

BS ISO 13322-1:2014



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Particle size analysis — Image analysis methods

Part 1: Static image analysis methods

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National foreword

This British Standard is the UK implementation of ISO 13322-1:2014. It supersedes BS ISO 13322-1:2005 which is withdrawn.

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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*



Reference number
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Foreword

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

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization*.

This second edition cancels and replaces the first edition (ISO 13322-1:2004), which has been technically revised.

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

- *Part 1: Static image analysis methods*
- *Part 2: Dynamic image analysis methods*

Introduction

The purpose of this part of ISO 13322 is to give guidance when using images for particle size analysis.

Image analysis is a technique that has gained popularity in different applications. The aim of this part of ISO 13322 is to give a standardized description of the technique used and its validation. This part of ISO 13322 does not describe specific instruments and is restricted to those parts of the acquisition of images that are relevant to the accuracy of the particle size analysis.

This part of ISO 13322 includes methods of calibration verification and recommends using a certified standard as a reference scale. However it is sensible to make some measurements on particles under study, or other reference objects, of known size so that the likely systematic uncertainties introduced by the equipment can be assessed.

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 measurements and it is important to obtain estimates for the uncertainty arising from each stage.

Essential operations are identified to ensure that measurements made conform to this part of ISO 13322 and are traceable.

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 where the velocity of the particles against the axis of the optical system of the imaging device is zero. The particles are appropriately dispersed and fixed in the object plane of the instrument. The field of view may sample the object plane dynamically either by moving the sample support or the camera provided this can be accomplished without any motion effects on the image. Captured images can be analysed subsequently.

This part of ISO 13322 concentrates upon the analysis of digital images created from either light or electron detection systems. It does not address the method of creating the image although the detection settings chosen together with its calibration are important to particle sizing accuracy. This part of ISO 13322 considers only image evaluation methods using complete pixel counts.

Both the type of distribution, (by number or by volume) together with the width of the particle size distribution has a very material influence upon the number of particles to be measured to secure the desired accuracy within the specified confidence limits. An example is shown in [Annex A](#).

Automation of the analysis is possible in order to measure sufficient particle numbers for a required degree of precision.

This part of ISO 13322 does not address the sample preparation. However, the sub sampling, dispersion and presentation of particles to be measured are a vital part of the operational chain of actions necessary to ensure accuracy and precision of any final result.

NOTE Further details about sampling and sample preparation can be found in ISO 14887 and ISO 14488.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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: The calculations of average particle sizes/diameters and moments from particle size distributions*

ISO 14488, *Particulate materials — Sampling and sample splitting for the determination of particulate properties*

3 Terms and definitions and list of symbols

3.1 Terms and definitions

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

3.1.1
area equivalent diameter

diameter of a circle having the same area as the projected image of the particle

Note 1 to entry: It is also known as the Heywood diameter or as the equivalent circular diameter.

3.1.2
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

3.1.3
contrast (of an image)

<particle size analysis> difference between the intensity of the particle image with respect to the background near to the particle

3.1.4
edge detection

methods used to detect transition between objects and background

Note 1 to entry: See *segmentation method* ([3.1.13](#)).

3.1.5
Feret diameter

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

3.1.6
field of view

field which is viewed by the viewing device

Note 1 to entry: The full image frame of a digital imaging device corresponds to its field of view.

SEE: [Figure 1](#).

3.1.7
grey image

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

3.1.8
image analysis

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

3.1.9
measurement field

field which is composed by the set of all measurement frames

SEE: [Figure 1](#).

3.1.10
measurement frame

selected area from the field of view in which particles are sized and counted for image analysis

SEE: [Figure 1](#).

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

raster pattern

scanning order of measurement frames in the total measurement field

SEE: [Figure 1](#).

3.1.13

segmentation method

strategy employed to separate the objects of interest from their surroundings

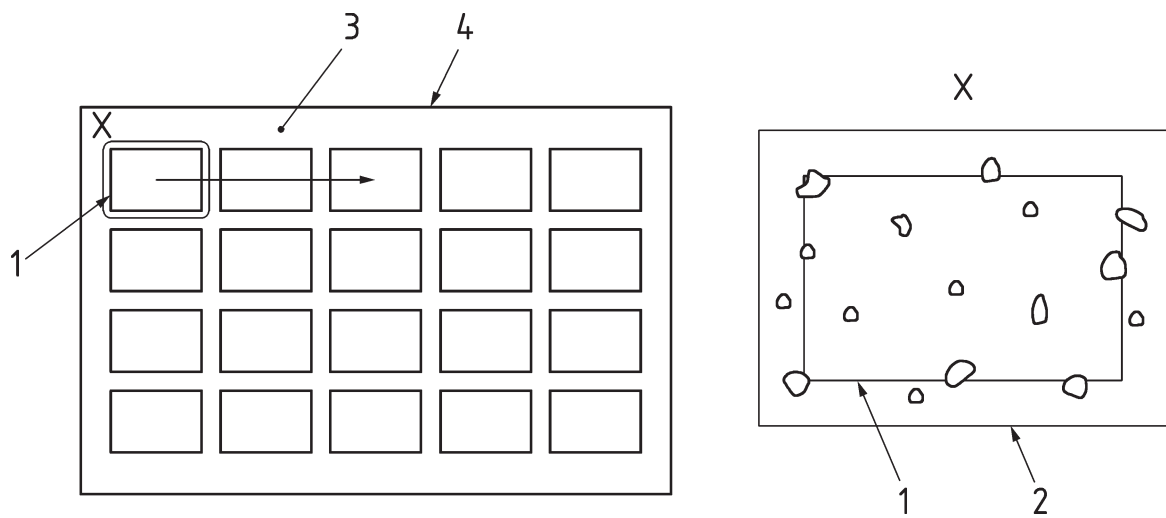
Note 1 to entry: Method of dividing the particle image from the background.

Note 2 to entry: See *edge detection* ([3.1.4](#)).

3.1.14

threshold

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



Key

- 1 measurement frame
- 2 field of view
- 3 raster pattern of measurement frames
- 4 measurement field
- X enlarged view of a field of view

Figure 1 — Relationship between the terms “field of view”, “measurement frame”, “raster pattern” and “measurement field”

3.2 Symbols

A_i	projected area of particle i
α_1	horizontal calibration factor
α_2	vertical calibration factor
d	minimum feature length
d_c	diameter of a circle
N	number of particles to be measured
n_c	measured number of pixels within a circle
n_j	numbers of particles in size interval Δx_j
P_i	probability that particle i exists in the measuring frame (also called Miles-Lantuéjoul factor)
φ_i	shape descriptor
σ	standard deviation
V_i	volume of particle i
$x_{A,i}$	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
$x_{Fmax,i}$	longest dimension of particle i , also called maximum Feret diameter
$x_{Fmin,i}$	shortest dimension of particle i , also called minimum Feret diameter
x_1	horizontal dimension of object
$x_{1,m}$	horizontal dimension of object in SI unit
$x_{1,p}$	horizontal dimension of object in pixel
x_2	vertical dimension of object
$x_{2,m}$	vertical dimension of object in SI unit
$x_{2,p}$	vertical dimension of object in pixel
$x_{10,3}$	particle size corresponding to 10 % of the cumulative undersize distribution by volume
$x_{90,3}$	particle size corresponding to 90 % of the cumulative undersize distribution by volume
Z_1	horizontal side length of the rectangular measurement frame
Z_2	vertical side length of the rectangular measurement frame

4 Preparation for image capture

4.1 Introduction

A pre-requisite for accurate particle size measurement using this method requires a full understanding of the settings and calibration applied within the image capture device as well as a consideration of the purpose for conducting the measurement.

The final settings and calibration of the image capture device need to be established via an iterative approach. The size range of the particles within an unknown test sample has an influence upon the settings required within the image capturing device. These remain unknown until the first image has been taken, the result observed and the necessary adjustments to the image capture device to achieve the desired accuracy of particle size measurement required. A fully trained operator shall conduct the assessment.

The imaging instrument should be set up and operated in accordance with the manufacturer's recommendations considering the conditions prevailing.

In order to achieve accurate particle size measurements it is preferred that the illumination be uniform over the total field of view and of a type designed to create images of high contrast. The magnification should be such as to provide a minimum number of pixels for the smallest particle consistent with the accuracy demanded and set to achieve a sharp focus. The number of pixels for the smallest dimension of a particle is relevant for cases where linear dimensions or combinations thereof are measured.

Distortion in the image might arise from a number of causes, but its presence and effect on the image may be determined by selecting known sized particles or other reference objects of similar optical properties at a number of points and orientations in the field of view. It is important to note that the measurements made provide only two-dimensional, X and Y, information.

4.2 Procedures

The operator should decide why the result of the image analysis is required. Is a size distribution by the number of particles in each size class required or is the volume of particles in each size class the requirement? What accuracy and precision is required for the final result? These decisions will have a significant influence upon the choice of settings and the method employed in conducting the measurement.

For each material to be analysed and for each instrument employed the person conducting the analysis shall ensure that the following procedures are followed.

- a) Ensure an adequate calibration for both the X and Y-axis of the measurement frame exists for the imaging instrument being employed, preferably by using a certified graticule or equivalent reference of equal standing.
- b) Ensure that the optical magnification employed is suitable and such as the image of the smallest particle to be measured covers a sufficient number of pixels to support the required accuracy of measurement.
- c) Ensure that the illumination method and setting of any focus is correctly established to give a good contrast and uniformity of illumination of any image gathered.
- d) Ensure that the number of particles within the measurement frame is such as to minimize the number of touching particles.
- e) Ensure that a sufficient number of images of separate aliquot samples are gathered to provide a suitable total number of particles with respect to the type of distribution, number or volume based, and the width of the particle size distribution (see ISO 14488) and that they contain an adequate statistical number of the largest particle of the target material (see [Annex A](#)).

- f) Some implementations of the image analysis technique employ a large area X, Y servo or manually controlled sample slide assembly. Such large slides enable many measurement frames of the particles deposited to be examined. Should the method of fully separate measurement frames be employed then any frame overlap shall be avoided. If the method of overlapping measurement frames, or other methods of analysing particles that interact with the measurement frame edge is used, then procedures shall be employed to ensure that each particle is only included into the total count for the appropriate size class, once. For more information, see [8.3](#).
- g) If appropriate, ensure that the image quality consisting of illumination, focus and magnification has not changed at the end of the measurement. The requirement for this step depends on the variability of the instrument employed.
- h) For the case when image analysis is to be used for certified reference material measurement at the end of the image gathering procedure, the calibration outlined in a) should be repeated and any measured deviation recorded.
- i) All the conditions, set up or established, for the target material shall be fully documented.

5 Sample preparation demands for method description

5.1 Sample splitting and reduction

As only a small amount of material is needed to prepare a test sample, this should be sub-divided from the whole sample in a manner that ensures that the test sample is representative of the whole as specified in ISO 14488.

5.2 Touching particles

In order to assess the degree of touching particles, a suitable optical resolution setting of the imaging system should be chosen. The optical resolution should also meet the criteria set out in [4.2 b\)](#).

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. Touching particles measured as one particle without a proper separation will introduce error.

It is often not possible to reliably detect touching particles by image analysis alone, but the influence of touching particles on the result can be investigated experimentally by increasing or decreasing the number of particles per image. If the number of particles cannot be changed, the influence on the results can be investigated using a reference material with similar size and shape.

5.3 Particle distribution

There should be an adequate distribution of particles in the field of view. It may be necessary to examine several fields of view if a large total particle count is required. The whole area of the measurement field should be examined to ascertain whether there is noticeable segregation of particles (by size). The requirements set out in [4.2 f\)](#) should be followed.

5.4 Number of particles to be counted

The number of particles to be analysed depends upon whether the final result is a particle size distribution by the number or by the volume of particles. Considerable care has to be exercised in order to ensure that the analysis is representative of the bulk sample as described in ISO 14488. This can be demonstrated by splitting the bulk sample into at least three test samples. Each test sample should contain sufficient particle numbers for a full measurement. Statistical analysis of the data will reveal the repeatability of the method including sampling and dispersion.

NOTE See [Annex A](#) for more information.

5.5 Particle suspending fluid

It is likely that a large number of particle measurements will be of particles presented in a gas where an adequate image contrast should be ensured. Should particle presentation require a liquid suspension then it is preferred that such liquids be clean, particle-free, transparent and have a refractive index as different as possible from the refractive index of particles to enhance the image contrast. Particles presented in a mixed optical background such as in biological specimens, may require dynamic particle by particle threshold selection.

WARNING — Automated particle by particle parameter selection applied for segmentation as envisaged when mixed optical backgrounds are required cannot be validated for true particle size and may result in particle size bias and reduced accuracy.

CAUTION — Particle systems of mixed optical properties may have inaccurate particle sizes attributed to them due to possible threshold setting errors introducing particle size bias.

6 Quality of captured images

6.1 General

It is important for the analysis that the particles in the captured images are well dispersed. The number of overlapping or touching particles reduces with the concentration of the particles in the frame (see 5.2). This is in conflict with the requirements of having a large particle number to obtain a high degree of precision. A compromise should be established.

The contrast achieved should be consistent with the level of accuracy required. The difference between the brightness of the particle and its background shall be a few times of the resolution of the grey level image from zero to maximum brightness signal.

The accuracy of the results is strongly affected by the number of pixels for each particle image. When only the projected area of the particles is measured as an average value, a few pixels may provide acceptable results for the smallest particles. Higher pixel numbers are required for accurate information about each individual particle.

6.2 Pixels per particle

Both the number of pixels forming the image of the particle and the relative position of the centring of the image with respect to the fixed pixel pattern can have a material influence upon the final particle size assessed from each particle image.

Image analysis can be a method of choice for the certification of reference materials. It also can be the method of choice for general measurements. The conditions required to achieve a defined accuracy and precision may be quite different for these two cases and warrant separate approaches.

6.2.1 Characterization of reference materials

Materials selected for reference materials are often spherical and having limited or mono dispersed size distributions. In order to characterize such materials to a high order of accuracy requires that each particle cover a substantial number of pixels. Errors in particle size assignment from digitized images arise from two sources^[9].

- a) The number of pixels covered by the particle image in combination with the relative position of the centring of the particle image with respect to the fixed, pixel pattern. A limited number of pixels per image results in a variation in the reported size as a result of image centring with respect to the fixed pixel array and due to the finite number of pixels. This results in a finite broadening of the particle size distribution even from mono-sized particles.
- b) The setting and control of the threshold of pixel detection. Any error in the setting of the threshold level to decide whether a pixel is included or excluded, results in a bias to the size reported. The

influence of the threshold setting shall be carefully examined as demonstrated in Reference [12]. Any automated threshold setting algorithm which cannot demonstrate not inducing any error or bias shall not be used for the purpose of characterization of reference materials.

For a circle of diameter, d_c , expressed in pixel units and covering a measured number of pixels, n_c , the standard deviation of n_c may be approximated by (see Reference [4]):

$$\sigma(n_c) = 0,68 \left(\frac{d_c}{2} \right)^{\frac{1}{2}} \quad (1)$$

The number of pixels, n_c , to form the image of a particle of diameter, d_c , can be estimated using Formula (1) for any standard deviation σ .

NOTE A larger number of pixels per particle also introduces a restriction upon the number of particles per image frame that can be counted per slide.

6.2.2 General particle sizing

In this more general case, a wider particle size range is most likely. For this a balance shall be achieved, whereby the largest particles are readily contained within the image frame restriction, while providing sufficient pixels to describe the smallest particle to the desired accuracy. The threshold level may be set as described in 8.2.

7 Image analysis

7.1 General

Modern image analyzers usually have algorithms available for enhancing the quality of the image prior to analysis and for separating touching particles. It is permitted to use enhancement algorithms provided that the measurements can be unambiguously associated with the particles in the original image and the enhancement can be verified as not introducing additional errors of particle size or likely to bias the final result. Irregularly shaped particles or particles with sharp corners should not be separated since this would distort the shape of the particles. All touching irregular shaped particles should be rejected from the measurement and a note should be made for the proportion of particles rejected from each measurement frame, see 8.4. Touching spherical particles may be separated, as this gives only minor distortion of the area of particles. A flow chart showing typical procedures used in carrying out measurements by image analysis is given in Figure C.1.

7.2 Size classes and magnification

The theoretical limit for resolution of objects by size using image analysis is one pixel, although the uncertainty of particle size increases with very low pixel numbers. Discretized representations should be stored particle-by-particle with a resolution of one pixel. Note that any compression of images might reduce the accuracy and the resolution. However, it is necessary to define the size classes for the final reporting of results; the desire for maximum dynamic range of sizes covered in each frame should be balanced by the necessity for accuracy, which is a function of the total number of particles counted, the dynamic range and the number of pixels included in the smallest objects to be considered. It is recommended that pixels be converted to SI length units prior to any reporting of size for quantitative analysis.

The magnification used should be such that the smallest particles counted have a projected area sufficient to cover an adequate number of pixels to meet the accuracy required. All particles measured should be sized and stored with a resolution of one pixel. The final results are to be reported by grouping the particles into size classes. For samples with a narrow size distribution, the grouping may be based on a linear progression and for samples with a wide size distribution; the grouping may be based on a logarithmic progression. The intervals for these progressions should be based on the dynamic range and

total number of particles counted. The particles assigned to a given class are those that have a diameter that is equal to or greater than the lower limit of the class interval and less than the upper limit.

8 Counting procedure

8.1 General

The particle size distributions should be determined by counting those particle images that have been accepted as passing the software selected criteria being employed for each measurement frame and then summing these over all of the frames.

8.2 Particle image edges

Several segmentation methods exist for particle contour detection. These are for instance:

- a) thresholding;
- b) edge detection.

NOTE 1 See [Annex B](#) for more information.

These methods should be tested with reference materials in order to find out what method and parameter set give the best approximation of the reference materials result and are suitable for the material that has to be characterized.

NOTE 2 In the field of optical microscopy, the optical appearance of a real particle depends on the refractive index of the particle, the refractive index of the surrounding medium, the surface structure, and the type of illumination. Furthermore the optical particle image on the sensor may be slightly out of focus and is digitally sampled on a discrete pixel grid. All these effects have influence on the particle image edges.

A threshold method can be established manually, if necessary.

EXAMPLE If a half-amplitude method is applicable, a small region of the background located a few pixels away from the boundary of a typical particle is selected to establish the background value. The amplitude of signal from pixels just fully responding to the particles presence is selected to establish the foreground value. The threshold level is to be set at the average of these two values; see Reference [2].

NOTE 3 Manual threshold levels may be subjectively checked by direct comparison of the threshold image with that of the original image. This subjective method does not constitute validation but readily detects incorrect settings.

A second option is to “auto-threshold” the image. Such an auto-threshold procedure shall be validated against a certified reference material having optical properties similar to that of the particles under test. This can be a certified reference material or a certified reticule. Ensure that the threshold applied is independent of particle size.

CAUTION — The use of a graticule or a reference material having different optical properties to that of the material under test especially when the particles are suspended in a liquid can lead to substantial bias in the reported particle size when this reference is used to establish a threshold level.

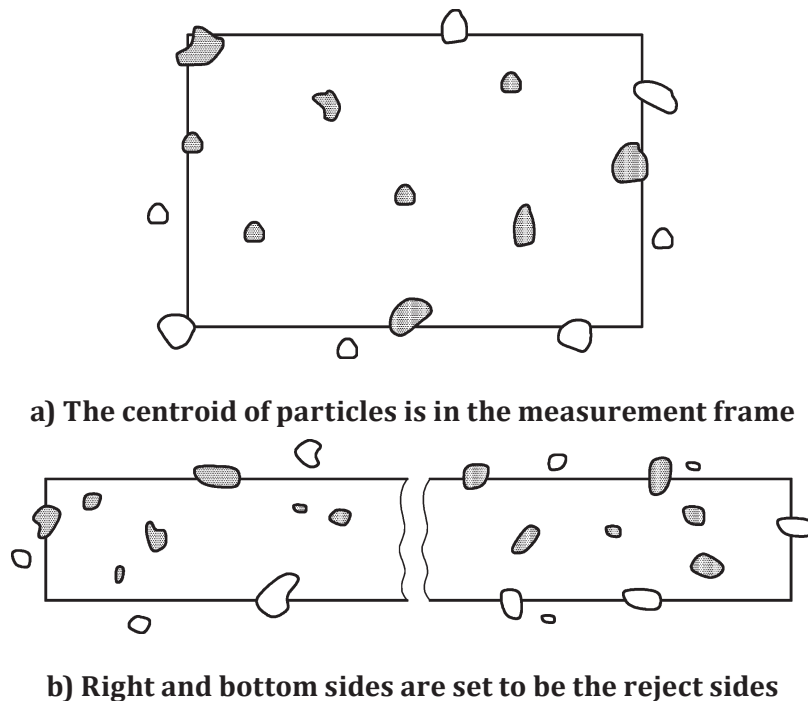
CAUTION — Failure to set an appropriate threshold level can lead to significant bias in the determination of the particles size. This bias depends on particle size. All particles are affected but the relative value of influence to the particles size increases with decreasing particle size under a given resolution.

8.3 Particles cut by the edge of the measurement frame

8.3.1 Method of counting all particles in a measurement frame

If all the objects that appear within the image frame (field of view) are accepted for measurement, the accuracy of the final distribution will be impaired because some of the objects will be cut by the edge of the image frame. To overcome this, a measurement frame is defined within the image frame. The measurement frame can be used in the following two ways.

- a) All the objects are allocated one pixel (e.g. the centroid) as the feature count point. Objects are accepted only when their feature count point lies within the measurement frame; see [Figure 2 a\)](#). The measurement frame can be of any shape provided that there is enough space between the edges of the two frames so that no accepted particle is cut by the edge of the image frame.
- b) A rectangular frame is used with the bottom and right edges defined as reject sides. Objects lying partially or wholly within the measurement frame and not touching the reject sides are accepted; see [Figure 2 b\)](#). There has to be sufficient space between the top and left edges of the two frames so that no accepted objects are cut by the edge of the image frame. This covers all eventualities except for particles intersecting two opposite sides of the frame; these would either be too large to be measured at the magnification or would be so acicular that it is unsuitable for classification by area anyway. Image analysis systems that reject all particles intersecting a frame edge use an effective frame size that is different for each size class and also different for each particle shape.



NOTE Shaded particles are included in count; unshaded particles are excluded from count.

Figure 2 — Treatment of particles cut by the edge of the measurement frame

8.3.2 Method of neglecting particles cut by the edge of a measurement frame

All particles entirely inside the measurement frame are accepted for counting. All particles outside, or cut by the edge, are neglected. This creates the situation where the probability for a particle to be included in the measurement frame varies inversely with the size of the particle. This, therefore, introduces a bias that is greater the larger the size of particle considered. The probability, P_i , of a particle i having

a horizontal Feret diameter of object x_{F1} and a vertical Feret diameter of object x_{F2} in a rectangular measurement frame of size Z_1 by Z_2 is given by Formula (2) (see References [3][5]):

$$P_i = \frac{(Z_1 - x_{F1})(Z_2 - x_{F2})}{Z_1 Z_2} \quad (2)$$

The population of particles in the measurement frame should, therefore, be divided by the probability, P_i .

NOTE A very large number of frames may be required to minimize the error created by the edge effect influence, especially when the particles are no longer very small compared to the frame size.

EXAMPLE A square frame of size 100 units times 100 units is used for counting a population of particles of sizes ranging from two units to ten units. The count of the particles wholly in the measuring frame and the correction factors are shown in [Table 1](#).

Table 1 — Example of corrected count

Diameter x_i arbitrary unit	Raw count n_i	Probability P_i	Corrected count n_i/P_i
2	81	0,96	84
4	64	0,92	70
6	49	0,88	56
8	36	0,85	42
10	25	0,81	31

8.3.3 Analysis method of overlapping measurement frames

The analysis of overlapping measurement frames provides an alternative method of overcoming edge effects in order to include the maximum number of particles into the total valid particle count. This method requires that the stage movement to expose the next measurement frame be of sufficient accuracy and precision such that each particle can be assigned a positional index. This is an essential requirement to prevent duplication of particle counts.

During the image processing of a measurement frame, particles having interacted with the measurement frame edge are identified and their position listed for analysis in subsequent frames. All other particles wholly within the measurement frame are included into the count and listed as being counted. A degree of frame overlap was previously decided from knowledge of the size distribution and the magnification selected. The overlap should be such that each particle is counted in one of the measurement frames.

This method includes all of those particles in the measurement field which do not interact with the extreme edges of the field.

8.4 Touching particles

The slide preparation method should be chosen to give a minimum number of touching particles. Nevertheless it is inevitable that there will be touching particles in each measurement frame and some method for dealing with them is necessary.

However, the first difficulty is to have an automatic method of identifying touching particles. This can be done by:

- a) following the number of particles “created” by numerical separation procedure;
- b) some criterion such as the shape factor or even;
- c) by manual intervention; the statistical procedure for evaluating slides may also give some indication.

Numerical separation procedures are not recommended for separating particle aggregates into individual particles as they can change the size of the particles in the image. Particle size distributions obtained by such methods have no traceability. Such procedures for identifying aggregates can be investigated by comparing the results with the size counting performed on the original untreated image. Touching spherical particles may be separated, as this should give only minor distortion of the area of the particle.

Identification of touching particles on the basis of shape is not fool proof, in particular for compact overlapping agglomerates, and will not distinguish real out-of-shape or oversized particles. In cases where touching particles cannot be avoided, careful use of various techniques, e.g. fractal analysis to identify aggregates or model-based separation techniques may be used to separate the particles. This procedure should not be employed within certified reference material measurement procedures.

8.5 Measurements

The measurement of the perimeter of particles depends strongly on the image-analysis system used. Accordingly, the primary measurement is the projected area of each particle, expressed in pixels, then the longest and the shortest Feret diameters of each particle, $x_{Fmax,i}$ and $x_{Fmin,i}$, expressed in pixels. This method facilitates the definition of a shape factor with the greatest discrimination. It is therefore recommended that the primary values be:

- a) area of each object A_i ;
- b) longest dimension of each particle, maximum Feret diameter, $x_{Fmax,i}$;
- c) shortest dimension of each particle, minimum Feret diameter, $x_{Fmin,i}$;

These parameters are to be used to calculate the area equivalent diameter, $x_{A,i}$, in accordance with Formula (3) and the shape descriptor, φ_i , in accordance with Formula (4).

$$x_{A,i} = \sqrt{\frac{4A_i}{\pi}} \quad (3)$$

$$\varphi_i = \frac{x_{Fmin,i}}{x_{Fmax,i}} \quad (4)$$

Appropriate correction shall obviously be made if the equipment used is not based on square pixels. In order to compare the results obtained by the image analysis method with those obtained by the volumetric or mass-based method, the volume, V_i , of each particle i can be calculated from the projected area-equivalent diameter, $x_{A,i}$, of the particle according to Formula (5).

$$V_i = \frac{\pi(x_{A,i})^3}{6} \quad (5)$$

9 Calculation of the particle size results

Particle sizing, using the image analysis method results in a number distribution where the counts within each size class may have been corrected using the methods described in [Clause 8](#).

The calculation of mean particle diameters is described in the different parts of ISO 9276.

10 Calibration and traceability

10.1 General

The equipment is first calibrated to convert pixels into SI length units, e.g. nanometres, micrometres, millimetres, etc. for the final results. The calibration procedure shall include verification of the uniformity of the field of view. An essential requirement of the calibration procedure is that all measurements shall

be traceable to the standard metre. This can be done by calibration of the image analysis equipment with a certified standard stage micrometre. The calibration shall be done with the same illuminating and contrast conditions as the regular analysis.

EXAMPLE A certified chrome-on-glass reference stage graticule is available from the National Physical Laboratory (UK); certified spherical particle reference materials SRM 475 and SRM 484 are available from the National Institute of Standards Technology (US); also certified reference materials from commercial sources can ensure traceability to the SI.

10.2 Recommendations and requirements

10.2.1 Touching particles

Each object in an image frame should be counted and reported in the results together with its area, maximum and minimum Feret diameters.

If an excessive number of touching particles is observed, reduce the number of particles per image by dilution of the sample and repeat the measurement using more frames (see [8.4](#) and [5.2](#)).

10.2.2 Distortion of images

Distortion is identified as follows.

- a) Select a square on a multiple-square grid feature from a reference stage graticule. Place it at the centre and measure its width, x_1 , and its height, x_2 . The number of pixels included with the x_1 , x_2 , area shall be sufficient to achieve the desired calibration accuracy.
- b) Place it at each of the four corners and measure its width, x_1 , and its height, x_2 , at each of the four additional positions.
- c) Report the five values of x_1 , and x_2 with the final results.

10.2.3 Calibration

Each setting of the imaging instrument is calibrated as follows.

- a) Determine the correspondence between image size in pixels and the actual size in SI units using the multiple square grid feature on the reference stage graticule.
- b) Report the results as, α_1 calculated in accordance with Formula (6), and, α_2 calculated in accordance with Formula (7):

$$\alpha_1 = \frac{x_{1,m}}{x_{1,p}} \quad (6)$$

where

$x_{1,m}$ is the horizontal actual size of the object;

$x_{1,p}$ is the horizontal size of the object in pixels.

$$\alpha_2 = \frac{x_{2,m}}{x_{2,p}} \quad (7)$$

where

$x_{2,m}$ is the vertical actual size of the object;

$x_{2,p}$ is the vertical size of the object in pixels.

NOTE When using a matrix camera as well as a line scanner, either x_1 or x_2 and either α_1 or α_2 may be reported.

11 Accuracy

11.1 General

The correct operation of the instrument should be verified by a qualification procedure (see [4.2](#)).

11.2 Reference materials

To ensure accuracy, use traceable spherical certified reference materials (CRMs), e.g. particles with certified values that are traceable to results obtained by national metrology institutes, e.g. by direct calibration or by using CRMs produced by these institutes. This ensures that the instrument is correctly functioning as an analytical platform. Should any modifications or major maintenance be required, again use traceable CRMs to ensure the accuracy of the instruments.

For accuracy, traceable spherical CRMs are required, which are declared suitable for the image analysis technique and consist of a known distribution having a range of spherical particles with an $x_{90,3}/x_{10,3}$ ratio of at least 1,5 and also having common density and optical properties. They should be produced according to ISO Guide 34[6] and have certified values for percentage mass fraction or percentage volume fraction that are traceable to the SI and have been assigned by a metrologically valid procedure.

It is essential that a robust procedure is available that fully describes the sub-sampling and sample preparation. Such a procedure shall be followed in its entirety and the title and version number reported. An instrument demonstrates primary accuracy if the results obtained using fully traceable CRM's are demonstrated to be within acceptable limits.

Operational qualification may be demonstrated using non spherical (non-certified) reference materials suitable for image analysis techniques. The particle distribution should consist of a known range of particles with an $x_{90,3}/x_{10,3}$ ratio of at least 1,5.

For non-spherical materials, the aspect ratio shall be restricted to 1:3. The particle size distribution shall consist of documented values coming from image analysis in one or more instrument types according to an agreed, detailed operational procedure which has been shown to yield adequate and stable results over time. If the reference values come from other methods than image analysis, a significant bias may result. If sub-sampling is necessary, exercise due care while using a method that has been proven to yield adequate results (see ISO 14488). If a protocol for sampling, dispersion or measurement is not available, the procedure that is used shall be reported with the final results.

11.3 Instrument preparation

Follow the instructions and advice given in the instrument manual for preparation of the instrument. The functionality of the unit shall have passed the supplying manufacturer's operational qualification (OQ) test or equivalent, with the date and result of the test recorded. The analysis mode, if selectable, shall be suitable for this class of measurement (see [4.2](#)). A well-trained operator shall prepare the instrument and perform the qualification test. The result presentation software shall preferably be set so as to produce an output of the cumulative undersize distribution in accordance with ISO 9276-1.

11.4 Qualification test

The test protocol of the CRM shall be followed during the measurement. Analysis of at least three test portions observed with an adequate number of measurement frames is preferred, for which the average results shall be used. It is preferred that a live display of both the raw image and the digitized representation be made available.

11.5 Qualification acceptance

The qualification acceptance is to apply to CRM materials supplied as number or volume distributions. The 95 % tolerance limits stated for each size value of the CRM specification form a set of maximum and minimum values that define the stated parameter. The qualification test shall be accepted as fulfilling the requirements of this part of ISO 13322 if the resulting measured particle size distribution achieves the following criteria:

- a) the number of accepted particles that are measured exceeds the number required for the desired accuracy within the specified confidence limits;
- b) the number of touching particles is below specified limits; using spherical CRMs their influence on the result may be reduced by using shape descriptors as filters;
- c) the reported values of the particle diameter between the 10th and 30th percentiles of the cumulative undersize distribution by mass or volume do not exceed the quoted maximum or minimum values for the reference material over this percentile range by more than 3 % relative;
- d) the reported values of the particle diameter between the 30th and 70th percentiles of the cumulative undersize distribution by mass or volume do not exceed the quoted maximum or minimum values for the reference material over this percentile range by more than 2,5 % relative;
- e) the reported values of the particle diameter between the 70th and 90th percentiles of the cumulative undersize distribution by mass or volume do not exceed the quoted maximum or minimum values for the reference material over this percentile range by more than 4 % relative.

If a larger deviation is obtained, check all potential error sources and/or seek expert advice. If a higher accuracy is required for any reason, then a CRM should be chosen with a narrow confidence interval, and a total protocol for sampling, dispersion and measurement should be used that guarantees minimum deviations.

12 Test report

The test report should contain the following information:

- a) identification of the test specimen;
- b) reference to this part of ISO 13322 (ISO 13322-1:2014);
- c) complete description of the method used for sub-sample preparation, with full quantitative details of the nominal weights, volumes and compositions of particles and products used at each stage of the sub-sample preparation procedure;
- d) system used (hardware and software);
- e) size of the images;
- f) resolution;
- g) number of frames;
- h) total number of accepted particles.

Usable results should be reported in tables and graphs in accordance with ISO 9276-1 and ISO 9276-2.

A micrograph should be provided of a typical field for each of the samples and for each setting of the imaging instrument.

Annex A (informative)

Estimation of the number of particles to be counted for a given accuracy

A.1 General

A prediction of the minimum number of particles required to be measured in order to achieve a defined level of accuracy within a defined confidence interval has been provided by Masuda and Gotoh[1], Wedd[2] and Yoshida[13]. A key property is the type of the size distribution required. A size distribution described by the volume of particles in each size class requires a greater number of particles to be counted than for a distribution by the number of particles in each size class to achieve a finite accuracy. This is due to the cubic weighting with respect to particle size. It is required counting a thousand, 10 µm-diameter particles to have the same volume as a single 100 µm particle.

A.2 Calculation of the sufficient sample size for the estimation of mean particle diameters

A.2.1 Summary of the method

The value to be calculated, n^* , gives an estimation on how many particles should be sampled to get satisfactory results. More precisely, n^* is the number of particles required to get mean diameter measurement within δ relative error with a probability of P . Reference [1] provides a way, based upon the log-normal distribution by number, to calculate the sufficient number of particles, n^* when the relative error δ and the probability P are given. A short step by step guideline is given below.

An intermediate parameter u defined by the given probability P as described in Formula (A.1) is needed:

$$\phi(-|u|) = \frac{1-P}{2} \tag{A.1}$$

where ϕ is a cumulative distribution function of the standard normal distribution. For the most common probability values of P , the value of u is given in [Table A.1](#).

**Table A.1 — $u(P)$ for some typical probability values above $P = 50\%$
(values below 50% are too low for the confidence level)**

P (%)	50	68,3	75	80	90	95	97,5	99	99,5	99,8	99,9
u	0,67	1,00	1,15	1,28	1,64	1,96	2,24	2,58	2,81	3,09	3,29

Next, the standard deviation, s , of the population diameters has to be calculated. Assuming log-normal distribution by number, it can be derived from 84th and 50th sample percentile diameters using Formula (A.2):

$$s = \ln(s_g) = \ln(x_{84,0}) - \ln(x_{50,0}) \tag{A.2}$$

where s_g is a geometric standard deviation which characterizes the width for log-normal distributions independent on the weighting method for the diameter and \ln is the natural logarithm.

NOTE In Reference [1], the symbols σ and σ_g were used for the standard deviations. They have been adapted and changed to s and s_g as specified in ISO 9276-5 and are also used in [A.2](#).

Having standard deviation s from Formula (A.2) and u from [Table 1](#) (e.g. $u = 1,96$ for the $P = 95$ % probability level), an interim parameter ω is calculated:

$$\omega = u^2 \alpha^2 s^2 (2c^2 s^2 + 1)$$

where

$$c = \beta + \frac{\alpha}{2}$$

α, β are constants defined from a type of the mean diameter to be measured (discussed below).

It is now possible to substitute ω and the relative error δ (e.g. $\delta = 0,01$ for 1 % relative error, or $\delta = 0,0316$ for 3,16 % relative error) to get the final result for n^* :

$$n^* = \omega \delta^{-2} \tag{A.4}$$

[Table A.2](#) provides values for α and β depending on the measured mean diameter type.

Table A.2 — Parameters α and β for different diameter types

Type	α	β
Mass median diameter	6	0
Sauter diameter (mean volume-surface diameter)	5	0
Mean volume diameter	3	0

A.2.2 Example

This example illustrates the calculation of the sufficient number of particles needed to measure the mass median diameter (MMD) within 5 % error ($\delta = 0,05$) with 95 % probability ($P = 0,95$ and $u = 1,96$ from [Table A.1](#)). From [Table A.2](#), we have $\alpha = 6$ and $\beta = 0$. After substitution of α and β we get:

$$n^* = \left[36u^2 s^2 (18s^2 + 1) \right] \delta^{-2}$$

For this particular example, given u and δ , the sufficient number of particles can be calculated as follows:

$$n^* = 55319 \cdot s^2 (18s^2 + 1), \text{ where } s = \ln(s_g)$$

Geometric standard deviation s_g is usually known for the population or can be derived from percentile diameters as shown in Formula (A.2) and can be easily substituted to the formula above. For example, for $s_g = 1,1$, after rounding to integer, $n^* = 585$ is obtained, (see Table 2 in Reference [1]), for this narrow, almost monosized distribution.

A.3 Example using logarithmic normal probability functions for particle size distributions by volume

In this example the minimum number of particles, n^* , required to achieve an uncertainty (expressed as the standard deviation) of a fraction $Q_3 = 90$ % is predicted from given particle size distributions as described in ISO 14488.

The particle size distribution by volume is assumed to be represented by a normal probability density distribution described in terms of a dimensionless variable, z (see ISO 9276-5):

$$q_3^*(z) = \frac{1}{\sqrt{2\pi}} e^{-0,5z^2} \quad (\text{A.5})$$

The logarithmic normal probability distribution is a formulation in which z is defined as a logarithm of x scaled by two parameters, the mean size $x_{50,3}$ and either the dimensionless standard deviation, s , or the geometric standard deviation, s_g , that characterize the distribution:

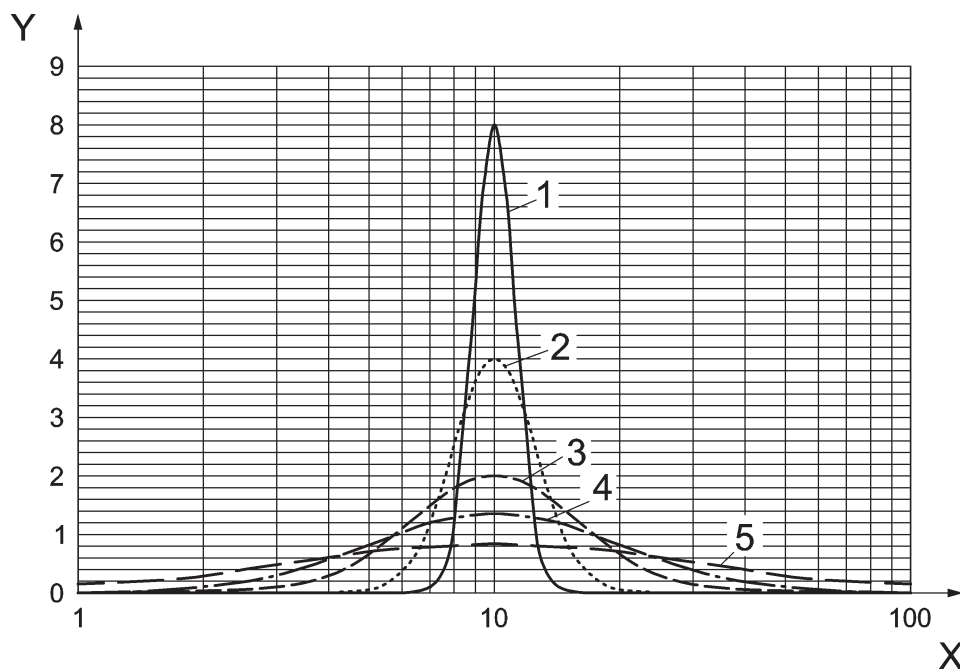
$$z = \frac{1}{s} \ln\left(\frac{x}{x_{50,3}}\right) = \frac{1}{\ln(s_g)} \ln\left(\frac{x}{x_{50,3}}\right) = \frac{1}{\lg(s_g)} \lg\left(\frac{x}{x_{50,3}}\right) \quad (\text{A.6})$$

The geometric standard deviation, s_g , is identical to $x_{84,3}/x_{50,3}$ and is a description for the width of the distribution. The value of $\lg(s_g)$ has been varied from 0,05 to 0,5 (see [Table A.3](#)). \lg is the logarithm to a base of 10.

Table A.3 — Conversion between values for different widths of the distributions used in the example

$\lg(s_g)$	$s_g = \frac{x_{84,3}}{x_{50,3}}$	$\frac{x_{90,3}}{x_{10,3}}$
0,01	1,02	1,06
0,05	1,12	1,34
0,1	1,26	1,80
0,2	1,58	3,26
0,3	2,00	5,87
0,5	3,16	19,12

The mean size, $x_{50,3}$, has been set to 10 μm and the volume-based size distributions have been calculated with a spreadsheet programme using 200 size classes equally spaced on the logarithmic scale between 1 μm and 100 μm . The logarithmic normal probability distributions, which normally extend from 0 to infinity, have been truncated and normalized using the defined class limits. The result is plotted in [Figure A1](#).



Key		X	particle size x (μm)
1	$\lg(s_g)$ 0,05	Y	frequency q_3^*
2	0,1		
3	0,2		
4	0,3		
5	0,5		

Figure A.1 — Logarithmic normal probability distributions

The spreadsheet programme has then been used to convert the volume-based frequency distributions into the corresponding cumulative number and volume distributions, $Q_0(x)$ and $Q_0(x)$. The conversion principles and equations given in the different parts of ISO 9276 have been used.

The number of particles n_0 that are assigned to a size class corresponding to the 90th and 99,9th percentiles of the volume distribution is calculated according to

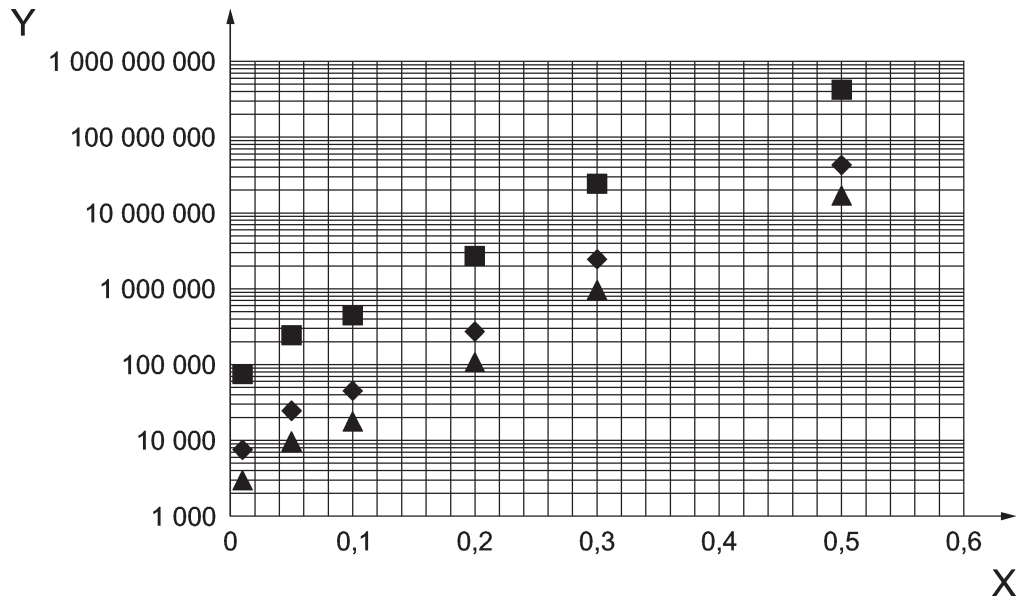
$$n_0 = n \cdot \left(Q_0 \left(x \Big|_{Q_3(x) \approx 99,9\%} \right) - Q_0 \left(x \Big|_{Q_3(x) \approx 90\%} \right) \right) \quad (\text{A.7})$$

where n is the total number of particles in the sample. The value of 99,9 % was chosen to determine the upper class limit. The nearest approximations for the abscissas, $x \Big|_{Q_3(x) \approx 99,9\%}$ and $x \Big|_{Q_3(x) \approx 90\%}$, and the corresponding values of Q_0 are read from the spreadsheet.

If the number n_0 of particles within a size class is very small in comparison to n , then Poisson statistics may be used for estimating the standard deviation of the particle number in that class (see ISO 14488):

$$\sigma(n_0) = \sqrt{n_0} \quad (\text{A.8})$$

If the relative uncertainty in the size class, $\sqrt{n_0} / n_0$, is set to a certain level (e.g. 1 %, 3,16 %, 5 %) the required number of particles, n_0 , in that size class can be calculated (i.e. an uncertainty of 1 % requires 10 000 particles to be measured in this class). The minimum number of particles n^* for the whole sample follows then directly by solving Formula (A.7) for n . The results for the different widths of the distributions and for different levels of uncertainty are shown in [Figure A.2](#).



Key

- Uncertainty 1 %
- ◆ 3,16 %
- ▲ 5 %
- X distribution width $\lg(s_g)$
- Y minimum particle number, n^*

NOTE For the distribution width in terms of $x_{84,3}/x_{50,3}$ or $x_{90,3}/x_{10,3}$ instead of $\lg(s_g)$ see [Table A.3](#).

Figure A.2 — Minimum number of particles, n^* , required to achieve an uncertainty (expressed as the standard deviation) of a fraction $Q_3 = 90\%$

Annex B (informative)

Common segmentation methods for particle edge detection

B.1 General

Segmentation aims at either

- a) determining which pixels belong to foreground objects, or
- b) finding a contour circumscribing each foreground object.

Many contour finding methods are sub-pixel oriented. This part of 13322 considers only methods having complete pixel counts.

The selection of the segmentation method depends on the physical process of image creation^[12].

The algorithms for size and shape calculation has to be adapted to the segmentation method in order to obtain identical results from the same particle images.

B.2 Methods

B.2.1 Thresholding

In this method, all pixels whose (grey-value) intensity is above a certain threshold value are considered part of a foreground object. All pixels whose (grey-value) intensity is below a certain threshold value are considered part of the background. An inverse binarization process is also possible. Such an image is usually displayed as a binary image using black and white pixels to distinguish the particles from the background.

Automated methods are using either the image histogram or the image itself to adjust the threshold value^{[10][11]}. The threshold selection can be performed locally in a region of an image or globally using the complete image. The optimum choice of the method depends on the imaging system and the optical properties of the particles.

The theoretical intensity response to a step function, which can be used to model the optical image of a high contrast edge of a particle, is described by Goodman^[8]. An imaging system using incoherent light leads to an optimum threshold half way between fore- and background level. For real particle systems and other types of imaging methods this type of threshold selection may not be applicable.

B.2.2 Edge detection

Edge detection algorithms can also be used to determine contours around objects. One problem with these methods is that they do not necessarily yield closed curves.

Annex C (informative)

Flow chart showing a typical image analysis method

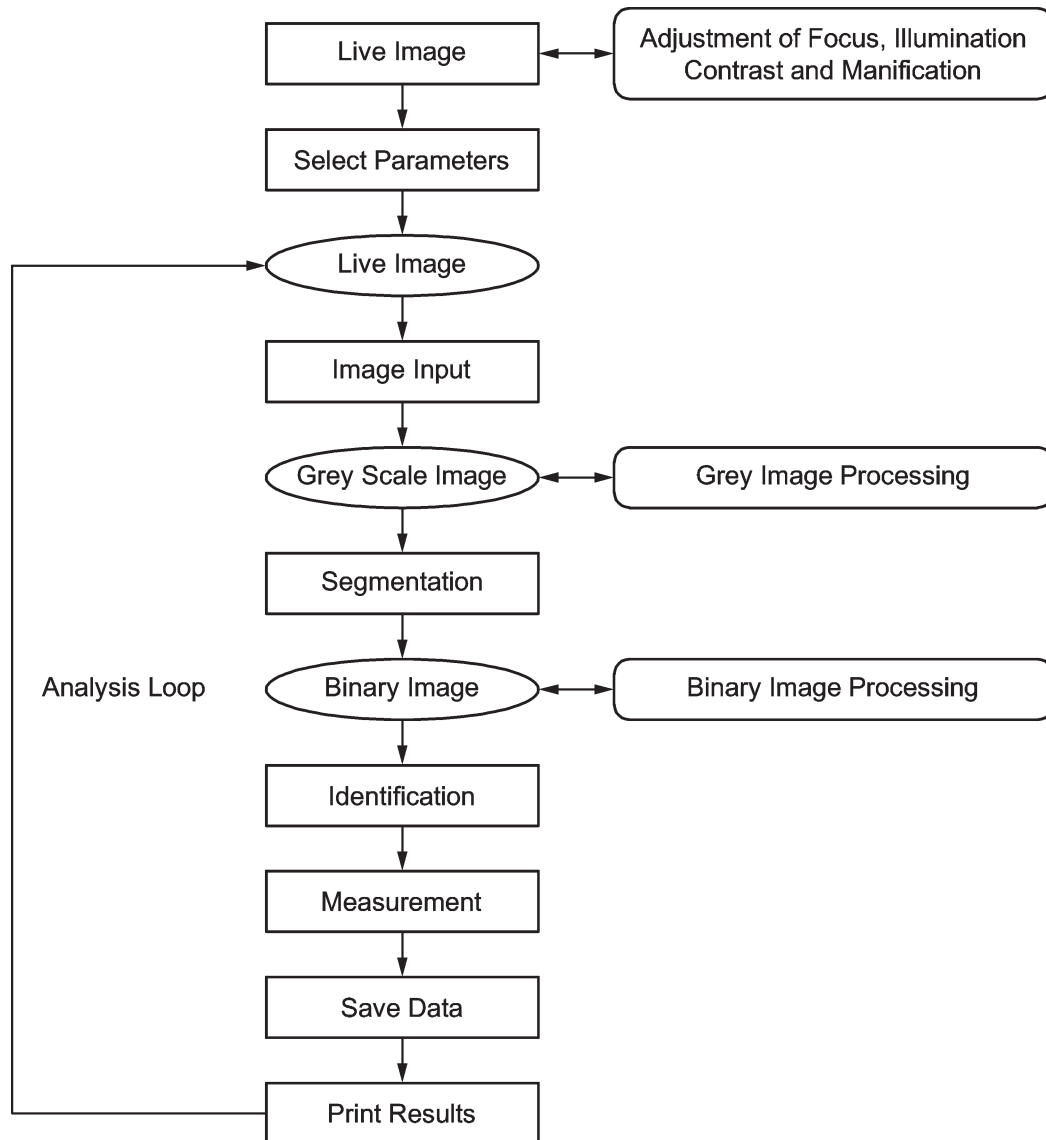


Figure C.1 — Flow chart of a typical image analysis method

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