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Particle size analysis — Image analysis methods —

Part 1: Static image analysis methods

Analyse granulométrique — Méthodes par analyse d'images —

Partie 1: Méthodes par analyse d'images statiques

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 13322-1 was prepared by Technical Committee ISO/TC 24, *Sieves, sieving and other sizing methods*, Subcommittee SC 4, *Sizing by methods other than sieving*.

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ISO 13322 consists of the following parts, under the general title *Particle size analysis — Image analysis methods*:

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— *Part 1: Static image analysis methods*

— *Part 2: Dynamic image analysis methods*

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Introduction

The purpose of this part of ISO 13322 is to give guidance for a measurement description and its validation when determining particle size by image analysis.

Image analysis is a technique used in very different applications on image material with variations in material properties. Hence, it is not relevant to describe an exact standard method for determination of particle size by image analysis. The aim of this part of ISO 13322 is limited to give a standardized description of the technique used and a standardized validation.

This part of ISO 13322 includes methods of calibration verification using a certified standard graticule as a reference or by using certified standard particles. It is sensible to make some measurements on particles, or other reference objects, of known size so that the likely systematic uncertainties introduced by the equipment can be calculated.

This part of ISO 13322 gives a recommendation for a precise description of the distribution including the number of analyzed particles and an analysis window to make sure that the obtained information is valid.

Measurement of particle-size distributions by microscopy methods is apparently simple, but because only a small amount of sample is examined, considerable care has to be exercised in order to ensure that the analysis is representative of the bulk sample. This can be demonstrated by splitting the original sample and making measurements on three or more parts. Statistical analysis of the data, for example using the Student's *t*-test, will reveal whether the samples are truly representative of the whole.

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Errors introduced at all stages of the analysis from sub-division of the sample to generation of the final result add to the total uncertainty of measurement and it is important to obtain estimates for the uncertainty arising from each stage. Indications where this is required are given at the appropriate point in the method.

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Because of the diverse range of equipment and sample preparation expertise available, it is not intended to give a prescriptive procedure where use of individual methods does not jeopardize the validity of the data. However, essential operations are identified to ensure that measurements made conform to this part of ISO 13322 and are traceable.

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Particle size analysis — Image analysis methods —

Part 1: Static image analysis methods

1 Scope

This part of ISO 13322 is applicable to the analysis of images for the purpose of determining particle size distributions. The particles are appropriately dispersed and fixed on an optical or electron microscope sample stage such as glass slides, stubs, filters, etc. Image analysis can recover particle images directly from microscopes or from photomicrographs.

Even though automation of the analysis is possible, this technique is basically limited to narrow size distributions of less than an order of magnitude. A standard deviation of 1,6 of a log-normal distribution corresponds to a distribution of less than 10:1 in size. Such a narrow distribution requires that over 6 000 particles be measured in order to obtain a repeatable volume-mean diameter. If reliable values are required for percentiles, e.g. D_{90} or other percentiles, at least 64 000 particles must be measured. This is described in Annex A.

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2 Normative references

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The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

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

3 Terms, abbreviated terms, definitions and symbols

3.1 Terms, abbreviated terms and definitions

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

3.1.1
view field
field which is viewed by a viewing device, e.g. optical microscope or electron scanning microscope

3.1.2
measurement frame
field in a view field in which particles are counted for image analysis

NOTE The set of measurement frames composes the total measurement field.

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3.1.3
binary image
digitized image consisting of an array of pixels, each of which has a value of 0 or 1, whose values are normally represented by dark and bright regions on the display screen or by the use of two distinct colours

3.1.4
edge finding
one of many edge detection methods used to detect transition between objects and background

3.1.5
Euler number
number of objects minus the number of holes inside the objects, which describes the connectedness of a region, not its shape

NOTE A connected region is one in which all pairs of points can be connected by a curve lying entirely in the region. If a complex two-dimensional object is considered to be a set of connected regions, where each one can have holes, the Euler number for such an object is defined as the number of connected regions minus the number of holes. The number of holes is one less than the connected regions in the set compliment of the object. It is important to report the Euler number together with the connectivity applied, i.e., 4-connectivity or 8-connectivity.

3.1.6
Feret diameter
distance between two parallel tangents on opposite sides of the image of a particle

3.1.7
equivalent circular diameter
ecd
diameter of a circle having the same area as the projected image of the particle

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NOTE It is also known as the Haywood Diameter.

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3.1.8
grey image
image in which multiple grey level values are permitted for each pixel

3.1.9
image analysis
processing and data reduction operation which yields a numerical or logical result from an image

3.1.10
numerical aperture
NA
product of the refractive index of the object space and the sine of the semi-aperture of the cone of rays entering the entrance pupil of the objective lens from the object point

3.1.11
pixel
picture element
individual sample in a digital image that has been formed by uniform sampling in both the horizontal and vertical directions

3.1.12
segmentation
⟨noun⟩ part into which something can be divided; subdivision or section

3.1.13
segmentation
⟨verb⟩ act of dividing something into segments

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3.1.14
threshold
grey level value which is set to discriminate objects of interest from background

3.2 Symbols

δ	error
θ	half-angle subtended by the particle at the objective lens
λ	wavelength, expressed in micrometres
μ	refractive index of the surrounding medium
φ	shape factor
A_i	projected area of particle i
d	minimum feature length
H_{cal}	horizontal calibration factor
K	constant numerically determined by the confidence limit
N	number of particles to be measured
n_i	numbers of particles of size X_i
P	probability
P_i	probability that particle i exists in the measurement frame (also called Miles-Lantuejoul factor)
V_{cal}	vertical calibration factor
V_i	relative volume of particle i
X_{A}	diameter of spherical particle i
X_{Ai}	area equivalent diameter of particle i
X_{F1}	horizontal Feret diameter of object
X_{F2}	vertical Feret diameter of object
X_i	dimension of particle i
$X_{i\,\mathrm{max}}$	longest dimension of particle i, also called maximum Feret diameter
$X_{i\,\mathrm{min}}$	shortest dimension of particle i, also called minimum Feret diameter
X_{LIL}	lower limit of a class interval
X_{mean}	mean of X_i
X_{UIL}	upper limit of a class interval
X_1	horizontal dimension of object

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$X_{1,m}$	horizontal dimension, expressed in micrometres
$X_{1,p}$	horizontal dimension, expressed in pixels
X_2	vertical dimension of object
$X_{2,m}$	vertical dimension, expressed in micrometres
$X_{2,p}$	vertical dimension, expressed in pixels
Z_1	horizontal side length of the rectangular measurement frame
Z_2	vertical side length of the rectangular measurement frame

4 Sample preparation demands for method description

4.1 General recommendations

4.1.1 General

The following recommendations provide a sampling of standard microscopy practices.

NOTE See References [4], [5] and [10] for additional suggestions.

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4.1.2 Sample subdivision

As only a small amount is needed to prepare a sample, the whole sample shall be subdivided in a manner that ensures that the portion taken is representative of the whole.

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The method used to subdivide the sample is likely to be dictated by the sample preparation method and will be decided by the laboratory performing the analysis.

Provided that the sample is well dispersed by the method and that there is no segregation of particles by size, the choice of method is left to the expertise of the laboratory, since any specialized equipment required by a particular method might not be available to all.

4.1.3 Touching particles

The number of particles touching each other should be minimized.

It is a prime requirement of the method that measurements shall be made on isolated particles. There should be as few particles as possible touching each other. Touching particles measured as one particle without a proper separation will introduce error.

4.1.4 Particle distribution

There should be an adequate distribution of particles on the sample support. The whole area of the preparation should be examined to ascertain whether there is noticeable segregation of particles (by size). Statistical comparison of the results on a frame-by-frame basis will test for uniform distribution of particles. This procedure is detailed in Clause 7.

4.1.5 Sample preparation

Electron microscope samples should be coated with a thin layer of metal (e.g. Au, Au/Pd, Pt/Pd) to reduce charging effects.

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Samples should be examined as soon as possible after preparation, and should contain an expiration date.

The sample preparation method used should be fully described in the final particle size analysis report by giving quantitative details of the nominal masses, volumes and compositions of particles and products used at each stage of the preparation procedure.

4.1.6 Number of particles to be counted

The number of particles measured should be determined based on the particle-size distribution and the desired confidence limits. Assuming the particles are log-normally distributed, the required number (N) of particles with a given error (δ) and a given confidence limit is estimated in accordance with Equation (1):

$$\log N = -2 \log \delta + K \quad (1)$$

where K is numerically determined by the confidence limit, particle distribution and other parameters; see References [1] and [2].

NOTE See Annex A for detailed information.

4.2 Suggested preparation methods

Several methods can be investigated for preparing samples for measurement. The following methods may be used. They are based on the assumption that a representative sample be used to give an adequate dispersion of the particles and a sharply contrasted image.

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4.2.1 Camphor-naphthalene (C-N) method

This method uses a eutectic mixture of 60 % mass fraction camphor and 40 % mass fraction naphthalene that melts at 32 °C and sublimates rapidly in a vacuum. To prepare the sample, a 1 g sample of the particles to be counted is kneaded by hand inside a plastic bag with the requisite amount of the C-N eutectic mixture. When the particles sample is fully disaggregated and well dispersed in the C-N by the heat of the hand, the plastic bag is cooled to solidify the resulting mixture. Small lumps of this solid mixture are then transferred to a microscope slide resting on a warm plate. The sample, when melted, is flattened under a cover-slip that is afterwards removed to allow the C-N eutectic to sublimate under vacuum.

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This technique was found to give good dispersion of irregular quartz particles and has the advantages that the particles are viewed in air, which results in a good contrast in the refractive index, and that the slides do not age. However, tests with glass beads have been unsuccessful, as the particles segregate on the slide, do not stick well and tend to roll off, making the method unusable; see Reference [3].

4.2.2 Paste-dilution method

A sample of about 1 g of particles is mixed with a viscous liquid (gelatine, sucrose or glycerol in water, collodion in amyl acetate) on a watch glass with a spatula to give a thick paste, thus ensuring mechanical disaggregation and dispersion. A sample of the paste is then taken with the point of a spatula and diluted in the same viscous liquid to a concentration such that, after homogenization, one drop of the resulting suspension, flattened under a cover-slip, will give the required number of particles on a microscope slide, that is, about 20 particles per view frame. Depending on the choice of liquid, the slides can have only a temporary life or might be able to be stored indefinitely. Using glycerol, this method has been successful for glass beads. It gives a good uniform dispersion and a reasonably contrasted image. The use of a cover-slip aids resolution with high-magnification objectives. However, the slides tend to dry out within an hour or so and repeat counts with the same slide are not possible; see Reference [4].

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4.2.3 Filtration methods

4.2.3.1 Powder or dry suspensions

A 1 g sample of particles is suspended in a suitable liquid and dispersed. A given volume of this suspension is then filtered to dryness on a suitable membrane. The concentration of the suspension and the membrane area of filtration are such that the particles are deposited in the required concentration for counting (about 20 particles per measurement frame). After air-drying, the membrane is cut into small sections which are attached by their edges to a microscope slide using an acetone-resistant glue (e.g. cyanoacrylate or "super-glue"). The gluing is to prevent the membrane from shrinking. The slide is then put in a closed container on a support over a free surface of liquid acetone, whose vapour renders the membrane transparent for viewing and particle-counting. The method has the advantage that the particles are viewed in air giving a good contrast in refractive index. Tests indicate that to avoid the membrane re-opacifying, it is preferable to perform the exposure to acetone very slowly over several hours; see Reference [5].

4.2.3.2 Liquid suspensions

A known volume of suspension, typically 100 ml, is vacuum-filtered, as described below, through a membrane of compatible material and known pore size, typically 0,8 µm cellulose nitrate for mineral oils. Particles larger than the pore size should appear well scattered across the membrane with little or no overlap. If the number of particles is too great and overlapping is excessive, the test should be repeated with a smaller known volume of suspension. Conversely, if the number of particles is too few, a greater volume of known amount should be used. The vacuum arrangement, for example a Millipore[1)] filtration system, consists of a membrane holder attached to an open flask, with a vacuum pump attached below the filter holder. A separate spray container with an integral filter attachment, typically 0,45 µm, is used with a compatible solvent to wash down the sides of the open flask to ensure that all particles are collected on the membrane for analysis, and to remove the liquid from the suspension, leaving a reasonably dry membrane for examination. The membrane should be examined as soon as possible; if there is a delay, it can be inserted between two pre-cleaned microscope slides. Appropriate glue for making the membrane transparent may be used; see Reference [6].

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4.2.4 Dry deposition method

Particles may be prepared for counting by dry deposition onto a slide covered with double-sided transparent adhesive tape. Care shall be taken that all the particles in a given sample effectively stick on the slide, so as to ensure that there is no selective capture of particles by size. A microscope slide is positioned in the bottom of a vacuum chamber having a volume of about 1 l. A conical metal plug is fitted in the top of the chamber and the particles to be analyzed are placed in a groove all around the plug. When the vacuum is released by lifting the plug, the particles are sucked as a cloud into the chamber and fall on the slide. Adhesion on the slide may be enhanced by using double-sided tape or a film of grease. This method also has the advantage that particles are viewed in air, resulting in a good contrast in refractive index.

5 Image capture

5.1 General

Particle-size data can be influenced by specific parameters affecting the image formation process. It is possible to distort the reported size, particularly of the smallest particles, by using inappropriate image-capture conditions, e.g. magnification, illumination, etc. Distortion in the image might arise from a number of causes, but its presence and effect on the image can be measured by selecting a recognizable object at a number of points and orientations in a frame of view. It is important to note that the measurements made provide only two-dimensional, X and Y, information. The imaging instrument should be set up and operated in accordance with its manufacturer's recommendations considering the conditions given below.

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1) Millipore is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 13322 and does not constitute an endorsement by ISO of this product.

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5.2 Procedures

At each operating condition used for the analysis, carry out the following steps.

a) Select a recognizable object in the image.

b) Place the feature in the centre and at the corners of the field of view in turn and measure its horizontal length (X_1).

c) Rotate the sample stage 90 degrees and repeat the measurements (X_2).

d) Record the values of X_1 and X_2 with the final result.

e) Calibrate the imaging instrument prior to the examination of samples using a certified graticule or equivalent.

f) If possible, mount the traceable calibration graticule together with the specimen in the imaging instrument.

g) Select the magnification in accordance with Annex B or Annex C and set the corresponding illumination and imaging conditions.

h) Place the calibration grating in the field of view, select a suitable area and focus it.

i) Obtain the image to be analyzed and then capture it either digitally or by use of a suitable photographic image.

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j) Record a significant number of measurement frames for each sample by scanning the sample in a raster pattern as indicated in Figure 1. Once this operation is started, no changes to the operating conditions should be made.

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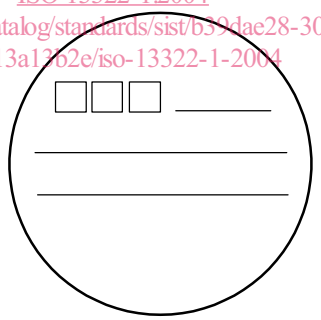

Figure 1 — Sample raster pattern

k) At the end of measurement, place the calibration graticule in the field of view and check the calibration once more. The comparison of two calibration images taken at the beginning and the end of the examination will provide a measure of the variability in instrument magnification.

l) Report the calibration constants obtained before and after the analysis together with the precise details of the microscope settings (working distance, spot size, electron microscope magnification, etc.).

5.3 Operating conditions for an image capture instrument

5.3.1 General

There are various imaging systems used for particle sizing. The setup for particle sizing using an electron or optical microscope is briefly described in 5.3.2 and 5.3.3.

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