## INTERNATIONAL **STANDARD**

First edition 2006-11-01

### **Particle size analysis — Image analysis methods —**

Part 2: **Dynamic image analysis methods** 

*Analyse granulométrique — Méthodes par analyse d'images — Partie 2: Méthodes par analyse d'images dynamiques* 



Reference number ISO 13322-2:2006(E)

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### **Foreword**

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ISO 13322-2 was prepared by Technical Committee ISO/TC 24, *Sieves, sieving and other sizing methods*, Subcommittee SC 4, *Sizing by methods other than sieving*.

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 provide guidance for measuring and describing particle size distribution, using image analysis methods where particles are in motion. This entails using techniques for dispersing particles in liquid or gas, taking in-focus, still images of them while the particles are moving and subsequently analysing the images. This methodology is called dynamic image analysis.

There are several image capture methods. Some typical methods are described in this part of ISO 13322.

### **Particle size analysis — Image analysis methods —**

### Part 2: **Dynamic image analysis methods**

### **1 Scope**

This part of ISO 13322 describes methods for controlling the position of moving particles in a liquid or gas and on a conveyor, as well as the image capture and image analysis of the particles. These methods are used to measure the particle sizes and their distributions, the particles being appropriately dispersed in the liquid or gas medium or on the conveyor. The practical limitations of the derived particle size are addressed when using this part of ISO 13322.

### **2 Normative references**

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 13322-1:2004, *Particle size analysis — Image analysis methods — Part 1: Static image analysis methods*

### **3 Terms, definitions and symbols**

### **3.1 Terms and definitions**

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

### **3.1.1**

### **flow-cell**

measurement cell inside which the fluid-particle mixture flows

### **3.1.2**

#### **orifice tube**

tube with an aperture through which a stream of fluid with dispersed particles flows

### **3.1.3**

#### **sheath flow**

clean fluid flow surrounding particle-laden fluid for directing particles into a specific measurement zone

### **3.1.4**

### **particle illumination**

continuous illumination for image capture device with an electronic exposure time controller, or illumination of short duration for synchronized image capture device

### **3.1.5**

#### **measurement volume**

volume in which particles are measured by an image analyser

### **3.1.6**

### **depth of field**

region where the sharpness of the edges of the images reaches the pre-set optimum

#### **3.1.7**

**image capture device**  matrix camera or line camera

### **3.2 Symbols**

- *a* moving distance of a particle during time *t*
- *Ai* projected area of particle *i*
- *b* measured diameter of binary image
- *t* exposure time
- *v* particle velocity
- *x* diameter of particle
- $x_{Ai}$  projected area equivalent diameter of particle  $i$
- *xi*max maximum Feret diameter of particle *i*
- *xi*min minimum Feret diameter of particle *i*
- $\varepsilon$  ratio of the measured particle diameter to the static particle diameter

### **4 Principle**

### **4.1 General**

A general diagram for dynamic image analysis is shown in Figure 1.



- 7 image capture device
- 8 image analyser
- 9 display

### **Figure 1 — Flow diagram for typical dynamic image analysis method**

### **4.2 Particle motion**

Moving particles can be introduced into the measurement volume by three means:

- a) particle motion in a moving fluid (e.g. particles in suspension, in an aerosol, in a duct, in an air jet, in a sheath flow, in turbulent flow or in a push-pull flow regime);
- b) particle motion in a still fluid, i.e. in an injection or free-falling system, where particles are intentionally moved by an external force (e.g. gravity, electrostatic charge);
- c) particle motion with a moving substrate, where particles are on the moving substrate (e.g. conveyor belt).

### **4.3 Particle positioning**

Particles are introduced into the measurement volume and an image is taken when particles reach the object plane. The depth of the measurement volume is determined by the depth of field of the optical system used.

Figure 2 shows an example of measurement volume.



#### **Key**

- 1 light source
- 2 camera
- 3 measurement volume

### **Figure 2 — Example of measurement volume**

The direction of observation (e.g. parallel or perpendicular) of the particles affects the interpretation of particle size and shape, as shown in Figure 3. However, this part of ISO 13322 is not concerned with the influence of particle shape on the overall measurement.



#### **Key**

- 1 measurement volume parallel to particle motion
- 2 measurement volume perpendicular to particle motion

#### **Figure 3 — Particle movement and direction of observation**

The focus of the image capture equipment shall be adjusted so as to acquire the exact image of the particles moving in the fluid. There are two recommended ways to achieve this:

- a) by controlling the position of the moving particles so that they pass only within the measurement volume of the image capture equipment;
- b) by illuminating the particles for a short time period (e.g. by flash light) or capturing the image of the moving particles when they pass through the measurement volume of the image capture equipment.

### **5 Operational procedures**

#### **5.1 General**

Modern image analysers usually have algorithms to enhance the quality of the image prior to analysis. It is acceptable to use enhancement algorithms provided that the measured results are traceable back to the original image.

### **5.2 Still image resolution**

The resolution of an image captured by a dynamic image analysis system depends not only on the optical system (lens magnification and camera resolution) but also on the lighting system and the velocity of the particles.

When a spherical particle of diameter *x* moves at a velocity *v*, the centre of the projected area of the particle moves a distance *a* during a time *t*, where *t* is either the strobe light emission time or camera shutter opening time (see Figure A.1), i.e.

$$
a = v \times t \tag{1}
$$

Without appropriate grey level handling, a shall not exceed either 0,5 pixel or  $x \times (s^2 - 1)$  pixel, where  $\varepsilon$  is the ratio of the measured particle diameter to the static particle diameter.

Grey level handling between pixel level and background level should ensure that the measured diameter of binary image *b* equals the diameter, *x*, of the static particle.

The total system resolution should be determined based on the particle size distribution and the desired confidence limits (see ISO 13322-1).

### **5.3 Calibration and traceability**

The equipment shall be calibrated to convert pixels into SI length units (e.g. nanometres, micrometres, millimetres) 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 back to the standard metre. This can be achieved by calibration of the image analysis equipment with a certified standard stage micrometre.

Movement of particles during the capture of particle images, especially for smaller particles, may introduce serious error in determining particle sizes. It is therefore recommended that the whole system be verified with a standard reference material under motion.

The calibration particles shall be selected to include the dynamic range of the entire system. It is recommended to calibrate with three sizes of certified particles, i.e. with values near the maximum, mid-point and minimum particle sizes to be measured with the system.

### **5.4 Size classes and magnification**

The theoretical limit for resolution of objects by size using image analysis is 1 pixel, and counts should be stored particle by particle, with the maximum resolution of 1 pixel. However, it is necessary to define size classes for the final reporting of the result, which is a function of the total number of particles, the dynamic range and the number of pixels included in the smallest considered objects. It is recommended that pixel size be converted to actual size prior to any reporting of size for quantitative analysis.

For a system in which not all the particles are measured, large particles may often be positioned on an edge of the image frame. Therefore, the magnification should be selected so that the maximum diameter of the largest particle does not exceed one-third of a shorter side of a rectangular image frame of the measuring area (see Annex B).

It is strongly recommended to address within the report any errors resulting from the loss of information of larger particles positioned at the edge of an image frame.

Optical resolution, where applicable, is normally better than electronic resolution.

### **5.5 Particle edges**

In an image, the particle edge shall be defined by a suitable threshold level. The technique for doing this depends on the sophistication of the image analysis equipment.

It is strongly recommended that the threshold level be adjusted by comparing the processed binary images with the original grey images, in order to ensure that they are a reliable representation of the original grey images.

### **5.6 Measurements**

The measurement of the perimeter of a particle is heavily dependent on the image analysis system used. It is recommended that the primary measurements are:

- a) the projected area of each particle in pixels  $(A_i)$ ,
- b) the longest dimension of each particle in pixels (maximum Feret diameter,  $x_{i\text{max}}$ ), and
- c) the shortest dimension of each particle in pixels (minimum Feret diameter,  $x_{ijmin}$ ),

thus allowing the definition of a shape factor with the greatest discrimination.

The projected area of each particle can be converted to the area equivalent circular diameter,  $x_{div}$ 

$$
x_{Ai} = \sqrt{\frac{4A_i}{\pi}}\tag{2}
$$

### **6 Sample preparation**

The number of particles in the dispersion medium shall be controlled so that overlapping images of particles are not generated.

### **7 Sample and measurement variability**

The measurement of the total number of particles or the total particle number count is possible under certain conditions. Such methods should ensure that no particles are lost or counted more than once.

The minimum number of particles to be counted shall be based upon the particle size distribution and the desired confidence limits (see ISO 13322-1).

To increase the confidence in the measurements, statistical parameters such as the mean diameter and standard deviation for a group of measurements can be calculated.

Annex C provides typical examples of sample feed and image capture systems.

### **Annex A**

(informative)

### **Particle velocity and exposure time recommended**

Special precautions are required when measuring small particles in motion by dynamic image analysis.

When a spherical particle of diameter  $x$  [pix] moves at a velocity  $v$  [pix/s] and the exposure time is  $t$  [s], the centre of area of the particle moves the distance *a* [pix] during this period, i.e.

 $a = v \times t$  (A.1)

The observed diameter of the particle *b* [pix] in the direction of motion is between  $(x + a)$  and  $(x - a)$ , depending on the threshold level used (see Figure A.1).

Consequently, when the image of a moving spherical particle is captured as a grey image and then converted into a binary image with a given threshold level, the shape appears to be a prolonged ellipsoid rather than circular. The maximum dimension of the binary particle image would be:

$$
b = x + a \tag{A.2}
$$

In order to make the results of dynamic particle measurement consistent with those obtained by static particle measurement, it is recommended that the difference between *x* and *b* be less than 0,5 pixel, i.e.

 $a = v \times t < 0.5$  (A.3)

However, if the measurement is performed only with large particles (e.g. *x* is larger than 10 pixel, with a given error in the measured area equivalent diameter), the difference between *x* and *b* (which is equal to *a*) can be calculated as follows:

$$
x_{A,\text{real}} = \sqrt{\frac{4 \times A_{\text{real}}}{\pi}} = x \tag{A.4}
$$

$$
A_{\text{real}} = \frac{\pi}{4} \times x^2 \tag{A.5}
$$

 $m_{\text{meas}} = \sqrt{\frac{4 \times A_{\text{meas}}}{2}}$ *A*  $x_{A,\text{meas}} = \sqrt{\frac{4 \times A_{\text{r}}}{\pi}}$ (A.6)

$$
A_{\text{meas}} = \frac{\pi}{4} \times x \times b \tag{A.7}
$$

where  $x_{A,real}$ ,  $x_{A,mean}$  are the area equivalent diameter of a static particle and the measured particle and  $A_{real}$ , *A<sub>meas</sub>* are the projected area of the static particle and the measured particle.

The ratio of the measured particle diameter to the static particle diameter  $\varepsilon$  is given by:

$$
\varepsilon = \frac{x_{A,\text{meas}}}{x_{A,\text{real}}} = \sqrt{\frac{b}{x}} = \sqrt{\frac{x+a}{x}} = \sqrt{1 + \frac{a}{x}}
$$
(A.8)

This equation can also be expressed as follows:

$$
a = x \left( \varepsilon^2 - 1 \right) \tag{A.9}
$$

For instance, when  $\varepsilon$  is less than 1,1 (which corresponds to a relative error of 10 % in particle diameter),

$$
\frac{a}{x} < 0.21\tag{A.10}
$$

Therefore, *a* can be as large as 2 pix when the minimum particle size measured is 10 pix, i.e.

$$
a = v \times t < 2,1 \tag{A.11}
$$

Figure A.1 illustrates particle image and threshold level, and Figure A.2 illustrates an extension for particles of arbitrary shape.



### **Key**

- *a* travelled distance during the exposure time [pix]
- *b* measured diameter of binary-imaged particle [pix]
- *v* direction of motion and velocity [pix/s]
- *x* diameter of static particle [pix]
- A particle position at start of image capturing
- B particle position at end of image capturing
	- threshold line
		- grey level

### **Figure A.1 — Particle image and threshold level**



#### **Key**

*A*err maximum error caused by the particle movement

- *A*real projected area of the static particle
- *a* travelled distance during the exposure time [pix]
- *direction of motion and velocity [pix/s]*

*x*<sub>F</sub> Feret diameter of projected area perpendicular to the direction of motion

- A particle position at start of image capturing
- B particle position at end of image capturing

### **Figure A.2 — Extension of Figure A.1 for particles of arbitrary shape**

### In Figure A.2,

$$
A_{\text{err}} = a \times x_{\text{F}} \tag{A.12}
$$

where  $x_F$  depends on the particle orientation relative to the moving direction.

$$
\varepsilon = \frac{x_{A,\text{meas}}}{x_{A,\text{real}}} = \sqrt{\frac{A_{\text{real}} + A_{\text{err}}}{A_{\text{real}}}} = \sqrt{1 + \frac{4 \times a \times x_{\text{F}}}{\pi \times x_{A,\text{real}}^2}}
$$
(A.13)

### **Annex B**

### (informative)

### **Maximum particle size recommended**

### **B.1 General**

Two methods are commonly used to correct the counts for any particles which touch the side of the measurement frame (see ISO 13322-1:2004, 6.3).

### **B.2 Guard line principle** [1]

The measurement area is a rectangular frame whose bottom and right-hand sides are defined as the reject sides. Particles which lie partially or wholly within the measurement frame and do not touch the reject sides are accepted. Particles which touch the reject sides or their extension lines (the guard lines) are rejected from the count. There shall be a sufficiently large space between the upper-left corner of the rectangular view frame and that of the measurement frame, such that no accepted particles are cut by the edges of view frame. If we consider a rectangular field of view with a smaller side length *L*, and a rectangular measurement frame with a smaller side length *Z*, as shown in Figure B.1, in order for the accepted particles not to be cut by the view frame, the particle size shall be smaller than or equal to  $L - Z$ , i.e.

$$
x \leqslant L - Z \tag{B.1}
$$

The ratio of the effective measurement frame area to the field of view area shall be *r*, where

$$
r = \frac{Z^2}{L^2} \tag{B.2}
$$

Equations (B.1) and (B.2) can be combined as follows:

$$
r \leqslant \left(1 - \frac{x}{L}\right)^2 \tag{B.3}
$$

Equation (B.3) gives the relationship between the ratio of the effective measurement frame area to the field of view area *r* and the maximum particle size  $\frac{x}{L}$ , measured by the Guard line method (see Figure B.2).



#### **Key**

- 1 field of view
- 2 measurement frame
- 3 guard lines
- 4 sufficient space between the top and left edges of the two frames
- *L* smaller side length of field of view
- *Z* smaller side length of measurement frame
- NOTE Shaded particles are accepted and unshaded particles are rejected.

**Figure B.1 — Guard line principle** 



#### **Key**

*r* ratio of effective area of the measurement frame to the area of the field of view

*x*  $\frac{\lambda}{L}$  ratio of the diameter of the particle, *x*, to the length of the shorter side of the rectangular field of view, *L* 

NOTE The same curve is used to estimate the probability of particles existing in the measurement frame (see Figure B.4).

#### **Figure B.2 — Ratio of effective measurement frame area to the view field area**

### **B.3 Miles-Lantuejoul method** [5]

All particles entirely within the measurement frame are accepted for counting. All particles outside, including those cut by the sides of the measurement frame, are rejected. The probability of particles being included in the measurement frame decreases as the diameter of particles increases. The probability  $P_i$  (Miles-Lantuejoul factor) of particle *i* existing within the measurement frame is determined by the particle diameter and the measurement frame size. The counted number of particles in the measurement frame should be weighted by dividing  $P_i$  according to particle size. For non-spherical particles, in the calculation of Miles-Lantuejoul factor  $P_i$ (see Figure B.3), the longest dimension of a particle is chosen as the particle diameter whilst the shorter side of rectangular measurement frame is chosen as the frame length.

If we consider a square measurement frame of size  $Z$ , then  $P_i$  for a particle with size  $x$  is calculated as follows:

$$
P_i = \frac{(Z - x)^2}{Z^2} = \left(1 - \frac{x}{Z}\right)^2
$$
 (B.4)

The probability  $P_i$  is plotted as a function of dimensionless particle size in Figure B.4.

Using the Guard line method, when a measurement frame is required whose area is larger than 50 % of the field of view, the ratio illustrated in Figure B.2 gives a value of  $\frac{x}{L} = 0.3$  when  $r = 0.5$ . This indicates that particles smaller than approximately one-third the size of the shorter side of the field of view can be correctly measured.

Similarly, using the Miles-Lantuejoul method (see Figure B.4), when the number of counted particles with a correction factor larger than 50 % is not to be corrected, particles smaller than approximately one-third the size of the shorter side of the measurement frame should be measured.

Therefore, whichever method is applied, particles smaller than approximately one-third the size of the measurement frame can be included in the measurement.

Measurements made without a measurement frame (i.e. where the field of view is the measurement frame) can follow the  $P_i$  correction method.  $-$  ,



#### **Key**

- 1 field of view
- 2 measurement frame
- *Z* side length of measurement frame

NOTE Shaded particles are accepted and unshaded particles are rejected.

**Figure B.3 — Particles in the measurement frame** 



### **Key**

- $P_i$ probability of particle *i* existing within measurement frame (Miles-Lantuejoul factor)
- $rac{x}{Z}$ *<sup>Z</sup>* ratio of particle diameter, *x*, to the side length of square measurement frame, *<sup>Z</sup>*

NOTE The same curve is used to estimate the ratio of effective measurement frame area to the view field area (see Figure B.2).

#### **Figure B.4 — Probability of particles existing in the measurement frame**

## **Annex C**

### (informative)

### **Typical examples of sample feed and image capture systems**

# **C.1 Sheath flow system**  --`,,```,,,,````-`-`,,`,,`,`,,`---

In this method, the positioning of all particles dispersed in the core flow is controlled by the sheath flow. The core flow and particles should be at the precise focal plane of the image capture equipment. The size and shape of the flow can be controlled to accommodate the sizes and shapes of the particles being measured. The sheath flow is illuminated by the light source for the duration required by the image capture equipment, in order to take a still image of the particles in the flow. The still image captured in this manner has all of the particles in the flow in focus. In order to create a sharply-focused image of the particles, the distance travelled by the particles shall be less than the resolution of the image capture equipment when the lamp is illuminated. When this method is used, fibrous particles tend to align with the direction of flow and are thus presented perpendicular to the imaging system.

Figure C.1 illustrates the sheath flow cell system.



### **Key**

- 1 sample 5 core flow
- 2 sample feed nozzle 6 light source
- 
- 
- 
- 
- 3 camera 7 sheath flow
- 4 lens 8 measurement volume

### **Figure C.1 — Sheath flow cell system**

### **C.2 Electrical sensing zone system**

In this method, the optical system of the image capture equipment is focused on the aperture of the orifice tube. An electrical signal is generated between two electrodes immersed in a conductive liquid on both sides of a small aperture, which is called the electrical sensing zone. When a particle passes through this zone, it changes the impedance between the two electrodes. This results in an electrical pulse of short duration, which generates a trigger signal to the stroboscopic lamp. An image of the particle is captured by the image capture equipment at the precise instant when it traverses the focal plane of the optical system.

With the electrical sensing zone method, one image per particle is captured each time the light source is illuminated. Particle images are always captured at a specific position on the image screen: if there is no coincidence error, the particles never overlap one another or extend beyond the sides of the screen. When this method is used, the orientation of particles and flow direction is perpendicular to the optical axis of the image capture equipment.

Figure C.2 illustrates the electrical sensing zone system.



**Key** 

- 1 light source
- 2 camera
- 3 measurement volume

![](_page_22_Figure_10.jpeg)

### **C.3 Circulating method**

In this method, particles are dispersed in a fluid and are continuously re-circulated. The flow rate can be controlled to accommodate various sizes and shapes of particles during the measurement. With reference to Figure C.3, the particles are illuminated by a controlled light source (1). The intensity of the light is set in accordance with the image capture system used.

The image capture device (2) is typically a CCD (charged coupled device) camera. Through the use of proper optics, provision should be made to ensure that all particles in the measurement volume (3) are in focus. There may be situations where out-of-focus particles are measured. These out-of-focus particles shall be rejected through the use of image processing techniques.

Figure C.3 illustrates the method for circulating particles.

![](_page_23_Picture_5.jpeg)

#### **Key**

- 1 light source
- 2 camera
- 3 measurement volume

### **Figure C.3 — Method for circulating particles**

### **C.4 Agitating method**

In this method, particles dispersed in a liquid in a cell are stirred, and an optical system with a short exposure time takes still images of the moving particles.

Figure C.4 illustrates the method of agitating particles.

![](_page_24_Figure_4.jpeg)

#### **Key**

- 1 light source
- 2 camera
- 3 measurement volume

**Figure C.4 — Method of agitating particles** 

### **C.5 Dynamic stop-flow image analysis method**

In conventional dynamic image analysis methods, particles move continuously throughout the experiment. For small particles, any motion during image acquisition causes blurring of the image, leading to errors in particle size determination. In this method, the sample is suspended in a fluid and passed through the flow cell. The sample flow is stopped momentarily during each image acquisition and resumed after the image is taken. To ensure that the same particles are not measured or seen twice, each image is taken after the sample has flown for a defined time gap from the previous image. The particles are illuminated by a controlled light source whose intensity is set in accordance with the image capture system (typically, a charge-coupled device). The light source may also be auto-adjusting, in order to ensure consistency of results when fluid colour or particle colour is a factor.

Figure C.5 illustrates the dynamic stop flow image analysis method.

![](_page_25_Figure_4.jpeg)

#### **Key**

- 1 light source
- 2 image capturing device
- 3 flow cell
- 4 sample flow direction
- 5 particles in these areas are skipped for image acquisition
- 6 image is taken
- 7 flow cell top

### **Figure C.5 — Dynamic stop flow image analysis method**

### **C.6 Free-falling system**

In this method, particle delivery is controlled by a vibrating feeder. The feeder disperses the particles and drops them onto the focal plane of the image capture equipment. With reference to Figure C.6, the width of the sample falling area and the falling velocity can be controlled to an appropriate value by the vibrating feeder, in order to ensure that the particles pass through the focal plane (3) of the image capture equipment (2).

The falling particles are illuminated by a lamp which has an intensity appropriate for the image capture equipment. The image capture equipment is either a shutter camera or a line scan camera. In an image captured in this manner, there may be particles that are out of focus, which may be rejected by image processing techniques.

Figure C.6 illustrates the free-fall system.

![](_page_26_Figure_5.jpeg)

#### **Key**

- 1 light source
- 2 camera
- 3 measurement volume

**Figure C.6 — Free-fall system** 

### **C.7 Measurement on a moving substrate**

With reference to Figure C.7, in this method the particles are placed on a conveyor belt (3) by means of appropriate isolation mechanisms, and are hence measured in their stable, preferred position by the incident light (1). A line camera (2) or a matrix camera may be used. When a transparent conveyor belt (e.g. belt of glass-slides) is used, illumination from behind can give a better resolution of two-dimensional particle images.

Figure C.7 illustrates measurement on a moving substrate.

![](_page_27_Figure_4.jpeg)

- 
- 1 light source
- 2 image capturing device
- 3 conveyor

**Figure C.7 — Measurement on a moving substrate** 

### **C.8 Measurement at a conveyor discharge point**

With reference to Figure C.8, in this method the material flow can be recorded by the transmitted or incident light procedure directly behind the conveyor (3) discharge point, by means of a line light source (1) and line camera (2). The particles can be assumed to have a stable, preferred position, and consequently a good approximation of their projected area can be recorded during the measurement.

Figure C.8 illustrates measurement at conveyor discharge points.

![](_page_28_Figure_4.jpeg)

**Key** 

- 1 light source
- 2 image capturing device
- 3 conveyor

**Figure C.8 — Measurement at conveyor discharge points** 

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