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Nanotehnologije - Meritve porazdelitve velikosti in oblike delcev s skenirno elektronsko mikroskopijo (ISO 19749:2021)

Nanotechnologies - Measurements of particle size and shape distributions by scanning electron microscopy (ISO 19749:2021)

Nanotechnologien - Messung der Partikelgrößenverteilung und Partikelformverteilung mit Rasterelektronenmikroskopie (ISO 19749:2021)

Nanotechnologies - Détermination de la distribution de taille et de forme des particules par microscopie électronique à balayage (ISO 19749:2021)

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

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

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Contents

	Page
Foreword	v
Introduction	vi
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
3.1 General terms.....	2
3.2 Core terms: image analysis.....	4
3.3 Core terms: statistical symbols and definitions.....	4
3.4 Core terms: measurands and descriptors.....	6
3.5 Core terms: metrology.....	8
3.6 Core terms: scanning electron microscopy.....	10
4 General principles	11
4.1 SEM imaging.....	11
4.2 SEM image-based particle size measurements.....	12
4.3 SEM image-based particle shape measurements.....	13
5 Sample preparation	13
5.1 Sample preparation fundamental information.....	13
5.2 General recommendations.....	14
5.3 Ensuring good sampling of powder or dispersion-in-liquid raw materials.....	14
5.3.1 Powders.....	14
5.3.2 Nanoparticle dispersions in liquids.....	15
5.4 Ensuring representative dispersion.....	15
5.5 Nanoparticle deposition on a substrate.....	15
5.5.1 General.....	15
5.5.2 Nanoparticle deposition on wafers and chips of silicon or other materials.....	16
5.5.3 Nanoparticle deposition on TEM grids.....	17
5.6 Number of samples to be prepared.....	18
5.7 Number of particles to be measured for particle size determination.....	18
5.8 Number of particles to be measured for particle shape determination.....	19
6 Qualification of the SEM for nanoparticle measurements	19
7 Image acquisition	19
7.1 General.....	19
7.2 Setting suitable image magnification and pixel resolution.....	23
8 Particle analysis	24
8.1 Particle analysis fundamental information.....	24
8.2 Individual particle analysis.....	25
8.3 Automated particle analysis.....	25
8.4 Automated particle analysis procedure example.....	26
9 Data analysis	27
9.1 General.....	27
9.2 Raw data screening: detecting touching particles, artefacts and contaminants.....	27
9.3 Fitting models to data.....	27
9.4 Assessment of measurement uncertainty.....	27
9.4.1 General.....	27
9.4.2 Example: Measurement uncertainty for particle size measurements.....	28
9.4.3 Bivariate analysis.....	29
10 Reporting the results	29
Annex A (normative) Qualification of the SEM for nanoparticle measurements	31
Annex B (informative) Cross-sectional titanium dioxide samples preparation	36

ISO 19749:2021(E)

Annex C (informative) Case study on well-dispersed 60 nm size silicon dioxide nanoparticles	38
Annex D (informative) Case study on 40 nm size titanium dioxide nanoparticles	46
Annex E (informative) Example for extracting particle size results of SEM-based nanoparticle measurements using ImageJ	55
Annex F (informative) Effects of some image acquisition parameters and thresholding methods on SEM particle size measurements	57
Annex G (informative) Example for reporting results of SEM-based nanoparticle measurements	61
Bibliography	71

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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.

Introduction

This document provides guidance for measuring and reporting the size and shape distributions of nanometer-scale particles using images acquired by the scanning electron microscope (SEM). This document applies to the SEM-based measurement of larger particles also. Nanoparticles are three-dimensional (3D) objects, but the SEM image is only a two-dimensional (2D) representation of the 3D shape from a certain viewing angle. The SEM image carries valuable information about the size and shape of particles. While the SEM image does contain a certain amount of 3D information, for sake of simplicity, this document does not deal with reconstructing 3D information. Rigorous three-dimensional characterization of nanoparticles would include size, shape, surface structure (e.g. texture), surface and internal material composition, and their locations in the investigated 3D volume. This document deals with two attributes of morphology, size and shape, for discrete and aggregated nano-objects (materials with at least one dimension in the nanometer-scale, i.e. within 1 nm to 100 nm). Suitable sample preparation is essential to obtaining high-quality electron microscope images and preferred techniques often vary with the sample material. It is equally important to make sure that the SEM itself is suitable to carry out the measurements with the required uncertainty. Typical guidance suggests that a large number, several hundreds or thousands of particles need to be measured for statistically sound size and shape distribution results. The actual number of nano-objects needed to be measured depends on the sample, the required uncertainty and on the performance of the SEM. Statistical evaluation of the data and the evaluation of uncertainty of the measurands are included as part of the measurement and reporting procedures.

This document contains measurement procedures, particle and data analysis and reporting clauses. In the Annexes, there are specific examples for measurements and guidance for the qualification of the SEM for reliable quantitative measurements. Automation of the image acquisition and data analysis can reduce cost and improve the quality of the results. Measurements of samples of discrete nanoparticles are generally easier to carry out with automated image acquisition and particle analysis systems. Measurements of complex discrete nanoparticles, and aggregates or agglomerates of nanoparticles may require operator-assisted image acquisition and analysis. Evaluation of particle shape is facilitated by many pertinent analysis software solutions that allow for automatic selection of various shape attributes as well.

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

1 Scope

This document specifies methods of determining nanoparticle size and shape distributions by acquiring and evaluating scanning electron microscope images and by obtaining and reporting accurate results.

NOTE 1 This document applies to particles with a lower size limit that depends on the required uncertainty and on the suitable performance of the SEM, which is to be proven first - according to the requirements described in this document.

NOTE 2 This document applies also to SEM-based size and shape measurements of larger than nanoscale particles.

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/IEC Guide 99, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

ISO 9276-1, *Representation of results of particle size analysis — Part 1: Graphical representation*

ISO 9276-2, *Representation of results of particle size analysis — Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions*

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

ISO 9276-5, *Representation of results of particle size analysis — Part 5: Methods of calculation relating to particle size analyses using logarithmic normal probability distribution*

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

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

ISO 16700, *Microbeam analysis — Scanning electron microscopy — Guidelines for calibrating image magnification*

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

ISO/TS 24597:2011, *Microbeam analysis — Scanning electron microscopy — Methods of evaluating image sharpness*

ISO 26824, *Particle characterization of particulate systems — Vocabulary*

ISO/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

ISO/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

ISO/TS 80004-3, *Nanotechnologies — Vocabulary — Part 3: Carbon nano-objects*

ISO/TS 80004-4, *Nanotechnologies — Vocabulary — Part 4: Nanostructured materials*

ISO 19749:2021(E)

ISO/TS 80004-6, *Nanotechnologies — Vocabulary — Part 6: Nano-object characterization*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/IEC Guide 99, ISO 9276-6, ISO 26824, ISO/TS 80004-1, ISO/TS 80004-2, ISO/TS 80004-3, ISO/TS 80004-4, ISO/TS 80004-6, and the following 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 General terms

3.1.1

nanoscale

length range from approximately 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from larger sizes are predominantly exhibited in this length range.

[SOURCE: ISO/TS 80004-1:2015, 2.1]

3.1.2

nano-object

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

[SOURCE: ISO/TS 80004-1:2015, 2.5, modified — Note 1 to entry and the source have been deleted.]

3.1.3

particle

minute piece of matter with defined physical boundaries

[SOURCE: ISO/TR 16197:2014, 3.10, modified — Notes 1, 2 and 3 to entry and the source have been deleted.]

3.1.4

primary particle

original source *particle* (3.1.3) of *agglomerates* (3.1.5) or *aggregates* (3.1.6) or mixtures of the two

[SOURCE: ISO 26824:2013, 1.4, modified — Notes 1, 2 and 3 to entry have 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: Agglomerate originates from the Latin “agglomerare” meaning “to form into a ball”.

Note 2 to entry: The forces holding an agglomerate together are weak forces, for example van der Waals forces or simple physical entanglement.

Note 3 to entry: Agglomerates are also termed secondary particles and the original source particles are termed *primary particles* (3.1.4).

[SOURCE: ISO 26824:2013, 1.2, modified — Note 1 to entry has been added.]

3.1.6

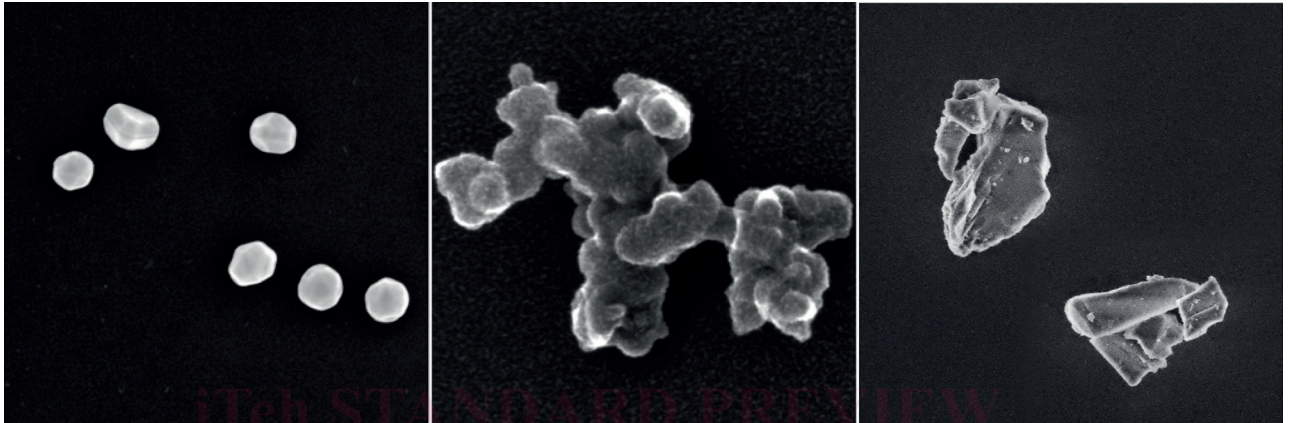
aggregate

particle (3.1.3) comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of 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, or otherwise combined former *primary particles* (3.1.4).

Note 2 to entry: Aggregate comes from the Latin “aggregat” meaning “herded together”.

Note 3 to entry: [Figure 1](#) shows examples of individual, aggregate and *agglomerate* (3.1.5) particles.



NOTE The images are projected views from certain angles of the 3D objects. Depending on the viewing angle, the observable size of particles can vary substantially.

Figure 1 — SEM images of individual gold (left) and carbon black aggregate (middle) and corundum agglomerate (right) particles

[SOURCE: ISO 26824:2013, 1.3, modified — Notes 2 and 3 to entry have been added.]

3.1.7

nanoparticle

nano-object (3.1.2) with all external dimensions in the *nanoscale* (3.1.1) 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 — Note 1 to entry has been deleted.]

3.1.8

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* (3.1.3) size. However, it is recognized that the symbol d is also widely used. Therefore, the symbol x may be replaced by d .

3.1.9

particle size distribution

distribution of the quantity of *particles* (3.1.3) as a function of *particle size* (3.1.8)

[SOURCE: ISO/TS 80004-6:2021, 4.1.2, modified — Notes 1 and 2 to entry have been deleted.]

ISO 19749:2021(E)**3.1.10****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 breadth.

[SOURCE: ISO/TS 80004-6:2021, 4.1.3, modified — Note 1 to entry has been added.]

3.1.11**analytical sample**

portion of material, resulting from the original sample or composite sample by means of an appropriate method of sample pretreatment and having the size (volume/mass) necessary for the desired testing or analysis

Note 1 to entry: The sample in analytical chemistry is a portion of material selected from a larger quantity of material. The term needs to be qualified, for example, bulk sample, representative sample, primary sample, bulked sample, test sample. The term 'sample' implies the existence of a sampling error, i.e. the results obtained on the portions taken are only estimates of the concentration of a constituent or the quantity of a property present in the parent material. If there is no or negligible sampling error, the portion removed is a test portion, aliquot or specimen. The term 'specimen' is used to denote a portion taken under conditions such that the sampling variability cannot be assessed (usually because the population is changing), and is assumed, for convenience, to be zero. The manner of selection of the sample should be prescribed in a sampling plan.

[SOURCE: ISO 11074:2015, 4.1.3, modified — Note 1 to entry has been added.]

3.2 Core terms: image analysis**3.2.1****binary image**

digitized image consisting of an array of *pixels* (3.2.2), 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 colors

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

3.2.2**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.]

3.2.3**pixel resolution**

number of imaging *pixels* (3.2.2) per unit distance of the detector

Note 1 to entry: The typical unit is sometimes expressed as dots per inch (dpi).

[SOURCE: ISO 29301:2017, 3.24, modified — the hyphen has been deleted in this term.]

3.3 Core terms: statistical symbols and definitions**3.3.1****arithmetic mean**

sum of values divided by the number of values

Note 1 to entry: See ISO 9276-1:1998 for other quantity measures and types of distributions.

3.3.2

standard deviation

measure of the dispersion of a series of results around their mean, equal to the positive square root of the variance and estimated by the positive square root of the mean square

[SOURCE: ISO 4259-1:2017, 3.21]

3.3.3

coefficient of variation

ratio of the *standard deviation* ([3.3.2](#)) to the *arithmetic mean* ([3.3.1](#))

[SOURCE: ISO 27448:2009, 3.11]

3.3.4

relative standard error

standard error ($SE_{\bar{x}}$) divided by the mean (\bar{x}) and expressed as a percentage

3.3.5

analysis of variance

ANOVA

technique which subdivides the total variation of a response variable into components associated with defined sources of variation

3.3.6

***p*-value**

probability of observing the observed test statistic value or any other value at least as unfavorable to the null hypothesis

Note 1 to entry: If the null hypothesis were true and if the experiment were repeated many times, a *p*-value is the probability that a value at least as extreme as the computed test statistic would be observed.

Note 2 to entry: In hypothesis testing, a statement claiming that the null parameter is the true parameter is called the null hypothesis. The purpose of a hypothesis test is to determine whether the data provide evidence against the null hypothesis. When a statistic is obtained that is very different from the null parameter, the null hypothesis can be rejected. An alternative, or research hypothesis, is a hypothesis that states that the true parameter is not (or is less than or is greater than) the null parameter; it is the hypothesis that corresponds to the research question. The goal of a hypothesis test is to reject the null hypothesis in favour of the research hypothesis.

[SOURCE: ISO/TR 14468:2010, 3.13, modified — Note 1 to entry has been modified and Note 2 to entry has been added.]

3.3.7

residual deviation

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

3.3.8

residual standard deviation

scatter of the information values about the calculated regression line

Note 1 to entry: It is a figure of merit, describing the *precision* ([3.5.3](#)) of the calibration.

Note 2 to entry: For this document, the *standard deviation* ([3.3.2](#)) of the method means the standard of deviation of the calibration procedure.

[SOURCE: ISO 8466-1:1990, 2.5, modified — the symbol has been deleted and the entire entry has been editorially revised.]

ISO 19749:2021(E)

3.3.9

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

Note 1 to entry: The quantile-quantile (q-q) plot is a probability plot, a graphical technique for determining if two data sets come from populations with a common distribution. A q-q plot is a plot of the quantiles of the first data set against the quantiles of the second data set. See ISO/TS 80004-6.

3.4 Core terms: measurands and descriptors

3.4.1

measurand

quantity intended to be measured

[SOURCE: ISO/IEC Guide 98-4:2012, 3.2.4]

3.4.2

Feret diameter

distance between two parallel lines which are tangent to the *perimeter* (3.4.5) of a *particle* (3.1.3)

[SOURCE: ISO 10788:2014, 2.1.4, modified — Note 1 to entry has been deleted.]

3.4.3

maximum Feret diameter

maximum length of an object whatever its orientation

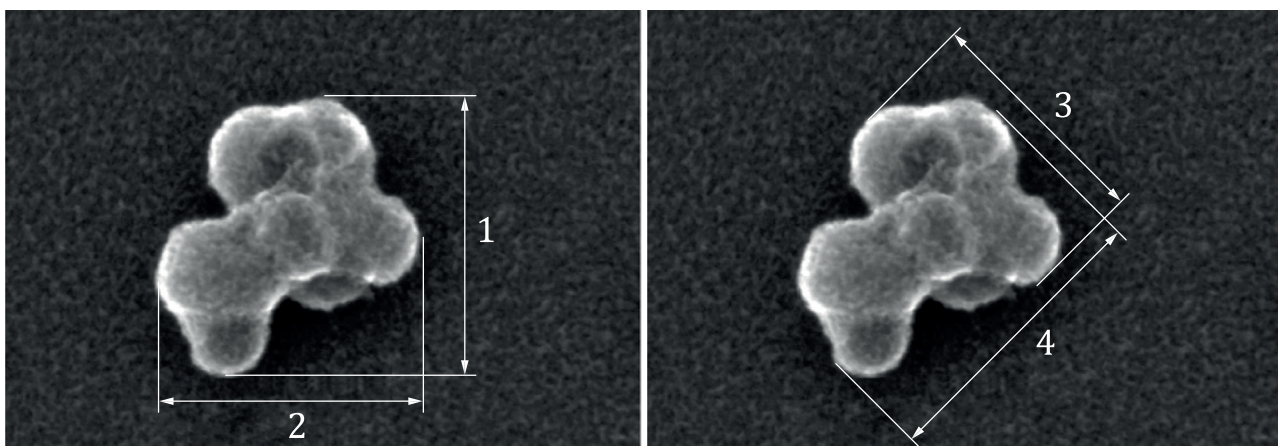
[SOURCE: ISO/TR 945-2:2011, 2.1, modified — the word "Féret" in the term has been changed to "Feret" and Note 1 to entry has been deleted.]

3.4.4

minimum Feret diameter

minimum length of an object whatever its orientation

Note 1 to entry: The *Feret diameter* (3.4.2) or Feret's diameter is a measure of an object size along a specified direction; it is applied to projections of a three-dimensional object on a two-dimensional plane, see [Figure 2](#). It is also called the caliper diameter.

**Key**

- | | | | |
|---|---------------------------|---|---------|
| 1 | vertical Feret diameter | 3 | breadth |
| 2 | horizontal Feret diameter | 4 | length |

Figure 2 — Horizontal Feret diameter (88 nm) and vertical Feret diameter (93 nm), and length (99 nm) and breadth (79 nm) of a carbon black particle

Note 2 to entry: The maximum Feret diameter x_{Fmax} is the “length” of the *particle* (3.1.3). The *minimum Feret diameter* (3.4.4) x_{Fmin} is the “breadth” of the particle.

Note 3 to entry: The Feret diameter depends on the orientation of the particle with respect to tangents, so a single measurement cannot always be representative. If all possible orientations are considered, for a convex particle with the particle *perimeter* (3.4.5) P : $P = \pi x_{Fmean}$. (Cauchy theorem). There is no such relation between P and x_{Fmean} for a concave object.

3.4.5 perimeter

total length of the object contour

[SOURCE: ISO/TR 945-2:2011, 2.3, modified — the symbol “P” has been deleted.]

3.4.6 convex hull

smallest convex set containing a given geometric object

[SOURCE: ISO 19123:2005, 4.1.2]

3.4.7 aspect ratio

ratio of the *minimum Feret diameter* (3.4.4) to the *maximum Feret diameter* (3.4.3)

3.4.8 ellipse ratio

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

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

3.4.9 extent

ratio of *particle* (3.1.3) area to the product of the *maximum Feret* (3.4.3) and the *minimum Feret* (3.4.4) diameters

3.4.10 compactness

degree to which the projection area A of the *particle* (3.1.4) is similar to a circle, considering the overall form of the particle with the *maximum Feret diameter* (3.4.3)

[SOURCE: ISO 26824:2013, 4.9, modified — the formula and Note 1 to entry have been deleted.]

3.4.11 convexity

ratio of the *perimeter* (3.4.5) of the *convex hull* (3.4.7) envelope bounding the *particle* (3.1.3) to its perimeter

3.4.12 circularity form factor

degree to which the projected area of the *particle* (3.1.3) is similar to a circle, based on its *perimeter* (3.4.5)

3.4.13 roundness

square of the *circularity* (3.4.12)

3.4.14 solidity

ratio of the projected area A to the area of the *convex hull* (3.4.7) A_C (envelope)