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Nanotechnologies — Measurements of particle size and shape distributions by transmission electron microscopy

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National foreword

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Nanotechnologies — Measurements of particle size and shape distributions by transmission electron microscopy

Nanotechnologies — Détermination de la distribution de taille et de forme des particules par microscopie électronique à transmission

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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 documents 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 229, *Nanotechnologies.*

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.

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Introduction

Characterization procedures for nanoparticles often include, but are not limited to, size, shape, surface structure (or texture), and surface chemistry. These measurements, combined with phase information, such as crystalline phase, constitute the morphology of the material. This document focuses on two attributes of morphology, size and shape distributions, for discrete, agglomerated and aggregated nano-objects (materials with at least one dimension in the nanoscale, 1 nm < a length dimension < 100 nm). Transmission electron microscopy, a standard tool for measurements on the nanoscale, provides two-dimensional images of particle projections. This generic workflow for measuring and evaluating particle size and shape distributions on the nanoscale includes sample preparation, instrument factors, image capture, particle analysis, data analysis, and reporting. Seven case studies have been included to illustrate how the generic protocol can be applied to different particle morphologies and sample types. Three discrete particle test samples are reported: spheroidal (gold nanospheres), a bimodal mixture of particle sizes (colloidal silicas), and a mixture of particle shapes (gold nanorods and gold nanocubes). Two aggregate test samples are reported: amorphous aciniform aggregates (carbon black) and aggregates of primary crystallites (titania). Measurements methods are also presented for low aspect ratio samples and nanoparticles with specific crystal habits. Several of the case studies are supported by interlaboratory collaborations conducted under the guidelines of the Versailles Project on Advanced Materials and Standards (VAMAS) for interlaboratory comparisons (ILCs)[42].

Three types of size and shape descriptors are considered. Size descriptors include those determined by linear or areal measurements. Shape descriptors include elongational descriptors, such as ratios of two length descriptors, and ruggedness descriptors, which represent surface irregularities.

The protocol emphasizes qualitative and quantitative analysis of data quality by the user. Qualitative comparisons of datasets include determining the similarity or differences between single descriptor means or multivariate means. Quantitative comparisons of datasets are based on difference or similarities between the parameters of reference models fitted to descriptor distributions. At least two parameters (mean and spread) and their uncertainties are needed to define a descriptor distribution. In some cases, these two quantitative parameters and their uncertainties may not be sufficient for characterization of particle size and shape distributions. Data visualization techniques, such as residual deviation and quantile plots, and data correlations, such as pairs of size and shape descriptors or fractal analysis, can provide additional ways to evaluate and differentiate test samples. Taken together, qualitative and quantitative quality metrics plus visualization and correlation tools permit users to tailor the protocol to their qualitative and quantitative quality targets.

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Nanotechnologies — Measurements of particle size and shape distributions by transmission electron microscopy

1 Scope

This document specifies how to capture, measure and analyse transmission electron microscopy images to obtain particle size and shape distributions in the nanoscale.

This document broadly is applicable to nano-objects as well as to particles with sizes larger than 100 nm. The exact working range of the method depends on the required uncertainty and on the performance of the transmission electron microscope. These elements can be evaluated according to the requirements described in this document.

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 9276-3, *Representation of results of particle size analysis — Part 3: Adjustment of an experimental curve to a reference model*

ISO 9276-6:2008, *Representation of results of particle size analysis — Part 6: Descriptive and quantitative representation of particle shape and morphology*

ISO 29301, *Microbeam analysis — Analytical electron microscopy — Methods for calibrating image magnification by using reference materials with periodic structures*

3 Terms, definitions and symbols

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

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1 Core terms — Particles

3.1.1
nano-object
discrete piece of material with one, two or three external dimensions in the *nanoscale* (3.1.2)

[SOURCE: ISO/TS 80004-2:2015, 2.2]

3.1.2
nanoscale
length range approximately from 1 nm to 100 nm

[SOURCE: ISO/TS 80004-1:2015, 2.1, modified — Note 1 to entry has been deleted.]

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3.1.3
particle
minute piece of matter with defined physical boundaries

[SOURCE: ISO 26824:2013, 1.1, modified — Notes 1, 2 and 3 to entry have been deleted.]

3.1.4
constituent particle
identifiable, integral component of a larger *particle* (3.1.3)

[SOURCE: ISO/TS 80004-2:2015, 3.3, modified — Note 1 to entry has been deleted.]

3.1.5
agglomerate
collection of weakly or medium strongly bound *particles* (3.1.3) where the resulting external surface area is similar to the sum of the surface areas of the individual components

Note 1 to entry: The forces holding an agglomerate together are weak forces, for example van der Waals forces or simple physical entanglement.

Note 2 to entry: Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-2:2015, 3.4]

3.1.6
aggregate
particle (3.1.3) comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components

Note 1 to entry: The forces holding an aggregate together are strong forces (for example, covalent bonds) or those resulting from sintering or complex physical entanglement.

Note 2 to entry: Aggregates are also termed secondary particles and the original source particles are termed primary particles.

Note 3 to entry: Entries 3.1.6 to 3.1.10 define elements of agglomerates and aggregates, some of which are illustrated in Figure 1. Constituent particles in an aggregate are tightly fused into a discrete entity (the aggregate), while the constituent particles in an agglomerate are weakly bound and generally easily dispersed under shear or mechanical stress.

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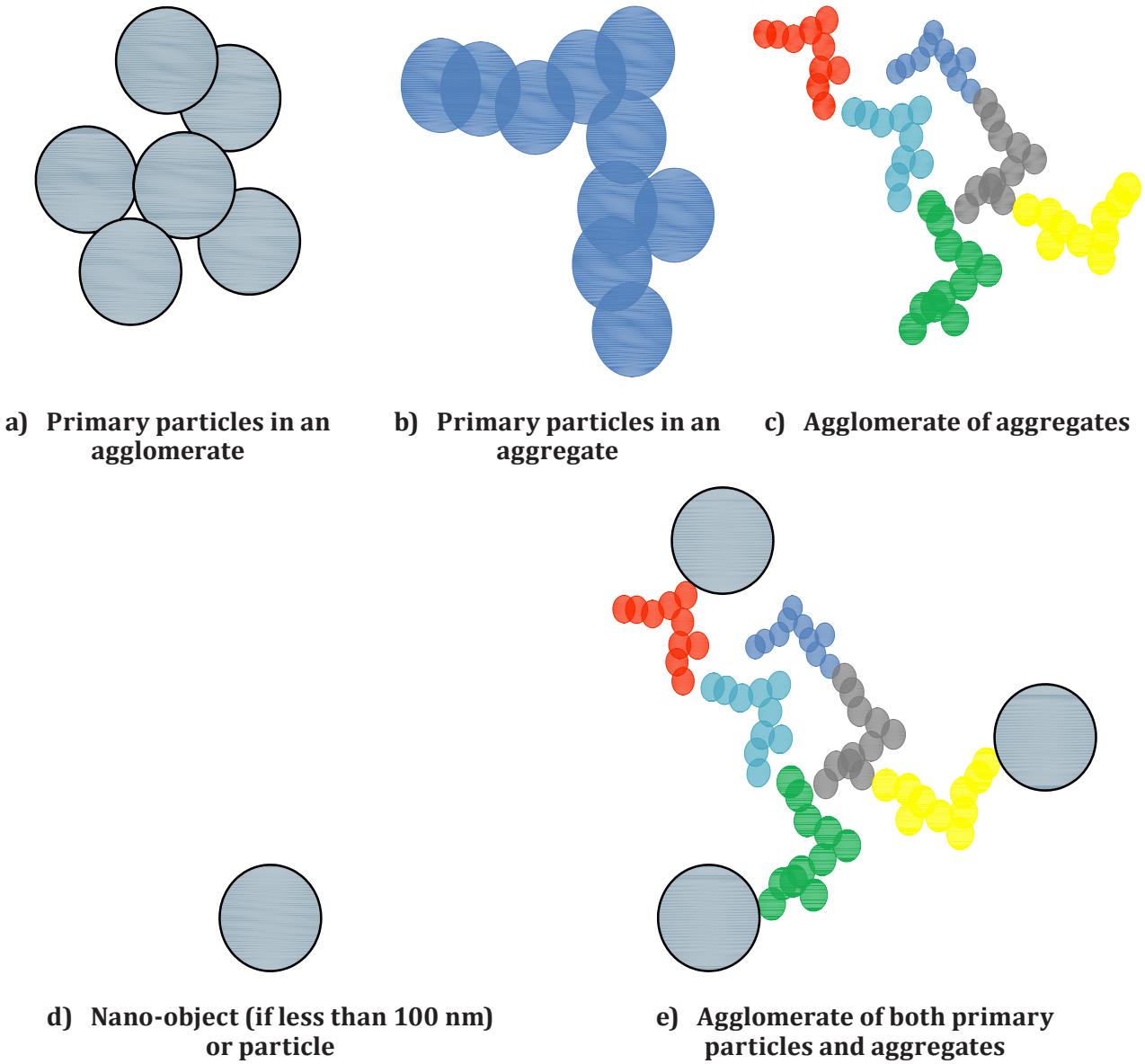

Figure 1 — Schematic showing elements of agglomerates and aggregates

[SOURCE: ISO/TS 80004-2:2015, 3.5, modified — In the definition, "may be significantly smaller" has replaced "is significantly smaller" and "calculated" has been added before "surface areas". In Note 1 to entry, "ionic bonds" in the example and the final phrase "or otherwise combined former primary particles" have been deleted. Note 3 to entry and Figure 1 have been added.]

3.1.7
nanoparticle
nano-object (3.1.1) with all three external dimensions in the *nanoscale* (3.1.2) where the lengths of the longest and shortest axes of the nano-object do not differ significantly

[SOURCE: ISO/TS 80004-2:2015, 4.4, modified — "three" has been added and Note 1 to entry has been deleted.]

3.1.8
nanorod
solid *nanofibre* (3.1.9)

[SOURCE: ISO/TS 80004-2:2015, 4.7]

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3.1.9
nanofibre
nano-object (3.1.1) with two similar external dimensions in the *nanoscale* (3.1.2) and the third dimension significantly larger

[SOURCE: ISO/TS 80004-2:2015, 4.5, modified — "similar" has been added and Notes 1, 2 and 3 to entry have been deleted.]

3.1.10
nanophase
physically or chemically distinct region or collective term for physically distinct regions of the same kind in a material with the discrete regions having one, two or three dimensions in the *nanoscale* (3.1.2)

Note 1 to entry: *Nano-objects* (3.1.1) embedded in another phase constitute a nanophase.

3.1.11
nanodispersion
material in which *nano-objects* (3.1.1) or a *nanophase* (3.1.10) are dispersed in a continuous phase of a different composition

[SOURCE: ISO/TS 80004-4:2011, 2.14]

3.1.12
particle size
x
dimension of a *particle* (3.1.3) 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, an area or a volume.

Note 2 to entry: The symbol x is used denote linear particle size. However, it is recognized that the symbol d is also widely used. Therefore, the symbol x may be replaced by d.

[SOURCE: ISO 9276-1:1998, 4.2, modified — Converted into a term and definition entry.]

3.1.13
particle size distribution
distribution of *particles* (3.1.3) as a function of *particle size* (3.1.12)

[SOURCE: ISO/TS 80004-6:2013, 3.1.2, modified — Note 1 to entry has been deleted.]

3.1.14
particle shape
external geometric form of a *particle* (3.1.3)

Note 1 to entry: Shape description requires two scalar descriptors, i.e. length and spread.

[SOURCE: ISO/TS 80004-6:2013, 3.1.3, modified — Note 1 to entry has been added.]

3.1.15
particle shape distribution
distribution of a specific *particle shape* (3.1.14) descriptor for a sample population

3.2 Core terms — Image capture and analysis

3.2.1
field of view
field that is viewed by the viewing device

[SOURCE: ISO 13322-1:2014, 3.1.6, modified — Note 1 to entry has been deleted.]

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3.2.2
measurement frame
selected area from the *field of view* (3.2.1) in which *particles* (3.1.3) are sized and counted for image analysis

[SOURCE: ISO 13322-1:2014, 3.1.10]

3.2.3
binary image
digitized image consisting of an array of *pixels* (3.2.4), each of which has a value of 0 or 1, whose values are normally represented by dark and bright regions on the display screen or by the use of two distinct colours

[SOURCE: ISO 13322-1:2014, 3.1.2]

3.2.4
pixel
smallest element of an image that can be uniquely processed, and is defined by its spatial coordinates and encoded with colour values

[SOURCE: ISO 12640-2:2004, 3.6, modified — Note 1 to entry has been deleted.]

3.2.5
pixel-resolution
number of imaging *pixels* (3.2.4) per unit distance of the detector

[SOURCE: ISO 29301:2017, 3.24, modified — Note 1 to entry has been deleted.]

3.2.6
pixel count
total number of *pixels* (3.2.4) per file, length, or area depending on the unit used

[SOURCE: ISO 19262:2015, 3.191]

3.2.7
micrograph
record of an image formed by a microscope

[SOURCE: ISO 10934-1:2002, 2.94]

3.2.8
artefact
artifact
unwanted distortion or added feature in measured data arising from lack of idealness of equipment

[SOURCE: ISO 18115-2: 2013, 5.6]

3.3 Core terms — Statistical symbols and definitions

3.3.1
coefficient of variation
C_v
ratio of the standard deviation to the arithmetic mean

Note 1 to entry: It is commonly reported as a percentage.

Note 2 to entry: For example, the coefficient of variation for a sample mean may be represented by:

$$c_v = \frac{s \cdot 100}{\bar{x}}$$

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where $\bar{x}$ is the descriptor's mean and s is the descriptor's standard deviation for several datasets. These "grand statistics" are used to evaluate descriptor data for interlaboratory comparisons.

[SOURCE: ISO 27448:2009, 3.11, modified — Notes 1 and 2 to entry have been added.]

3.3.2
standard error of estimation
σ_{est}
measure of dispersion of the dependent variable (output) about the least-squares line obtained by curve fitting or regression analysis

Note 1 to entry: The standard error of estimation may be determined by:

$$\sigma_{est} = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \bar{y})^2}{n-k}}$$

where

n is the number of data points;

k is the number of coefficients in the equation.

Note 2 to entry: The standard error of the mean may be determined by:

$$\sigma_{est,\bar{x}} = \frac{s}{\sqrt{n}}$$

Note 3 to entry: The standard error is the standard deviation of the sampling distribution of a statistic. The example is for a sample mean. Standard error of the mean is an estimate of how close the sample mean is to the population mean. This value decreases as the sample size increases.

[SOURCE: ISO 772:2011, 7.31, modified — The admitted term "residual standard deviation" has been deleted. Notes 1, 2 and 3 to entry have replaced the original Notes 1 and 2 to entry.]

3.3.3
relative standard error
RSE
standard error divided by its statistic

Note 1 to entry: It is expressed as a percentage.

Note 2 to entry: For example, the relative standard error of the mean is:

$$\mathrm{RSE}_{\bar{x}} = \frac{100 \cdot \sigma_{est,x}}{\bar{x}}$$

3.3.4
measurement bias
estimate of a systematic measurement error

Note 1 to entry: Bias is present when a statistic is systematically different than the population parameter it is estimating.

$\Delta m = |c_m - c_{crm}|$: the absolute difference between the mean measured value and the certified value. Bias of the normal mean of this study would be the average of the individual absolute differences between a measured mean and the certified reference material mean.

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$$\text{bias} = \frac{\sum_{i=1}^{n} \Delta_{m,i}}{n}$$

[SOURCE: ISO/IEC Guide 99:2007, 2.18, modified — Notes 1 and 2 to entry have been added.]

3.3.5
residual
difference between the observed value of the response variable and the estimated value of the response variable

3.3.6
residual standard deviation
description of the scatter of the information values about the calculated regression line

Note 1 to entry: It is a figure of merit, describing the *precision* (3.5.5) of the calibration.

[SOURCE: ISO 8466-1:1990, 2.5]

3.3.7
quantile plot
graphical method of comparing two distributions where the quantiles of the empirical (data) distribution are plotted on the y-axis while the quantiles of the theoretical (reference) distribution with the same mean and variance as the empirical distribution are plotted on the x-axis

3.4 Core terms — Measurands

3.4.1
measurand
quantity intended to be measured

[SOURCE: ISO/IEC Guide 99:2007, 2.3, modified — The notes have been deleted.]

3.4.2
image descriptor
descriptor extracted from one image

[SOURCE: ISO/IEC 15938-13:2015, 2.1]

3.4.3
Feret diameter
distance between two parallel tangents on opposite sides of the image of a *particle* (3.1.3)

Note 1 to entry: The *maximum Feret diameter* (3.4.4) is used in this document.

[SOURCE: ISO 13322-1:2014, 3.1.5, modified — Note 1 to entry has been added.]

3.4.4
maximum Feret diameter
maximum length of an object whatever its orientation

[SOURCE: ISO/TR 945-2:2011, 2.1, modified — Note 1 to entry has been deleted.]

3.4.5
minimum Feret diameter
minimum length of an object whatever its orientation

3.4.6
perimeter
total length of the object contour

[SOURCE: ISO/TR 945-2:2011, 2.3]

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3.4.7
equivalent circular diameter
diameter of a circle having the same area as the projected image of the *particle* (3.1.3)

EXAMPLE The ecd is:

$$\text{ecd} = \sqrt{\frac{4 \cdot A}{\pi}}$$

where A is the area of the particle.

[SOURCE: ISO 13322-1:2014, 3.1.1, modified — Note 1 to entry has been deleted and the example has been added.]

3.4.8
equivalent perimeter diameter
d_{epd}
diameter of a circle having the same *perimeter* (3.4.6) as the projected image of the *particle* (3.1.3)

Note 1 to entry: It may be calculated as follows:

$$d_{\text{epd}} = \frac{P}{\pi}$$

where P is the length of the perimeter.

3.4.9
convex hull
smallest convex set containing a given geometric object

[SOURCE: ISO 19123:2005, 4.1.2]

3.4.10
aspect ratio
ratio of the *minimum* (3.4.5) to the *maximum Feret diameter* (3.4.4)

Note 1 to entry: It may be calculated, for example, as follows:

$$\text{aspect ratio} = \frac{x_{\text{Fmin}}}{x_{\text{Fmax}}}$$

where

x_{Fmin} is the minimum Feret diameter;

x_{Fmax} is the maximum Feret diameter.

[SOURCE: ISO 26824:2013, 4.5, modified — Note 1 to entry has replaced the original Notes 1 and 2 to entry.]

3.4.11
ellipse ratio
ratio of the lengths of the axes of the Legendre ellipse of inertia

Note 1 to entry: For example, the ellipse ratio can be the ratio of the minor and major axes of the Legendre ellipse fitted to the *particle* (3.1.3); elliptical shape factor, thus:

$$\text{ellipse ratio} = \frac{x_{\text{Lmin}}}{x_{\text{Lmax}}}$$

where

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x_{Lmin} is the length of the minor axis of Legendre ellipse of inertia;

x_{Lmax} is the length of the major axis of Legendre ellipse of inertia.

[SOURCE: ISO 26824:2013, 4.4, modified — Note 1 to entry has been replaced.]

3.4.12
extent
bulkiness
ratio of particle area to the product of the *Feret* (3.4.3) and the *minimum Feret diameters* (3.4.6)

Note 1 to entry: For example, the extent may be calculated as:

$$\text{extent} = \frac{A}{x_{\mathrm{Fmin}} \cdot x_{\mathrm{Fmax}}}$$

where

x_{Fmin} is the minimum Feret diameter;

x_{Fmax} is the maximum Feret diameter.

[SOURCE: ISO 9276-6:2008, 8.1.3, modified — Converted into a term and definition entry. The definition has been added.]

3.4.13
compactness
degree to which the projection area A of the *particle* (3.1.3) is similar to a circle, considering the overall form of the *particle* (3.1.3) with the *maximum Feret diameter* (3.4.4)

Note 1 to entry: For example, the compactness may be calculated as:

$$\text{compactness} = \frac{\sqrt{\frac{4 \cdot A}{\pi}}}{X_{\mathrm{Fmax}}}$$

where

A is the area of the particle;

x_{Fmax} is the maximum Feret diameter.

[SOURCE: ISO 9276-6:2008, 8.1.3, modified — Converted into a term and definition entry. In the definition, "projection area A of the particle" has replaced "particle (or its projection area)" and "with the maximum Feret diameter" has been added.]

3.4.14
convexity
ratio of the *perimeter* (3.4.6) of the *convex hull* (3.4.9) envelope bounding the *particle* (3.1.3) to its perimeter

Note 1 to entry: For example, the convexity may be calculated as:

$$\text{convexity} = \frac{P_{\mathrm{C}}}{P}$$

where

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P_c is the length of the perimeter of the convex hull (envelope) bounding the particle;

P is the length of the perimeter.

[SOURCE: ISO 9276-6:2008, 8.2, modified — Converted into a term and definition entry. The definition has been added.]

3.4.15
circularity
C
degree to which the projected area of the *particle* (3.1.3) is similar to a circle, based on its *perimeter* (3.4.6)

Note 1 to entry: For example, the circularity may be calculated as:

$$C = \frac{x_a}{x_p} = \sqrt{\frac{4 \cdot \pi \cdot A}{P^2}}$$

where

x_a is the area-equivalent diameter of a particle;

x_p is the perimeter-equivalent diameter of particle;

A is the area of the particle;

P is the length of the perimeter.

[SOURCE: ISO 26824:2013, 4.12, modified — "projected area of the particle" has replaced "projection area of the particle *A*", "based on its perimeter" has replaced "considering the smoothness of its perimeter *P*". The formula and Note 1 to entry have been replaced.]

3.4.16
roundness
square of the *compactness* (3.4.13)

3.4.17
solidity
ratio of the projected area *A* to the area of the *convex hull* (3.4.9) A_C (envelope)

Note 1 to entry: For example, the solidity may be calculated as:

$$\text{solidity} = \frac{A}{A_C}$$

[SOURCE: ISO 26824:2013, 4.13, modified — Note 1 to entry has been added.]

3.5 Core terms — Metrology

3.5.1
repeatability condition of measurement
condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time

[SOURCE: ISO/IEC Guide 99:2007, 2.20, modified — The admitted term "repeatability condition" and the notes have been deleted.]

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3.5.2
intermediate precision condition of measurement
condition of measurement, out of a set of conditions that includes the same measurement procedure, same location, and replicate measurements on the same or similar objects over an extended period of time, but may include other conditions involving changes

[SOURCE: ISO/IEC Guide 99:2007, 2.22, modified — The admitted term "intermediate precision condition" and the notes have been deleted.]

3.5.3
reproducibility condition of measurement
condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects

Note 1 to entry: The different measuring systems may use different measurement procedures.

Note 2 to entry: A specification should give the conditions changed and unchanged, to the extent practical.

[SOURCE: ISO/IEC Guide 99:2007, 2.24, modified — The admitted term "reproducibility condition" has been deleted.]

3.5.4
measurement accuracy
closeness of agreement between a measured quantity value and a true quantity value of a *measurand* (3.4.1)

Note 1 to entry: The concept "measurement accuracy" is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement uncertainty.

Note 2 to entry: The term "measurement accuracy" should not be used for measurement trueness and the term "measurement precision" should not be used for "measurement accuracy", which, however, is related to both these concepts.

Note 3 to entry: "Measurement accuracy" is sometimes understood as closeness of agreement between measured quantity values that are being attributed to the measurand.

[SOURCE: ISO/IEC Guide 99:2007, 2.13, modified — The admitted terms "accuracy of measurement" and "accuracy" have been deleted. In Note 1 to entry, "measurement uncertainty" has replaced measurement error".]

3.5.5
precision
measurement precision
closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

Note 1 to entry: Measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or *coefficient of variation* (3.3.1) under the specified conditions of measurement.

Note 2 to entry: The "specified conditions" can be, for example, *repeatability conditions of measurement* (3.5.1), *intermediate precision conditions of measurement* (3.5.2), or *reproducibility conditions of measurement* (3.5.3) (see ISO 5725-3:1994).

Note 3 to entry: Measurement precision is used to define measurement repeatability, intermediate measurement precision, and measurement reproducibility.

Note 4 to entry: Sometimes "measurement precision" is erroneously used to mean *measurement accuracy* (3.5.4).

[SOURCE: ISO/IEC Guide 99:2007, 2.15, modified — "precision" has been made the preferred term and "measurement precision" the admitted term.]

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3.5.6
combined standard measurement uncertainty
standard measurement uncertainty that is obtained using the individual standard measurement uncertainties associated with the input quantities in a measurement model

Note 1 to entry: In cases of correlations of input quantities in a measurement model, covariances shall be taken into account when calculating the combined standard measurement uncertainty; see also ISO/IEC Guide 98-3:2008, 2.3.4.

3.5.7
expanded measurement uncertainty
U
product of a *combined standard measurement uncertainty* (3.5.6) and a factor larger than the number one

Note 1 to entry: The factor depends upon the type of probability distribution of the output quantity in a measurement model and on the selected coverage probability.

Note 2 to entry: The term "factor" in this definition refers to a coverage factor.

Note 3 to entry: Expanded measurement uncertainty is termed "overall uncertainty" in paragraph 5 of Recommendation INC-1 (1980) (see the GUM) and simply "uncertainty" in IEC documents.

[SOURCE: ISO/IEC Guide 99:2007, 2.35, modified — The admitted term "expanded uncertainty" has been deleted. The symbol "*U*" has been added.]

3.5.8
Type A evaluation of measurement uncertainty
evaluation of a component of measurement uncertainty by a statistical analysis of measured quantity values obtained under defined measurement conditions

[SOURCE: ISO/IEC Guide 99:2007, 2.28, modified — The admitted term "Type A evaluation" and the notes have been deleted.]

3.5.9
Type B evaluation of measurement uncertainty
evaluation of a component of measurement uncertainty determined by means other than a *Type A evaluation of measurement uncertainty* (3.5.8)

[SOURCE: ISO/IEC Guide 99:2007, 2.29, modified — The admitted term "Type B evaluation", the note and the examples have been deleted.]

3.5.10
reference material
RM
material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

[SOURCE: ISO Guide 30:2015, 2.1.1, modified — The notes to entry have been deleted.]

3.5.11
certified reference material
CRM
reference material (RM) (3.5.10) characterized by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

[SOURCE: ISO Guide 30:2015, 2.1.2, modified — The notes to entry have been deleted.]

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3.6 Core terms — Transmission electron microscopy

3.6.1
transmission electron microscopy
TEM
method that produces magnified images or diffraction patterns of the sample by an electron beam, which passes through the sample and interacts with it

[SOURCE: ISO/TS 80004-6:2013, 3.5.6]

3.6.2
scanning transmission electron microscopy
STEM
method that produces magnified images or diffraction patterns of the sample by a finely focused electron beam, scanned over the surface and which passes through the sample and interacts with it

[SOURCE: ISO/TS 80004-6:2013, 3.5.7, modified — The notes to entry have been deleted.]

3.6.3
accelerating voltage
potential difference applied between the filament and anode in order to accelerate the electrons emitted from the source

[SOURCE: ISO 22309:2011, 3.3, modified — Note 1 to entry has been deleted.]

3.6.4
bright-field transmission electron microscopy
bright-field TEM
TEM technique of electron illumination and imaging in which the direct electron beam passes through the sample and the image is formed only by the transmitted wave, by selecting the wave using an objective aperture on the back focal plane

Note 1 to entry: Generally, the portions of the sample that are thicker or that have a higher atomic number (Z) appear darker against a brighter background. In this mode, the contrast, when considered classically, is formed directly by occlusion and absorption of electrons in the sample. Thicker regions of the sample, or regions with a higher atomic number, will appear dark, while regions with no sample in the beam path will appear bright, hence the term "bright-field".

Note 2 to entry: This will be included in a vocabulary on analytical electron microscopy, which is under preparation by ISO/TC 202/SC 1.

[SOURCE: ISO/TS 10797:2012, 3.3]

3.6.5
dark-field transmission electron microscopy
dark-field TEM
TEM technique of electron illumination and imaging in which the direct electron beam passes through the sample and the image is formed only by diffracted wave, by selecting the wave using an objective aperture on the back focal plane

Note 1 to entry: Crystalline parts of the sample disperse the electrons of the direct beam into discrete locations in the back focal plane. By the placement of apertures in the back focal plane, i.e. the objective aperture, desired portions of the reflections can be selected, thus only those parts of the sample that are causing the electrons to scatter to the selected reflections will be imaged. If the selected reflections do not include the unscattered beam, then the image will appear dark wherever no sample scattering to the selected peak is present – hence the term "dark-field".

Note 2 to entry: Modern TEMs are often equipped with sample holders that allow the user to tilt the sample to obtain specific diffraction conditions. The wave that caused scattering and reflection (for example, Bragg reflection) in a crystalline sample will form a dark-field image by selecting a specific diffraction wave through objective apertures placed on the back focal plane of objective lens.

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Note 3 to entry: High-angle annular dark-field imaging (HAADF) is highly sensitive to variations in the atomic number of atoms in the sample and produces so-called *Z*-contrast images, which yield useful information on the presence of metals on nanotubes and catalyst residues, even when these small metal *particles* (3.1.3) are imbedded within amorphous carbon or catalyst support while otherwise invisible in bright-field imaging mode.

Note 4 to entry: This will be included in a vocabulary on analytical electron microscopy, which is under preparation by ISO/TC 202/SC 1.

[SOURCE: ISO/TS 10797:2012, 3.4]

3.7 Statistical symbols, measurands and descriptors

3.7.1 Statistical symbols

C_v coefficient of variation

$q(x)$ differential distribution (density distribution)

$Q(x)$ cumulative distribution

p statistic returned from analysis of variance or bivariate analysis

RSE relative standard error

s standard deviation of the descriptor, x, or the descriptor parameter

s^2 variance of the descriptor or the descriptor parameter

σ_{est} standard error of estimation

x value of the descriptor or descriptor parameter

$\bar{x}$ mean of the descriptor or descriptor parameter

$X(x)$ transform of the descriptor (e.g. $X(x) = x$ for a normal distribution, $X(x) = \ln x$ for a lognormal distribution)

$Y(Q)$ transform of Q plotted on the y-axis, i.e. Y = inverse of the distribution. Other transforms are listed in ISO 9276-3:2008, Table 1.

NOTE Quantity measures and types of distributions are defined in ISO 9276-1:1998.

3.7.2 Measurands and descriptors

NOTE Measurands or descriptors for size and shape measurements can be directly proportional to length (Category 1) or proportional to length squared (Category 2).

3.7.2.1 Size descriptors and symbols — Category 1

P length of the perimeter

P_c length of the perimeter of the convex hull (envelope) bounding the particle

x_a area-equivalent diameter of a particle [also known as “equivalent circle diameter (ECD)”]

x_{Fmax} maximum Feret diameter; corresponds to the “length” of the particle

x_{Fmin} minimum Feret diameter; corresponds to the “breadth” of the particle

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x_{Lmax} length of the major axis of Legendre ellipse of inertia,

x_{Lmin} length of the minor axis of Legendre ellipse of inertia

x_p perimeter-equivalent diameter of particle

$$x_p = \frac{P}{\pi}$$

3.7.2.2 Size descriptors and symbols — Category 2

A area of the particle

A_c area of the convex hull (envelope) bounding the particle

L^2 area computed by the multiplication of two lengths

NOTE Volume-equivalent diameter, x_V, and surface-equivalent diameter, x_S, are not included as they are three-dimensional descriptors.

3.7.2.3 Shape descriptor symbols — Category 1

AR aspect ratio

ER ellipse ratio

NOTE 1 ISO elongation shape descriptors for image analysis are defined such that their values scale from zero to one, rather than the inverse definition, which is quite common throughout the rest of the literature. The ISO definitions limit the descriptor variable range, making probability and cumulative density plots more compact.

C circularity

NOTE 2 ISO elongation shape descriptors for image analysis are defined such that their values scale from zero to one, rather than the inverse definition, which is quite common throughout the rest of the literature. The ISO definitions limit the descriptor variable range, making probability and cumulative density plots more compact.

4 Stakeholder needs for TEM measurement procedures

This document addresses the following needs of the user community for size and shape distributions:

a) commercially available materials should be measured;

b) the capture, measurement and analysis of the data should be automated as much as practical;

c) workflows should facilitate decisions about image capture, particle measurement and data analysis;

d) case study examples should illustrate:

   1) the identification of touching particles, unselected particles and artefacts;

   2) the selection of size and shape descriptors with high repeatability and reproducibility;

   3) the estimation of measurement uncertainties for distribution parameters, i.e. mean and spread;

   4) the differentiation between samples using size and shape descriptors;

   5) the use of data visualization tools to augment measurement uncertainty results.

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Figure 2 illustrates the workflow for the measurement of particle size and shape distributions that addresses stakeholder needs. It is important to identify any particles that are not targets of the investigation. It may also be necessary or preferred to remove touching particles. Data repeatability, intermediate precision or reproducibility should be quantitated, depending on the application. Fitting distributions to data provides values for the mean and spread of reference distributions, for which measurement uncertainties can be estimated. In some cases, the individual numerical results (mean, spread and their uncertainties) alone cannot differentiate between models and/or data. Visualization tools and correlations can provide additional information about the test sample.

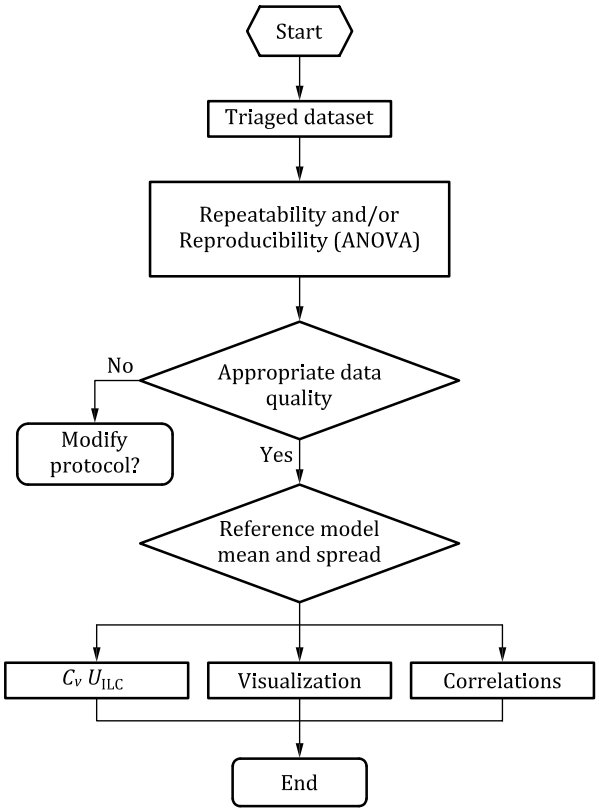

Figure 2 — Workflow for measurement of particle size and shape distributions by TEM

5 Sample preparation

5.1 General

The procedural steps (sample preparation, instrument set-up and calibration and image capture) are highly interdependent. All contribute to generating high quality datasets of size and shape descriptors. Statistical tools, such as analysis of variance, fitting distribution models to the data and bivariate analysis, can help the researcher tune the entire procedure to provide the accuracy or measurement uncertainty needed for the specific sample.

Often, a large number of nanoparticles will need to be imaged and analysed to obtain a particle size distribution. Particle analysis time can be reduced and data quality can be improved when image analysis software is used. When procedures are developed for unknown samples, it can be useful to go through the entire process to determine which factors are critical for the sample at hand. Analysis will be facilitated when image analysis software is used. Both commercial and open source software are available. The images shall be of sufficient quality such that individual particles can be resolved and their dimensions measured. An important objective of sample preparation is to generate uniform distributions of particles across mounting substrates.

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5.2 Sample sources

Samples will typically be supplied as powders or suspensions. Powder samples are often dispersed in liquids prior to their deposition on a substrate for TEM evaluation. Suspensions often need to be diluted in order to image discrete particles. Key sample preparation objectives are:

— use a representative sample of the powder or liquid;

— minimize agglomeration in the dispersion used for mounting the sample;

— minimize particle-particle associations on the support;

— distribute the sample uniformly across the support (see ISO 9276-3);

— select a support that enhances the contrast between particle and background.

Health effect information of various nanoparticles is still being assembled. It is likely that some nanoparticles are hazardous to health while others may not be hazardous or may not cause adverse health effects following exposure. Sample preparation should be done wearing appropriate personal protective equipment, including disposable gloves, safety glasses, laboratory coats, filter respirators, etc. Sample preparation should be carried out in a vented fume hood equipped with suitable air filters.

5.3 Use a representative sample

5.3.1 General

During manufacturing processes for engineered nanomaterials, the nanoparticles may exist in several colloidal states. For example, chemical vapour deposition or physical vapour deposition processes can generate “seeds” that can become nanoparticle aerosols. Rapidly cooling liquid nanoparticles can solidify, fusing together at their surfaces prior to solidification, yielding aggregated products that have dimensions greater than one micrometre but originate from many particles. Since the original primary particles are often not recognizable in the aggregate, the term “constituent particle” is used to describe the particles that can be discerned inside an aggregate.

5.3.2 Powder samples

Powder samples need to be taken carefully, as material can redistribute under the vibrations and forces of transportation. The samples also need to be handled carefully, particularly if they are aggregated and can fracture with mechanical action. ISO 14887:2000 and ISO 14488:2007 provide guidance on how to take and handle powder samples. Most powders and nanoparticles will be negatively charged in the gas phase, so loading dry samples on substrate surfaces, which are also usually negatively charged, often results in large agglomerates that are difficult to image. Powder samples are usually prepared for TEM imaging by dispersing them in a liquid phase, which is then deposited on the measurement substrate or grid.

5.3.3 Nanoparticle dispersions in liquids

In other manufacturing systems, the nanoparticle precursors are dispersed to colloidal dimensions first in a liquid phase, followed by conversion to liquid sols. Stable colloidal dispersions in the liquid phase usually require either electrostatic or steric stabilization methods in order to prevent agglomeration. Concentrated nanoparticle dispersions shall be diluted in order to reduce touching nanoparticles on the measurement substrate or grid. The stability of colloidal dispersions affects whether they remain as discrete particles or agglomerate, aggregate or flocculate. Colloid stability depends on the particle surface chemistry, coatings or adducts on the particles, solvent chemistry, and inorganic and organic ligands in the fluid phase. Sonication can be effective in dispersing some nanoparticles in liquids, as shown in the some of the case studies.

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5.4 Minimize particle agglomeration in the sample dispersion

Forces that affect the stability of colloidal systems include:

— excluded volume repulsion;

— electrostatic interaction;

— van der Waals forces;

— entropic forces ;

— steric interactions.

Mutual repulsion of like electrical charges on nanoparticle colloids leads to electrostatic stabilization. Electrical double layers can form at the nanoparticle/liquid interface. Due to their small size, nanoparticles can have very large surface areas per volume (mass), so repulsive surface forces can overcome buoyancy forces that might lead to particulate settling. Steric stabilization of colloids occurs when coatings, such as polymers or oligomers, are attached to the nanoparticle surfaces. These coatings prevent the nanoparticles from approaching close enough that attractive forces would become effective, thus preventing agglomeration.

Many commercial nanoparticle products have surface coatings or surface functionalizations that are designed to control their dispersion in specific media. Destabilization of nanoparticle colloids in liquid dispersions can occur via a variety of mechanisms. The common methods for controlling the interactive forces between particles are selection of the dispersing liquid and the dispersing agent (see ISO 14887 and Reference [22]). Selection of the dispersing liquid is usually preferred if the chosen liquid reduces or prevents particle agglomeration, as dispersing agents coat the nanoparticles. Depending on the size ratio between the nanoparticle and the dispersing agent, the agent may affect the imaging of particle surfaces and cross-sections. Also, dispersing aids can desorb from the nanoparticles or may induce nanoparticle agglomeration with changes in temperature and liquid phase composition; they shall be chosen with care.

5.5 Selection of the mounting support

Several common methods for depositing nanoparticles on measurement substrates are available (see Reference [22]). A preferred support has most of the following characteristics:

— flat over the field of view selected;

— uniform and low background intensity (bright field) across the field of view;

— good contrast between particles and background.

Different types of samples often require different specimen preparation strategies, i.e. other commonly applied standards are based on grid-on-drop and on-grid centrifugation. Particle composition, particle surface chemistry, dispersing solvent, acidic or basic conditions, mounting support modifications and other factors contribute to the quality of the mounting support and mounting technique. Therefore, specimen preparation, including all consumables and chemicals, shall be described in great detail in the analysis report.

6 Instrument factors

6.1 Instrument set-up

ISO 29301:2017, 6.3, provides guidance on the operating conditions for electron microscopes. The TEM instrument parameters shall be selected[22] to provide high quality images with good contrast between background and particle. To contribute to the TEM image contrast, the nanoparticles shall scatter electrons, which depend on the sample composition and crystalline phase orientation.

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6.2 Calibration

6.2.1 General

ISO 13322-1:2014, Clause 10, has generic recommendations on calibration and metrological traceability.

6.2.2 Calibration standards

Since TEMs have a wide range of magnification and many operating modes, the actual magnification at any set of instrument settings may differ from the indicated magnification by up to 10 %. Calibration of the instrument to the SI unit of length (the metre) at optical conditions similar to those used for analysis is preferred. Calibrants shall fulfil all the requirements of a certified reference material (CRM), i.e. they shall be homogenous and stable, come with (a) certified value(s) and associated uncertainty and metrological traceability statement, come with a material certificate, etc. Standards should be run periodically to provide verification of correct instrument operation within manufacturer specifications and to validate measurement procedures. Typical examples are shown in Table 1. Be sure that the standard sample used has not passed its expiration date.

Table 1 — Typical calibration standards

Standard size nm	**Material**
2 160 lines/mm	Gratings; linear or cross-grating
2 to 100	Polystyrene, gold and silica nanoparticles
> 10	Crystals of catalase enzyme (bovine liver catalase crystals have lattice spacings of 8,75 nm and 6,85 nm[23])

When selecting a calibration technique for a TEM particle size analysis procedure, preference should be given to particle-based CRMs rather than the more traditional line-width standards. For example, calibration against a lattice spacing may only be useful at a magnification that is used to image the particles. These magnifications may not be appropriate for a number of nanoparticles. The calibration validity should be checked on a regular basis. Such quality control should be done with standards/CRMs that are different than the one used for calibration. Furthermore, the verification checks, as well as the (routine) particle size analyses shall be done under similar conditions (in terms of magnification, probe current, acceleration voltage, etc.) as used during calibration.

6.2.3 General calibration procedure

As the calibration strongly depends on the electron optics condition, it shall be done under optimum lens conditions using a suitable CRM. Then, the actual particle size measurement should be done under the same conditions of the calibration with a reference material (RM), e.g. objective lens current and the other lens parameters and specimen height.

The objective lens shall be calibrated to the eucentric axis. The specimen shall be set to the eucentric position using Z-axis movement. The “wobbler” functions can be used as the focus aid. When the sample is positioned on the eucentric axis, the image at the first image plane will have the minimum error in its length (see Figure 3). While the sample is positioned on the eucentric axis, tilting the sample around this axis is the same of tilting on first image plane, so the sample image doesn’t move on the fluorescent screen. If the sample position is not at the eucentric axis, there will be errors in its measured dimensions. Both RM calibrations and sample measurements shall be done with the specimens positioned on the eucentric axis.

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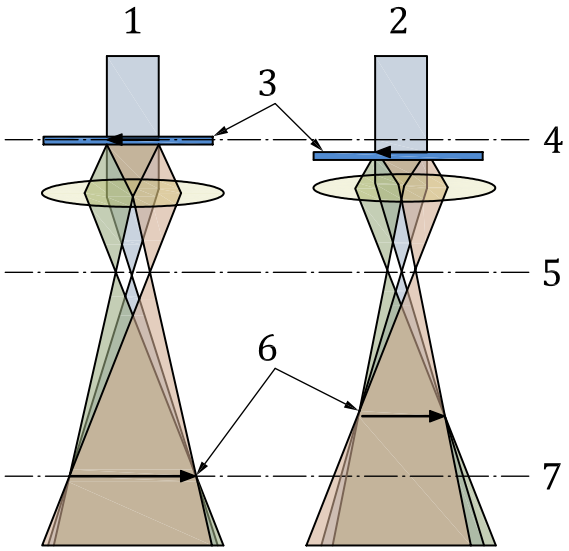

Key

1 on axis
2 off axis
3 specimen
4 eucentric axis
5 back focal plane
6 image
7 first image plane

NOTE Left hand side: proper alignment. Right hand side: sample displaced from the eucentric axis.

Figure 3 — Example of scale size error due to the specimen plane not coinciding with eucentric axis

TEM projection lenses need to be switched to make accurate measurements across the entire size range of a particle distribution. The lens ranges will depend on the TEM model, for example, low magnification < 100 000 times and high magnification ≥ 100 000 times. In each range, linearity of scaling will be achieved. However, there are discontinuities between calibration lines for different lenses: scale calibration needs to be done for each projection lens used to acquire images. Figure 4 demonstrates such differences in scaling calibration lines for the case of three projection lens systems. Calibration equations change between each lens system resulting in discontinuities (offsets) between the lines for different lenses. Details of scale calibration are provided in ISO 29301.

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