INTERNATIONAL STANDARD

ISO
19749

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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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 jointly by Technical Committee ISO/ TC 229, *Nanotechnologies*, and Technical Committee IEC/TC 113, *Nanotechnology for electrotechnical products and systems*.

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. This document provides a detailed list of other shape parameters for size, macroshape descriptors, geometrical descriptors, proportion descriptors, and mesoshape descriptors*

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

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

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

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/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 9276-6, ISO 26824, ISO/TS 80004-1, ISO/TS 80004-2, ISO/TS 80004-3, ISO/TS 80004-4, ISO/TS 80004-6, ISO/IEC Guide 99, 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 80004-3:2015, 3.1.3, 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.
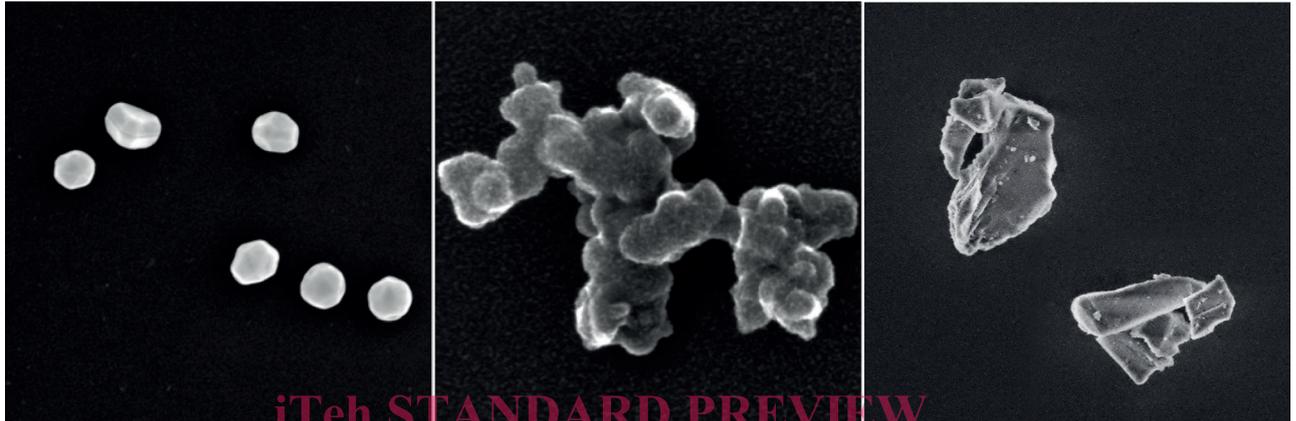
NOTE   The images are projected views from certain angles of the 3D objects. Depending on the viewing angle, the observable size of particles can vary substantially.

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 *particles* ([3.1.3](#)) as a function of *particle size* ([3.1.8](#))

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

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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 80004-6:2013, 3.1.3, 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_x) divided by the mean ($\overline{x}$) and expressed as a percentage

3.3.5
analysis of variance
ANOVA
technique which subdivides the total variation of a response variable (2.2) 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 — Notes 1 and 2 to entry have 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. It is a figure of merit, describing the *precision* ([3.5.3](#)) of the calibrationNote 1 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.]

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 Reference [21].

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".]

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.
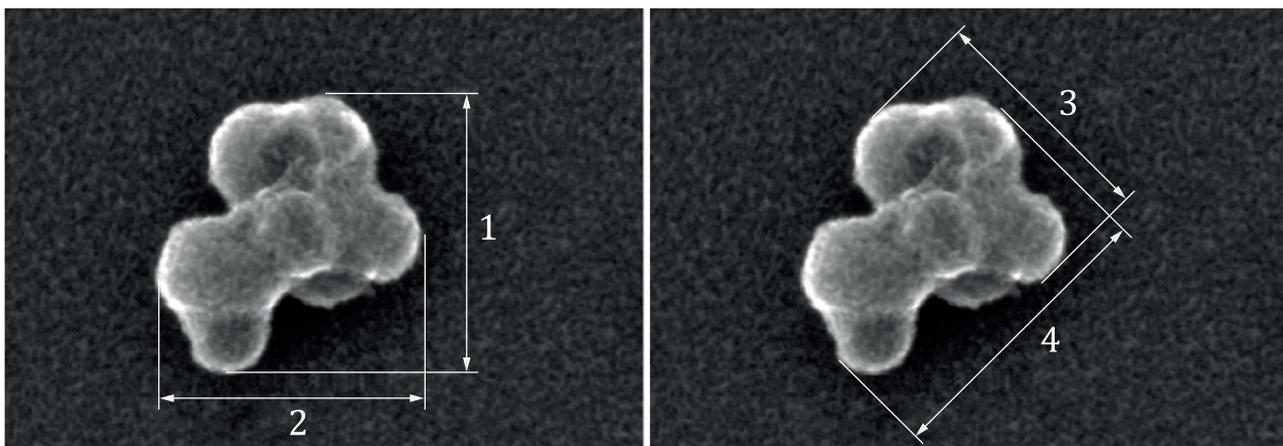
Key

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: $P = \pi\, x_{\mathrm{Fmean}}$. (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" been added.]

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 has 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)

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 — Notes 1 and 2 to entry have been deleted.]

3.5.2
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 of 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* (3.5.4).

Note 2 to entry: The term "measurement accuracy" should not be used for measurement trueness and the term measurement *precision* (3.5.3) 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 second and third terms have been deleted.]

3.5.3
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* (3.3.2), variance, or *coefficient of variation* (3.3.3) under the specified conditions of measurement.

Note 2 to entry: The specified conditions can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement (see ISO 5725-1:1994).

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.

[SOURCE: ISO/IEC Guide 99:2007, 2.15, modified — the first term has been deleted.]

3.5.4
measurement uncertainty
non-negative parameter characterizing the dispersion of the quantity values being attributed to a *measurand*, (3.4.1) based on the information used

Note 1 to entry: Measurement uncertainty includes components arising from systematic effects, such as components associated with corrections and the assigned quantity values of measurement standards, as well as the definitional uncertainty. Sometimes estimated systematic effects are not corrected for but, instead, associated measurement uncertainty components are incorporated.

Note 2 to entry: The parameter may be, for example, a *standard deviation* (3.3.2) called standard measurement uncertainty (or a specified multiple of it), or the half-width of an interval, having a stated coverage probability.

Note 3 to entry: Measurement uncertainty comprises, in general, many components. Some of these may be evaluated by *Type A evaluation of measurement uncertainty* (3.5.7) from the statistical distribution of the quantity values from series of measurements and can be characterized by standard deviations. The other components, which may be evaluated by *Type B evaluation of measurement uncertainty* (3.5.8), can also be characterized by standard deviations, evaluated from probability density functions based on experience or other information.

Note 4 to entry: In general, for a given set of information, it is understood that the measurement uncertainty is associated with a stated quantity value attributed to the *measurand* (3.4.1). A modification of this value results in a modification of the associated uncertainty.

[SOURCE: VIM:JCGM 200:2012, 2.26]

3.5.5
combined standard measurement uncertainty
standard *measurement uncertainty* (3.5.4), a non-negative parameter characterizing the dispersion of the quantity values being attributed to a *measurand* (3.4.1), based on the information use, is obtained using the individual standard measurement uncertainties associated with the input quantities in a measurement model

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

Note 2 to entry: Measurement uncertainty includes components arising from systematic effects, such as components associated with corrections and the assigned quantity values of measurement standards, as well as the definitional uncertainty. Sometimes estimated systematic effects are not corrected for but, instead, associated measurement uncertainty components are incorporated.

Note 3 to entry: The parameter maybe, for example, a *standard deviation* (3.3.2) called standard measurement uncertainty (or a specified multiple of it), or the half-width of an interval, having a stated coverage probability.

Note 4 to entry: Measurement uncertainty comprises, in general, many components. Some of these maybe evaluated by *Type A evaluation of measurement uncertainty* (3.5.7) from the statistical distribution of the quantity values from series of measurements and can be characterized by standard deviations. The other components, which maybe evaluated by *Type B evaluation of measurement uncertainty* (3.5.8), can also be characterized by standard deviations, evaluated from probability density functions based on experience or other information.

Note 5 to entry: In general, for a given set of information, it is understood that the measurement uncertainty is associated with a stated quantity value attributed to the measurand. A modification of this value results in a modification of the associated uncertainty.

[SOURCE: VIM:JCGM 200:2012, 2.31]

3.5.6
expanded measurement uncertainty
U
product of a *combined standard measurement uncertainty* (3.5.5) 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 second term "expanded uncertainty" to "U".]