} \nabla c(x_n, t) \quad (\text{A.3})$$

Since the velocity of particles $v(x_n, t)$ is proportional to the force applied in the regime of low Reynolds numbers for the liquid, the $v(x_n, t) = \sigma F(x_n, t)$ where constant σ is the mobility of particles.

For spherical particles with diameter d , σ is given by the inverse of the Stokes drag:

$$\sigma = \frac{1}{3\pi\eta d} \quad (\text{A.4})$$

According to Fick's law $J(x_n, t) = -D\nabla c(x_n, t)$ where the diffusion constant D is given by:

$$D = \frac{k_B T}{3\pi\eta d} \quad (\text{A.5})$$

Assuming that, during a normal diffusion process, particles cannot be created or destroyed, the flux of particles into a volume shall be equal to that out of the volume. This leads to a continuity formula:

$$\frac{\partial}{\partial t} c(x_n, t) + \nabla J(x_n, t) = 0 \quad (\text{A.6})$$

This therefore leads to the following differential formula:

$$\frac{\partial}{\partial t} c(x_n, t) = \nabla (D\nabla c(x_n, t)) \quad (\text{A.7})$$

Assuming that there is no spatial variation of the diffusion constant D , the diffusion formula is recovered:

$$\frac{\partial}{\partial t} c(x_n, t) = D\nabla^2 c(x_n, t) \quad (\text{A.8})$$

The above diffusion formula has two main features: it is linear and it is separable. This means that the diffusion processes in three orthogonal directions are independent of each other and the solution to the formula can be written as:

$$c(x_n, t) = X(x) \cdot Y(y) \cdot Z(z) \cdot T(t) \quad (\text{A.9})$$

By substituting for $c(x_n, t)$ in the diffusion formula one obtains a separable form for all four dimensions.

$$\frac{\partial}{\partial t} c = D \left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2} \right) \quad (\text{A.10})$$

This leads to the separate form:

$$\frac{1}{DT} \frac{\partial T}{\partial t} = \left(\frac{1}{X} \frac{\partial^2 X}{\partial x^2} + \frac{1}{Y} \frac{\partial^2 Y}{\partial y^2} + \frac{1}{Z} \frac{\partial^2 Z}{\partial z^2} \right) \quad (\text{A.11})$$

This form can be further split to demonstrate that the left-hand side and the right-hand side of this formula can only be equal if they are both equal to the same constant.

$$\frac{1}{DT} \frac{\partial T}{\partial t} = - (m_1^2 + m_2^2 + m_3^2) \quad (\text{A.12})$$

This allows the whole [Formula \(A.11\)](#) to be rewritten in a linear operator form:

$$\mathfrak{S} = \frac{\partial}{\partial t} - D \nabla^2 \text{ with } \mathfrak{S}[c] = 0 \quad (\text{A.13})$$

By using the main property of linearity, one can represent the solution to this formula as a superposition of all solutions of the formula. This allows us to use Fourier transform and Green's function methods to show that the diffusion in three dimensions is simply the simultaneous 1-dimensional diffusion process in three orthogonal directions. This statement has the impact on the evaluation of the statistics of particle diffusion and it can be shown that in n dimensions, the root-mean-square (RMS) motion of the particles can be represented as:

$$\overline{(x)} = \sqrt{\langle \bar{x}^2 \rangle - \langle \bar{x} \rangle^2} = \sqrt{2nDt} \quad (\text{A.14})$$

where averages are ensemble averages, assuming same particles are being observed. The fact that the time average over a given period is equal to the space average, which is what the result of PTA measurements is meant to be is based on the theorem by George Birkhoff.^[12] In general, the time average and space average can be different, but if the transformation is ergodic and the measure is invariant, then the time average is equal to the space average almost everywhere. In 2017, Hartman and Kirby^[10] demonstrated for the PTA measurement that the time averaging over $t \rightarrow \infty$ is equivalent to ensemble averaging for $N \rightarrow \infty$.

This means that for every unrestricted diffusion dimension there is a $\sqrt{2Dt}$ contribution to the RMS value of the particle position. In most PTA measurements, the positions of particles in the x and y directions are tracked (in 2D). The RMS value is therefore 2D. Note that the movement of the particles in the sample is 3D and it is only the projection of that movement that is recorded during the tracking. Evaluating the RMS positions independently in x and y directions allows one to reconstruct the vertical component under the assumption that the diffusion movement and particle statistics is equivalent in all three dimensions, as it was proven in [Annex A](#).

$$d_x = \frac{k_B T}{3\pi\eta D_x} = \frac{2k_B T t}{3\pi\eta \overline{(x)}^2} \quad (\text{A.15})$$

where d_x is the spherical diameter of the tracked particle due to the x direction of movement. The same result (d_y) should be available from the movement in the y direction. Again, the average is ensemble average, assuming the same particles are being observed.

The assumption of the spherical symmetry of the particle can set a limitation on the applicability of the above derivation to the sizing of particles with aspect ratios different from 1. The statistics of movement of fibres or plates can be different from that of a spherical particle due to the Stokes' drag.

The derivation also assumes that the particles are not created or destroyed during the measurement time, t . This can therefore impose a limitation on the studies of agglomeration, aggregation or dissolution where particles change shape, size or disappear altogether. The key is that the particle state (size or shape) should not change during the observation time, t .

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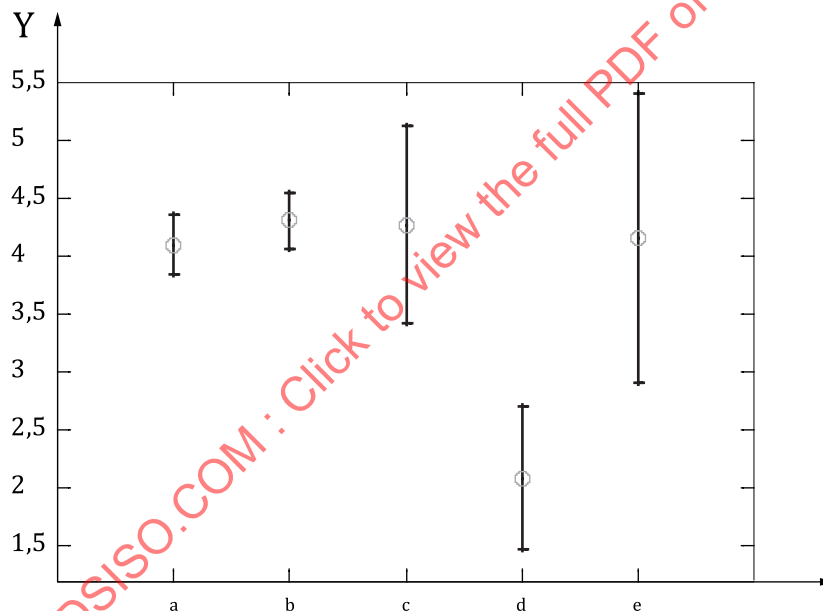
Annex B

(informative)

Comparative study of number concentration evaluation of gold nanoparticles in suspension

The results of the PTA were compared to other established particle counting and sizing techniques in a comprehensive study.^[13] In this study, small-angle X-ray scattering (SAXS) and single particle inductively coupled plasma mass spectroscopy (spICP-MS) were compared to PTA, differential centrifugal sedimentation (DCS), ultraviolet visible spectroscopy (UV-vis), and electrospray-differential mobility analysis with a condensation particle counter (ES-DMA-CPC) for the purpose of particle number concentration measurements and evaluation of relevant uncertainties (see [Figure B.1](#)). An attempt was made to measure gold nanoparticles with nominal diameters of 5 nm, 10 nm, 30 nm, 100 nm, 250 nm and 500 nm, however the samples larger than 100 nm displayed strong sedimentation limiting their use for most of the techniques used in this study. A 5 nm sample was below the LOD of several techniques including that of PTA.

The data was obtained in the VAMAS TW34 Project 10.^[14]



Key

Y $C/\text{ml}^{-1} \times 10^9$

a spICPMS

b PTA

c UV-vis

d DCS

e nominal

Figure B.1 — Results of the VAMAS TW34 Project 10 on number counting of 100 nm gold particles

The study found that while PTA can detect 10 nm gold particles, it was not possible to measure their concentration reliably. Particles of 30 nm and 100 nm were analysed by this technique. The following table compares PTA with reference methods and shows it agrees with the latter within uncertainties for both samples.

Table B.1 — Number concentration of the gold suspensions

Method	C_{Au10}/mL^{-1} (a)	C_{Au30}/mL^{-1} (b)	C_{Au100}/mL^{-1} (c)
SAXS	$(7,08 \pm 1,13) \cdot 10^{12}$	$(1,85 \pm 0,13) \cdot 10^{11}$	-
spICP-MS	-	$(1,80 \pm 0,14) \cdot 10^{11}$	$(4,10 \pm 0,26) \cdot 10^9$
PTA	-	$(1,78 \pm 0,08) \cdot 10^{11}$	$(4,31 \pm 0,24) \cdot 10^9$
UV-vis	$(7,64 \pm 1,53) \cdot 10^{12}$	$(1,88 \pm 0,38) \cdot 10^{11}$	$(4,27 \pm 0,85) \cdot 10^9$
DCS/DLS	$(8,42 \pm 2,53) \cdot 10^{12}$	$(1,61 \pm 0,48) \cdot 10^{11}$	$(2,08 \pm 0,62) \cdot 10^9$
ES-DMA-CPC	$(9,03 \pm 0,32) \cdot 10^{11}$	$(3,22 \pm 0,12) \cdot 10^{10}$	-
Nominal	$(4,84 \pm 1,45) \cdot 10^{12}$	$(1,76 \pm 0,53) \cdot 10^{11}$	$(4,16 \pm 1,25) \cdot 10^9$

[Table B.1](#) shows the comparison of number concentrations and corresponding standard uncertainties acquired with reference for:

- a) Au10;
- b) Au30;
- c) Au100 samples.

Only “nominal” did not have a full uncertainty budget.