BS EN ISO 19749:2023
Incorporating corrigendum April 2023

BSI Standards Publication

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

Documents
Document Sharing Hub

Full document details: BS EN ISO 19749:2023

National foreword

This British Standard is the UK implementation of EN ISO 19749:2023. It is identical to ISO 19749:2021. It supersedes BS ISO 19749:2021, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee NTI/1, Nanotechnologies.

A list of organizations represented on this committee can be obtained on request to its committee manager.

Contractual and legal considerations

This publication has been prepared in good faith, however no representation, warranty, assurance or undertaking (express or implied) is or will be made, and no responsibility or liability is or will be accepted by BSI in relation to the adequacy, accuracy, completeness or reasonableness of this publication. All and any such responsibility and liability is expressly disclaimed to the full extent permitted by the law.

This publication is provided as is, and is to be used at the recipient's own risk.

The recipient is advised to consider seeking professional guidance with respect to its use of this publication.

This publication is not intended to constitute a contract. Users are responsible for its correct application.

© The British Standards Institution 2023
Published by BSI Standards Limited 2023

ISBN 978 0 539 23732 0

ICS 07.120

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 July 2021.

Amendments/corrigenda issued since publication

Date	Text affected
30 April 2023	This corrigendum renumbers BS ISO 19749:2021 as BS EN ISO 19749:2023

Documents
Document Sharing Hub

Full document details: BS EN ISO 19749:2023

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 19749

March 2023

ICS 07.120

English Version

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

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

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

This European Standard was approved by CEN on 10 March 2023.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

© 2023 CEN All rights of exploitation in any form and by any means reserved worldwide for CEN national Members.

Ref. No. EN ISO 19749:2023 E

Documents
Document Sharing Hub

Full document details: BS EN ISO 19749:2023

European foreword

The text of ISO 19749:2021 has been prepared by Technical Committee ISO/TC 229 "Nanotechnologies" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 19749:2023 by Technical Committee CEN/TC 352 "Nanotechnologies" the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2023, and conflicting national standards shall be withdrawn at the latest by September 2023.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

Any feedback and questions on this document should be directed to the users' national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Endorsement notice

The text of ISO 19749:2021 has been approved by CEN as EN ISO 19749:2023 without any modification.

AVA Documents
Document Sharing Hub

Full document details: BS EN ISO 19749:2023

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**

AVA Documents
Document Sharing Hub

© ISO 2021 – All rights reserved

Full document details: BS EN ISO 19749:2023

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

© ISO 2021 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

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.

© ISO 2021 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

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.

© ISO 2021 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

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*

AVA Documents
Document Sharing Hub

© ISO 2021 – All rights reserved

Full document details: BS EN ISO 19749:2023

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.]

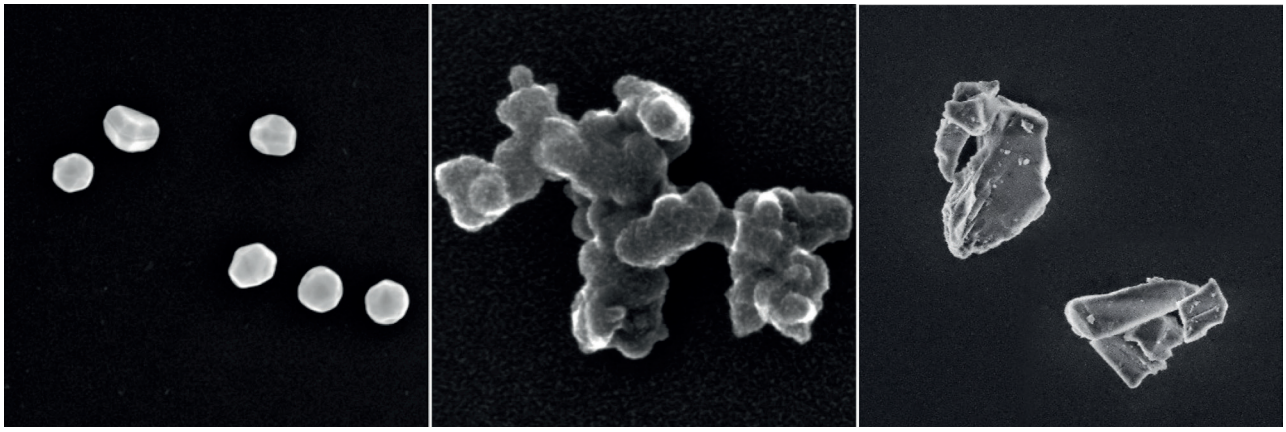
© ISO 2021 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

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 2021 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

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.

© ISO 2021 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

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 ($\bar{x}$) 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 2021 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

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)

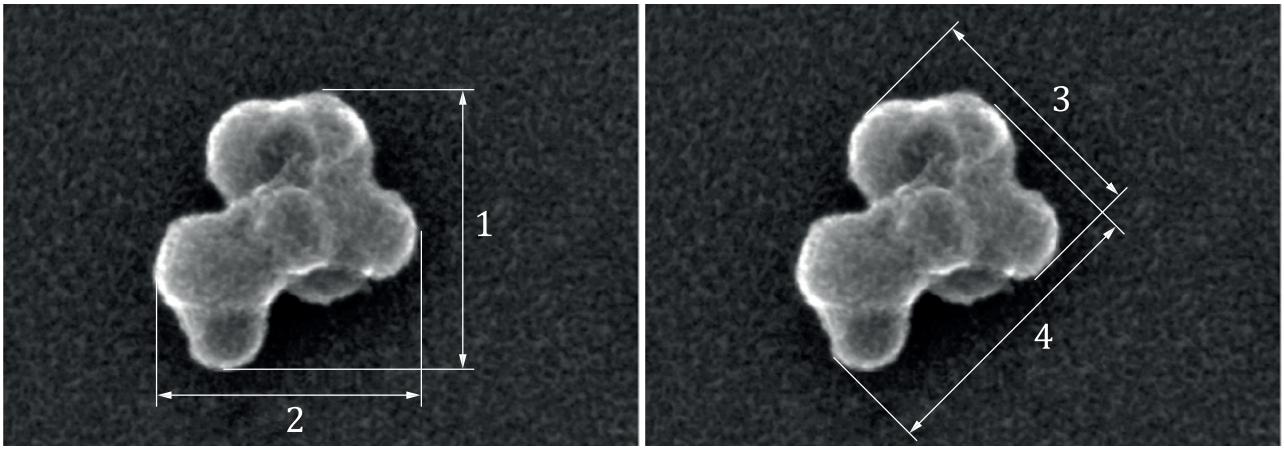
[SOURCE: ISO 10788:2014, 2.1.4, modified — Note 1 to entry has been deleted.]

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

© ISO 2021 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

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)

© ISO 2021 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

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.

© ISO 2021 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

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: 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: 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" has been changed to "*U*".]

© ISO 2021 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

3.5.7
Type A evaluation of measurement uncertainty
evaluation of a component of *measurement uncertainty* (3.5.4) by a statistical analysis of measured quantity values obtained under defined measurement conditions

[SOURCE: ISO/IEC Guide 99:2007, 2.28, modified — the second term and the Notes 1, 2 and 3 to entry have been deleted.]

3.5.8
Type B evaluation of measurement uncertainty
evaluation of a component of *measurement uncertainty* (3.5.4) determined by means other than a *Type A evaluation of measurement uncertainty* (3.5.7)

[SOURCE: ISO/IEC Guide 99:2007, 2.29, modified — the second term, the example and the Note 1 to entry have been deleted.]

3.5.9
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

3.5.10
certified reference material
CRM
reference material (3.5.9) characterized by a metrologically valid procedure for one or more specified properties, accompanied by a reference material 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 1, 2, 3 and 4 to entry have been deleted.]

3.6 Core terms: scanning electron microscopy

3.6.1
scanning electron microscope
SEM
instrument that produces magnified images of a specimen by scanning its surface with a well-focused electron beam

Note 1 to entry: A conventional SEM utilizes an electron source filament either made from W or LaB_6 materials that are heated to produce a source of electrons by thermionic emission. The electron beam probe sizes are in the range of 3 nm to 4 nm, which is not sufficient to resolve the smallest nanometer-scale details. The range of useful analysis is generally under 100 000 times magnification. Conventional SEMs typically operate at high *accelerating voltages* (3.6.2) (5 kV to 30 kV) and often require non-conducting samples to be coated with a thin layer of conductive metal (gold, gold-palladium, chromium, osmium) for imaging or carbon for analytical purposes (only).

Note 2 to entry: A field emission scanning electron microscope (FESEM) has an extremely sharp cathode tip that generates a smaller diameter probe size compared to a conventional SEM, even at low accelerating voltages (0,5 kV to 5 kV). In FESEMs, electron beam probe sizes can be 0,5 nm or less, expanding the useful magnification range by an order of magnitude higher. Non-conducting materials can be imaged without applying a conductive coating using low or very low accelerating voltages. A FESEM is sometimes referred to as a high-resolution SEM (HRSEM).

Note 3 to entry: Variable pressure SEM (VPSEM) or controlled pressure SEM which can operate with a pressure in the specimen chamber from 1 Pa up to 5,000 Pa so that direct secondary emission is no longer detectable and images are obtained with the detection of electrons by gas amplification, environmental SE detector, ions, photoemission or using other signals such as backscattered (BSE) or absorbed current. This type of SEM can be also used with a normal vacuum in the chamber.

© ISO 2021 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

3.6.2
accelerating voltage
potential difference applied between the filament and the anode to accelerate the electrons emitted from the source

[SOURCE: ISO 23833:2013, 5.1]

3.6.3
secondary electron
SE
electron emitted from a specimen as a result of bombardment by the primary electrons

Note 1 to entry: By convention, an electron with energy less than 50 eV is considered as a secondary electron.

[SOURCE: ISO 22493:2014, 3.4]

3.6.4
secondary electron image
SEI
scanning electron beam image in which the signal is derived from a detector that selectively measures *secondary electrons* (3.6.3) (electrons having less than 50 eV) and is not directly sensitive to backscattered electrons

[SOURCE: ISO 23833:2013, 4.4.11]

3.6.5
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

Note 1 to entry: In SEMs the energy of the primary electron beam is significantly lower than in traditional transmitted electron microscopes (TEMs), but still useful for STEM imaging and measurements of *nanoparticles* (3.1.7).

[SOURCE: ISO/TS 80004-6:2021, 4.5.7, modified — Notes 1 to entry has been modified and Note 2 to entry has been deleted.]

4 General principles

4.1 SEM imaging

Particles are three-dimensional. The SEM provides a projection, a view from a certain angle of the three-dimensional particles. The digital SEM image is a two-dimensional matrix of locations identified by horizontal and vertical parameters and the intensity of a signal at those locations. This matrix can be viewed as an image on the computer screen.

SEM imaging and analysis is especially useful for nanoparticle visualization and measurements of various particle size and shape distribution measurands. Imaging the nanoparticles is the first step to visualize their presence, but without suitably good repeatability in imaging, the SEM cannot provide useful information for particle size measurements. Depending on the resolving power of the SEM, it is possible to image well sub-10 nm particles. Figure 3 shows an example for various size nanoparticles with the approximate sizes for two of them. The horizontal field width is 300 nm.

There are 50 atoms in 1 nm^3 Si; a 1-nm diameter Si sphere particle contains about 26 atoms, a 2-nm diameter Si sphere particle contains about 210 atoms, so small nanoparticles cannot generate large signals. To achieve sufficiently noise-free images, proper setting of the SEM and the imaging parameters is indispensable.

© ISO 2021 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

Many modern SEMs have scanning transmission imaging capability. The images of nanoparticles obtained in the SEM in this scanning transmission electron microscopy working mode are very similar to images taken in the transmission electron microscope (TEM) and in scanning transmission electron microscope (STEM), which generally work at much higher accelerating voltages. STEM images taken in the SEM can be particularly useful for particle size and shape measurements. Obviously for this working mode, the same sample preparation requirements apply as for traditional TEM or STEM investigations. A similar ISO document is available on the size and shape measurements of particles with these techniques, which has information and examples for sample preparation and imaging useful for SEM-based measurements.

4.2 SEM image-based particle size measurements

Size measurements determine a one-dimensional (1D) parameter, a number related to the size of a three-dimensional (3D) particle, e.g. the circular equivalent diameter in a projected view image. SEM images can contain some volume-related, additional information also. Depending on the instrument settings and the sample material, more signal electrons might be generated by a spherical particle than by a flat one, even if they have the same size and shape projected image.

For the determination of particle size, relevant sections of ISO 13322-1, ISO 9276-1, ISO 9276-2, ISO 9276-3 and ISO 9276-5 shall be used.

Depending on the resolving power of the SEM, it is possible to measure the size and size distribution of sub-10 nm particles. The smallest measureable nanoparticles start at 1-nm size for the highest resolution SEMs that have a resolution of 0,4 nm or better. Other instruments, especially those that are equipped with conventional tungsten electron source, have a spatial resolution of a few nanometers, cannot suitably image and measure the smallest nanoparticles. The actual minimum value of the measurable nanoparticle size depends on the instrument, its environment, and the sample as well. When reliable sizing of the nanoparticles — due to lack of sufficiently high resolution — is not possible, particle counting still might be feasible. For this, it might be sufficient to detect the presence of nanoparticles. Signal intensity might also be used to determine size-related information with reduced accuracy. For example, in Figure 3, the approximately 2-nm size gold particle gives less intense signal than the 10-nm size. The detection limit of the presence of very small size nanoparticles shall be determined with comparisons of the results of higher-resolution and more sensitive SEM or TEM or other measurements, using suitable samples.

Additionally, the measurement error also depends on the resolving power of the SEM. Generally, sharper, more focused electron beam will result in images that show more details of particles that appear smaller, while lack of focus or resolving power makes them appear larger. This does not necessarily mean that good-quality size and shape distribution measurements cannot be carried out. Proper calibration using relevant reference samples with known particle sizes could help to significantly reduce measurement uncertainty.

© ISO 2021 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

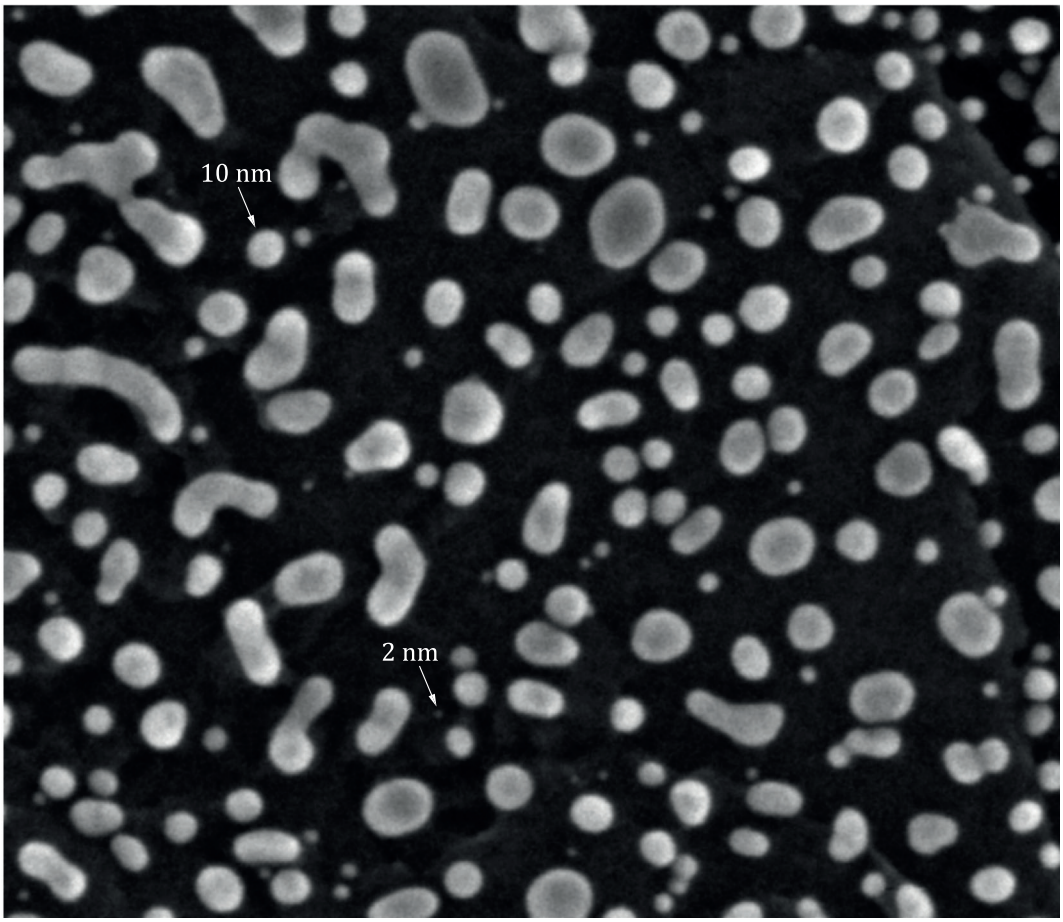


Figure 3 — SEM image of gold nanoparticles on carbon substrate showing large variation of size and shape (300 nm HFW)

4.3 SEM image-based particle shape measurements

Shape measurements determine a two-dimensional function related to projection, a view from a certain angle of the three-dimensional particle. ISO 9276-6 shall be used in SEM image-based particle shape measurements.

Accurate determination of the shape nanoparticles requires higher resolving power images than simpler size measurements. ISO 9276-6:2008 states that: "The optimum resolution shall not and cannot be stated absolutely but shall rather be related to the size of the element features to be determined (e.g. agglomerate branches, roughness scale). Analysis of image parameters is generally based on a digitized image. The process of digitizing an image can result in information loss because of the transformation of the continuous features innate to the particle into discrete elements of finite size — the finite resolution. For pixel errors, smaller than 5 % for a circle, the necessary pixel numbers per particle range from 100 to 200; for robust parameters, like projection area and ellipse ratio, up to 5,000 for parameters using the perimeter."

5 Sample preparation

5.1 Sample preparation fundamental information

The quality of SEM nanoparticle size and shape measurement results strongly depends on sample preparation. The relevance of the result obtained on prepared samples to the whole amount of the raw nanoparticle material can be ensured by proper sampling and with careful preparation. There

© ISO 2021 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

is no single best method for preparation of all nanoparticles, rather methods determined by the characteristics of nanoparticles in the raw nanoparticle material.

The measurand, quantity or property which is intended to be measured is also fundamental in sample preparation. Depending on exactly what information with what certainty shall be determined, the best sample preparation method might be different. It is up to the user to decide the most appropriate one.

The information on the health effects of various nanoparticles is not yet complete. It is possible that some nanoparticles are hazardous to health while others are not or do not cause adverse health effects following exposure. For this reason, it is recommended to always wear appropriate personal protective equipment, including disposable gloves, safety glasses, laboratory coats, filter respirators and take appropriate precautions when handling nanomaterial. Sample preparation should be carried out in a vented fume hood equipped with suitable air filters.

Tools used for preparation and handling nanoparticles shall be cleaned with filtered de-mineralized water and stored dry. The use of commercial cleaning agents formulated specifically for optical components is acceptable to remove residues, but care shall be taken to remove all traces of the cleaning detergent as this can impact the nanomaterial properties. If available, store tools under high-efficiency particulate air (HEPA) filtered air (e.g. in a clean enclosure/bench).

5.2 General recommendations

Nanoparticle samples are typically powders or suspensions. Powder samples are often dispersed in liquids prior to their deposition on a substrate for electron microscopy evaluation. Suspensions often need to be diluted to image discrete particles. Typically, out of a large volume of raw sample material analytical samples are taken that contain a smaller number of nanoparticles. Out of these samples a number of particles are separated and deposited on a substrate surface. Key sample preparation objectives are: use a representative sample of the raw 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 substrate and select a substrate that enhances the contrast between particle and background.

The prepared samples shall be relevant, i.e. the size and shape distribution of nanoparticles of the small amount needed to prepare samples for measurements, the original raw sample material shall be divided in a manner that ensures that the portion taken will be representative of the whole original sample material. This is the so-called sampling. The method used to divide the sample shall be determined by the sample preparation method and shall be decided by the laboratory performing the measurements.

The dispersion of the nanoparticles over the surface so that particles do not touch each other, i.e. the particles are clearly visible in the images with their boundaries easily discernible. There should be as few particles as possible touching each other or piling up. Measuring touching particles as one particle without proper separation will introduce a measurement error. It is also desired that the particles to be not far from each other, so many nanoparticles can be observed across the acquired images.

5.3 Ensuring good sampling of powder or dispersion-in-liquid raw materials

5.3.1 Powders

It is essential to sample powder samples carefully, particularly if they are aggregated as material can redistribute under the vibrations and forces of transportation. ISO 14887:2000, ISO 14703:2008, 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 and measure. Powder samples are prepared for SEM and STEM imaging by dispersed in a liquid phase, which is then deposited on the measurement substrate or grid.

© ISO 2021 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023

5.3.2 Nanoparticle dispersions in liquids

Nanoparticles can be dispersed in colloidal liquids or liquid sols. Stable colloidal dispersions in the liquid phase usually require either electrostatic or steric stabilization methods to prevent agglomeration. Concentrated nanoparticle dispersions shall be diluted 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.

For all raw samples, it is essential to minimize particle agglomeration in the sample dispersion, and sample preparation.

5.4 Ensuring representative dispersion

Forces that affect the stability of colloidal systems include: excluded volume repulsion, electrostatic interaction, van der Waals forces, entropic forces and 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 compared to their volume (mass), so repulsive surface forces can overcome buoyancy forces that might lead to particulate settling. Steric stabilization of particles occurs when coatings, such as surfactants, polymers or oligomers, are attached to the nanoparticle surfaces. These coatings prevent agglomeration of nanoparticles by keeping them apart enough that attractive forces would not become effective.

There are commercial nanoparticle preparation products that have surface coatings or functionalized surfaces, which are designed to control particle 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 using surfactants (see ISO 26824). Selection of the dispersing liquid is usually preferred, as surfactants could cover the surface of nanoparticles with thin layer of material. Depending on the size of the nanoparticles, this surfactant coating can affect the results of the characterization and measurements of the nanoparticles. Also, surfactants are susceptible to desorption from the nanoparticles or can also induce nanoparticle agglomeration with changes in temperature and liquid phase composition; it is essential to choose them with proper care.

Sample dispersion with ultrasonic treatment (sonication) is frequently required to minimize aggregation and agglomeration.

Particles can appear very different in size and shape, depending on the viewpoint. In the determination of their size and shape, it is indispensable to assess and use the information about how the 3D particles were deposited on the 2D sample surface. It is similarly important to assess and use the information related to the angle from which the images used for analysis were taken.

5.5 Nanoparticle deposition on a substrate

5.5.1 General

An important objective of sample preparation is to generate a uniform distribution of particles across the entire measurement substrate. Several common methods for depositing nanoparticles on measurement substrates are described, for example, in ISO/TS 24597, ISO 26824 and Reference [17]. The knowledge about and the number of methods of particle deposition are growing; it is advisable to explore and use the methods in pertinent literature.

Several common methods for depositing nanoparticles on measurement substrates are available (see ISO/TS 80004-3).

A preferred support has most of the following characteristics: relatively flat of the frame size selected, uniform and low background intensity across the frame size, and good contrast between particles and

© ISO 2021 – All rights reserved

AVA Documents Document Sharing Hub
Full document details: BS EN ISO 19749:2023

background. Many nanoparticles will need to be imaged and analysed to obtain a high-quality data on the particle size distribution. Particle analysis time can be reduced and data quality can improve if image analysis software is used. When preparation methods 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.

Commercial or open-source image analysis software is used to analyse the acquired images. 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 a uniform distribution of particles across the entire measurement substrate.

5.5.2 Nanoparticle deposition on wafers and chips of silicon or other materials

This is one of the easiest and often the most economical way of preparing sample for SEM measurements. Blank silicon wafers and chips have good electrical conductivity and are sufficiently flat and allow for high-quality secondary and backscattered electron-based imaging and measurements. The chips shall be cleaned to remove dust and silicon fragments from their surfaces. The chips are placed into a small beaker filled with a 9:1 solution of water (H_2O) and ammonium hydroxide (NH_4OH). The beaker is then exposed for 5 min with low intensity ultrasonic cleaning with a non-heated clean water bath. Then the chips are rinsed with clean water and an ethanol (C_2H_6O) jet and dried with a blast jet of pressurized oil-free nitrogen gas. Alternatively, one could use an anion-active or non-ionic surfactant and clean water, but this will require more rinsing. The shiny surface of the chips at the end of this step shall be completely free from any visible particles or discoloration. Another procedure is described in Reference [17].

— Etch and clean the surface using a strong cleaning agent, such as the so-called acidic piranha solution (mixture of 3:1 ratio of 30 % hydrogen peroxide (HOOH) solution added to concentrated sulphuric acid (H_2SO_4 vitriol)) [35r] or a mixture of ethanol (420 ml), sodium hydroxide, NaOH (70 g) and water (280 ml).

— The surface can be positively charged by using poly(l-lysine) or silane coupling agents. A variety of silanes can be used. For example, immerse the surface in a solution of aminopropyltrimethoxysilane (APTES, 1 % w/v) dissolved in 95 % ethanol and 5 % water mixture, followed by four ethanol rinses. The negatively charged nanoparticles adhere to the positively charged surface so that most of them are well separated. The substrates are dried and heated to 100 °C to remove surface water.

It is not unusual that a positively or negatively charged capturing layer of molecules (e.g. poly-L-lysine) can be used well for grabbing and adhering the nanoparticles to the surface of the chip. Within several minutes of room air exposure, an approximately 1,3 nm thin, so called “native oxide” forms on the surface of the Si chips, which can be sufficient for the preparation of the capturing layer. However, it has been shown that a thicker oxide layer is preferable. A one-minute wet oxygen plasma treatment of the Si chips forms thicker layer of silicon dioxide. The chips with their shiny surface facing up are to be treated in a plasma etcher with 40 W of power at 2×10^{-1} millibar (20 Pa) oxygen or air pressure.

The oxygen plasma oxidizes and removes organic contaminants from the chip surface and increases the thickness of the silicon dioxide layer. This layer is needed for the following chemical derivatization. However, the silicon dioxide, which as a bulk material is an insulator, should not be so thick (thicker than 4 nm to 5 nm) that the chip surface loses its ability to conduct away charge generated by the primary electron beam of the microscope. Figure 4, a) shows sample preparation examples for simple dilution and droplet deposition and air drying type of sample preparation of nanoparticles on the left and the same raw material prepared with the procedure described above,[17] yields much more desirable samples to measure on the right. The sample on the left has the particles too close to each other, surrounded by some residual colloidal material that is also on the surface of the Si substrate producing a noisy uneven background. The use of capturing layer allows for thorough rinsing away the gooey colloidal material, which results in clean nanoparticle surfaces, and better, more even contrast between the particles and the substrate.

© ISO 2021 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 19749:2023