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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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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.

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.

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.]

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

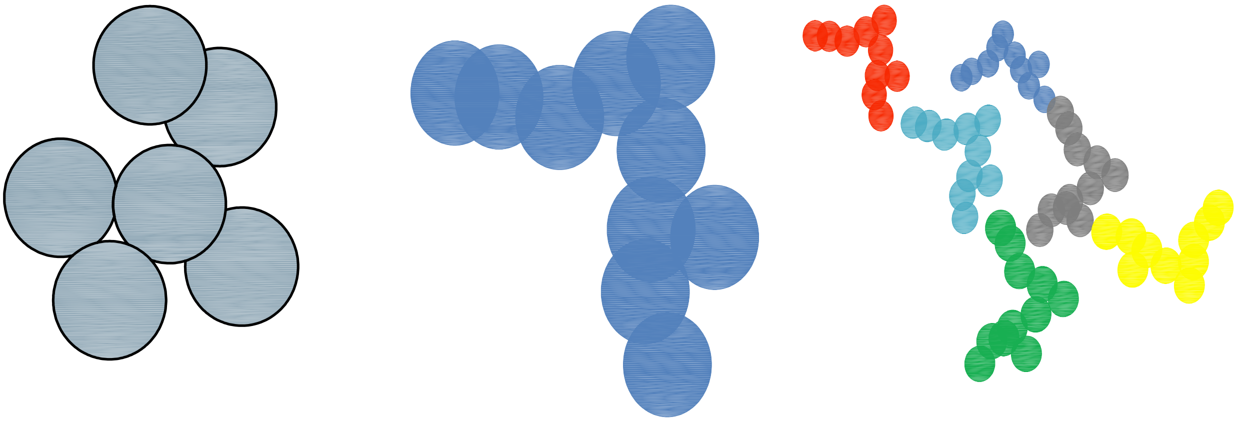
aggregate

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

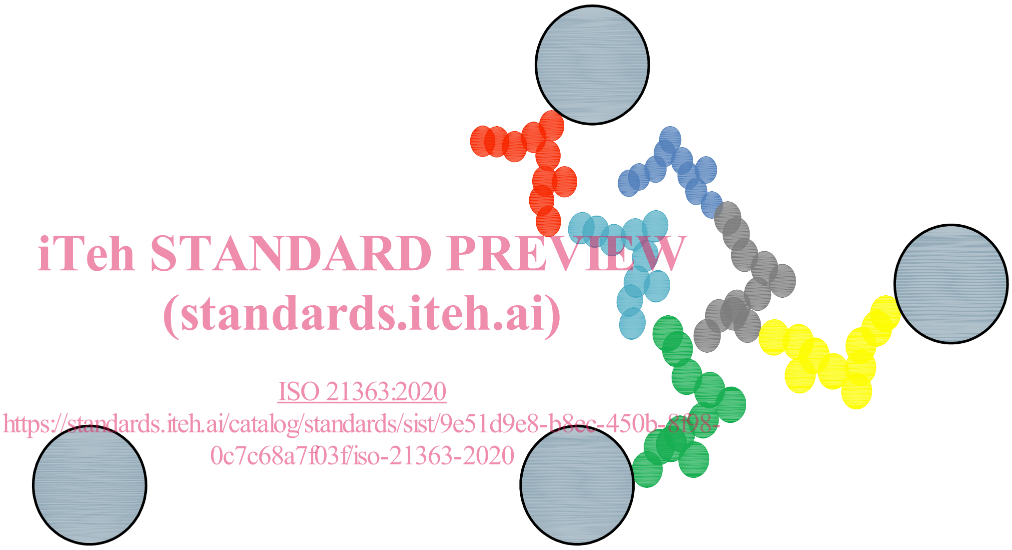
Note 1 to entry: The forces holding an aggregate together are strong forces (for example, covalent bonds) or those resulting from sintering or complex physical entanglement.

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.



a) Primary particles in an agglomerate **b) Primary particles in an aggregate** **c) Agglomerate of aggregates**



d) Nano-object (if less than 100 nm) or particle **e) Agglomerate of both primary particles and aggregates**

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]

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.]

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:

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

where $\overline{x}$ 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:

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

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:

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

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:

$$\text{RSE}_{\overline{x}} = \frac{100 \cdot \sigma_{\text{est},x}}{\overline{x}}$$

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.

$\Delta m = \left|c_{\text{m}} - c_{\text{crm}}\right|$: 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.

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

[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]

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

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:

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

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