BRITISH STANDARD

BS 3406-4: 1993

Methods for determination of particle size distribution —

Part 4: Guide to microscope and image analysis methods

Committees responsible for this British Standard

The preparation of this British Standard was entrusted by the General Mechanical Engineering Standards Policy Committee (GME/-) to Technical Committee GME/29, upon which the following bodies were represented:

BCIRA
British Cement Association
British Coal Corporation
British Laboratory Ware Association
Coated Abrasives Manufacturers' Association
Guild of Metal Perforators
Institution of Chemical Engineers
Institution of Mining and Metallurgy
Mechanical Handling Engineers Association
Ministry of Defence
NABIM
Society of Chemical Industry
Society of Cosmetic Scientists
Woven Wire Association

The following bodies were also represented in the drafting of the standard, through subcommittees and panels:

Aluminium Federation
China Clay Association
Department of Trade and Industry (National Physical Laboratory)
GAMBICA (BEAMA Ltd.)
Health and Safety Executive
Royal Pharmaceutical Society of Great Britain
Royal Society of Chemistry
Sunderland Polytechnic (Magnet Centre)
University of Bradford
University of Technology, Loughborough
Coopted members

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Contents

Foreword

This revision of BS 3406-4 has been prepared under the direction of the General Mechanical Engineering Standards Policy Committee and supersedes BS 3406-4:1963 which is withdrawn.

It is one of a series of methods for determining the size distribution of particles.

Other Parts of BS 3406 are:

— *Part 1: Guide to powder sampling;*

— *Part 2: Recommendations for gravitational liquid sedimentation methods for powders and suspensions;*

— *Part 3: Air elutriation methods (obsolescent);*

— *Part 5: Recommendations for electrical sensing zone method (the Coulter principle);*

— *Part 6: Recommendations for centrifugal liquid sedimentation methods for powders and suspensions;*

— *Part 7: Recommendations for single particle light interaction methods.*

The particle size distribution of a powder is an important basic property that can distinguish it from other samples of the same composition. Vast quantities of powdered materials are sold or processed and specifications often include average particle size and/or particle size distribution. Even when other bulk properties are specified, compliance with the specification may be achieved by varying the particle size distribution. Particle size analysis is also used extensively to monitor environmental conditions and in numerous areas of scientific research. In all these contexts the reproducibility and comparability of results is of paramount importance and the use of standardized techniques is essential.

Representative sampling and correct dispersion of particulate material are prerequisites for the determination of size distribution. The procedures described in BS 3406-1 have been selected to give test portions representative of the bulk.

The techniques used to measure particle size distribution are many and are varied both in principle and in their degree of technical complexity and automation. In principle, any property that depends on particle size can be used as a means of measuring particle size. In practice, the methods that are widely used are both experimentally convenient and based upon a physical principle having a well defined relationship to particle size.

The choice of the most suitable method will depend on the following considerations:

a) the purpose for which the analysis is required, e.g. quality control, research, specification requirements;

b) the size range and other properties of the particles, e.g. density, solubility, refractive index;

c) aggregation and dispersion characteristics of the powder;

d) the amount of material available for analysis;

e) the method by which the sample has been collected;

f) the conditions under which the powder is to be used, for example the state of dispersion in the test should be related to the state of dispersion in the application;

g) the resources available.

Microscopy is the only method in which direct observation is made of the size and shape of the particles. Most methods of particle size measurement ascribe a single dimension to each particle, usually expressed as the diameter of the spherical particle equivalent to some aspect of its properties, e.g. volume, surface, settling velocity, light scattering. Physical properties of the particles, such as solubility, friability, refractive index and conductivity, preclude the use of some methods. Nevertheless the same particles can be analysed using a range of techniques and if different size parameters are determined, different size distributions will result. The intercomparison of results is meaningful only when the same parameters of a representative sample are measured using reliable equipment and standard operating procedures. Standardization between laboratories is best established by the use of certified reference materials, for example those from the Community Bureau of Reference (BCR).

Microscopy is a widely used method of particle size analysis. One of its main benefits is that it allows the characterization of the shape of particles and the detection of agglomerates and contaminants. A sample being prepared for analysis by a non-microscope, automatic method should be examined under the microscope to ensure that it is suitable and well enough prepared. Microscope measurements are usually expressed as the diameter of a sphere of equivalent projected area. The first edition of BS 3406-4 in 1963 described the use of an eyepiece graticule with a series of circular spots drawn on it, against which the images of the particles were compared and classified. The only microscope covered by the standard was the light microscope fitted with a graduated draw tube. Much has changed since 1963; the electron microscope with its high resolution and great depth of field is routinely used, the image analyser has taken much of the effort out of taking measurements and the desktop computer allows complicated data analysis to be carried out on large amounts of data. This revision of the standard reflects these changes although many of the principles remain unaltered.

Microscopy is an apparently simple method of particle size analysis but in practice great care is needed if accurate results are to be achieved. The quality of the final result will suffer from the addition of uncertainties introduced at all stages of the method from sample preparation through to data analysis.

It has been assumed in the drafting of this British Standard that the execution of its provisions is entrusted to appropriately qualified and experienced people.

It is expected that users of this standard will be conversant with the appropriate measuring instrument manufacturer's instruction manual.

WARNING. This British Standard may involve the use of substances and/or procedures that may be injurious to health if adequate precautions are not taken. It refers only to technical suitability and does not absolve the user from legal obligations relating to health and safety at any stage. Attention is drawn to the specific hazards described in clause **5**.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations. In particular, attention is drawn to Health and Safety Executive Control of Substances Hazardous to Health Regulations (1988) (COSHH).

Summary of pages

This document comprises a front cover, an inside front cover, pages i to iv, pages 1 to 32, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

Introduction

This standard covers the measurement of particle size by light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Light microscopy is suitable for the measurement of particles in the size range from 3 μm to 1 mm, SEM from 20 nm to 1 mm and TEM from 2 nm to 1 μm. Microscopy uses a very small test portion. This has advantages if the laboratory sample is itself small or if the particles are expensive. The disadvantage with a small test portion is that even greater care is needed to produce a representative sample. Microscopy is particularly suited to making measurements on particles that are collected on a filter by a sampler if the filter can then be placed directly under the microscope.

Image analysis (IA) has removed a considerable amount of the hard work involved in making manual measurements. The IA will make measurements all day, the last one being as good as the first. The IA introduces its own uncertainties into measurements. In the same way that samples for size analysis by methods other than microscopy should be looked at with a microscope first, so too, test portions for automatic microscope analysis should be examined by eye before starting measurements. IAs are often equipped with the facility to control focus, delineation and image brightness automatically. These are useful functions but they have to be used with care because they can have unwanted effects if presented with an unusual image. A good understanding of the IA and its limitations is needed.

In this standard the particle size is expressed as the equivalent circle diameter. Except in the case of spherical particles the size distribution arrived at is unique to microscopy and cannot be related directly to the results of other sizing techniques.

This standard includes methods of calculating the mean and standard deviation of the particle size distribution from the measured data. The use of IAs to make the measurements, and microcomputers to calculate the statistics, means that large amounts of data can be generated and processed quickly. The use of many measurements reduces the effect of random uncertainties but has no effect on systematic uncertainties. 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 enables a realistically required confidence interval to be estimated.

Despite all the innovations in microscope methods, the fundamentals remain the same. There is no substitute for representative sampling and good microscopy.

1 Scope

This Part of BS 3406 recommends methods for using microscopes to measure the size of particles in the range from 2 nm to 1 mm. Making measurements with light microscopes, scanning electron microscopes and transmission electron microscopes is described both with and without the use of automatic and semi-automatic image analysers. A system is established for calculating the number of fields of view that have to be analysed to achieve a specified accuracy, and methods are given for the estimation of uncertainties. Guidance is provided on sample preparation, practical microscopy and the choice of equipment for image analysis.

This Part of BS 3406 does not cover the measurement of particle shape or the use of stereorological methods.

This standard does not cover the measurement of asbestos fibres. Asbestos fibres are covered by the European Reference Method (ERM) as given in MDHS 39[1)].

Specialized techniques applicable to the study of cell biology or haematology are outside the scope of this Part of BS 3406.

2 References

2.1 Normative references

This Part of BS 3406 incorporates, by reference, provisions from specific editions of other publications. These normative references are cited at the appropriate points in the text and the publications are listed on the inside back cover. Subsequent amendments to, or revisions of, any of these publications apply to this Part of BS 3406 only when incorporated in it by updating or revision.

[1)] Available from the Health and Safety Exective.

2.2 Informative references

This Part of BS 3406 refers to other publications that provide information or guidance. Editions of these publications current at the time of issue of this standard are listed on the inside back cover, but reference should be made to the latest editions.

3 Definitions

For the purposes of this standard, the definitions given in BS 2955:1958 apply, together with the following.

3.1
auto-delineation

the process which attempts to predict the true edge of unsharp images of particles by reference to the local grey level values. This is used to improve the accuracy of measurements and to make them less sensitive to incorrect grey level thresholding

3.2
binary image

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

3.3
detection

the process by which the digitized grey level image is converted into a binary image representing the particles to be measured. The simplest form of detection involves setting a grey level value above which a pixel will be counted as being part of the particle; all other pixels will then be counted as belonging to the background

NOTE Segmentation and thresholding are commonly found synonyms for detection.

3.4
digitization

the process of converting the continuously varying voltage output of a video signal into a discrete array of pixels whose values correspond to the intensities of the parts of the original image that they represent

3.5
equivalent circle diameter

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

3.6
image frame

the total area of the specimen displayed on the monitor at a given time

3.7
measurement frame

a subframe within the image frame over which the measurements are made

3.8
mouse

a device to allow interaction, by means of the operator's hand, with the computer

3.9
numerical aperture

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

NOTE The resolving power is proportional to the numerical aperture.

4 Abbreviations and symbols

4.1 Abbreviations

For the purposes of this standard, the following abbreviations apply.

BCR	Community Bureau of Reference
ECD	Equivalent circle diameter
IA	Image analysis/analyser
NA	Numerical aperture
SEM	Scanning electron microscope/microscopy
TEM	Transmission electron microscope/microscopy

4.2 Symbols

For the purposes of this standard, the following symbols apply.

Symbol	Quantity
A_r	Total area of measurement of r^{th} class
a_r	Field of view for one measurement
B	Average number of particles per unit area
c	Integer used to calculate class interval
d_r	Geometric mid-class ECD
$E()$	Expectation error of ()
i	Integer
j	Number of classes
k_i	Constant dependent on particle shape and density
m_r	Number of particles in the rth class
M_i	Expectation error of m_i [= $E(m_i)$])
n_r	Number of fields of view of r^{th} class
N	Total number of particles measured
P_i	Expectation error of p_i [= $E(p_i)$]
P_r	Number size proportion (100 P_r = percentage number in r^{th} class)
Q_i	Expectation error of q_i [= $E(q_i)$]
q_r	Volume size proportion (100 q_r = percentage number in r^{th} class)
r	Size class number
$S()$	Standard error in ()
T	Particle thickness
t	Number proportion
u_i	Mean of x_i
u	Sum of all u_i
V_i	Variance of x_i
V	Sum of all V_i
var()	Variance of ()
w_i	Confidence interval width for the i^{th} particle
×	Magnification, e.g. × 40
x_i	Independent random variable
Y_{app}	Apparent length of eyepiece graticule
Y_{ref}	Reference length of required graticule
Y_{req}	Required apparent length of eyepiece graticule
Y_{true}	True length of eyepiece graticule

Symbol	Quantity
z_i	$x_i - u_i$
z	Sum of all z_i
a	Angular aperture of SEM probe
β	Expected number of particles per unit area
θ	Half angle subtended by particle at objective
λ	Wavelength
μ	Reference index of the surrounding medium
ψ	Desired percentage accuracy for i^{th} particle
φ	Phase shift

5 Health and safety

Good laboratory practice should apply to the use of chemicals and in the preparation of samples, especially toxic dusts. To prevent inhalation, these materials should be handled in a suitable fume cupboard or glove box.

6 Sample preparation

6.1 General

Samples should be subdivided in accordance with BS 3406-1:1986. There are only general rules for sample preparation for the microscope, and no specific rule will cover every possible situation. It is important always to remember the following.

a) The energy of dispersion during sample preparation in the laboratory should be as closely equivalent as possible to the energy of dispersion in product use. This is particularly important for agglomerates, which may behave as single particles, and for fragile particles.

b) Microscopical inspection is recommended to assess the degree of dispersion that has been achieved prior to image analysis.

c) It should be accepted that it is not possible to prepare perfect samples routinely and repetitively. A procedure should be devised to ensure maximum reproducibility. Generally, the simpler the procedure, the smaller the error, and hence the greater the reproducibility. When the mounting technique is chosen, it should be rigidly adhered to so that no bias exists between samples.

d) In circumstances where a mounting medium is used, ideally it should be chosen to give good image contrast between the particles and that medium. It should not react or dissolve the particles. Regular blank checks for cleanliness should be performed.

e) The mounting surfaces should always be clean before use. For example, high quality slides and coverslips should be used and should be degreased with solvent and dried with a jet of filtered air.

f) It is essential that the test portion is not excessively loaded with particles since particle contact or overlap will give misleading results. Partial compensation for overlap can be gained using adjustments of focus in optical microscopy or of the depth of field in scanning electron microscopy. It is important to note that particles which apparently touch on the image analysis screen will, unless separated, be treated as a single particle. Some image analysis systems implement manual or automatic de-agglomeration routines that can be used to separate particles, but overlapping particles would have an apparent size that is less than the true size and this will inevitably produce misleading results.

6.2 Dispersion of laboratory sample

Sampling from liquids is generally the easiest method, providing the laboratory has a suitable piece of equipment for uniform dispersion. Providing the criteria listed in **6.1** apply, the simplest way is to agitate and stir the suspension in a low power ultrasonic bath and to extract suitable aliquots. For less robust particles or agglomerates, an alternative method is shown in Figure 1 in which an air jet circulates the suspension through a sampling tube which can be closed and withdrawn to provide samples for analysis. The microscopist has a choice of suspending the material on a filter [see **6.3** a)], using a straight-sided cavity slide (see Figure 2) to receive an aliquot [see **6.3** b)], using a rod to drop the suspension onto a microscope slide [see **6.3** c)], or smearing a paste [see **6.3** d)].

For sub-micrometric particles see the SEM method given in **6.3** a).

6.3 Mounting of test portion

The test portion should be mounted by one of the following methods.

a) *Mounting by filtration*

NOTE 1 This method is suitable for light microscopy scanning electron microscopy and transmission electron microscopy.

If the filter, or its support, is not completely dry before commencing the filtration, the particles will not be evenly distributed. Large-holed filter supports should be avoided or their effects minimized by the use of backing pads to ensure an even distribution of particles. It should be recognized that, if the particles are irregularly shaped, they will adopt preferred orientation on their largest face. The speed of filtration should be such that preferential settling of particles is avoided. Pressure filtration is quicker than vacuum filtration as a pressure of several atmospheres can be used. The pore size of the filter should be suitable for the collection of the particles to be sized and should not result in loss of particles or deep penetration of particles into the filter.

For light microscopy, the particles can be assessed by reflected light or, if the filter can be made transparent by a suitable process, by transmitted light. The clearing process should not involve the movement of particles on the filter surface. There should be good optical contrast between the particle and the surrounding medium.

For scanning electron microscopy, a smooth surfaced filter such as a polycarbonate membrane filter should be chosen. A suitable conducting film (e.g. gold or carbon) should be coated onto the preparation.

For analysis by transmission electron microscopy, the filter is prepared for examination by plasma ashing, carbon coating and solvent dissolution. The process is not suitable for organic particles.

b) *Mounting by straight-sided cavity slide*

NOTE 2 This method is suitable for light microscopy.

The refractive index of the mounting medium should be very different from that of the particles to ensure good optical contrast. Care should be taken to ensure that there is no preferential loss of a size class when the coverslip is added. The density of the liquid should be less than the density of the particles to encourage the particles to lie in one plane of focus at the bottom of the preparation, but this does not aid distribution in the initial mixing. Large particles will settle more quickly than small particles. The technique can therefore involve considerable changes of focus during analysis to ensure that all of the particles are sized. If the liquid is subject to viscosity variations with temperature, this will influence the settling rate. The settling of particles will also involve preferential orientation.

c) *Mounting by a drop of suspension on a microscope slide*

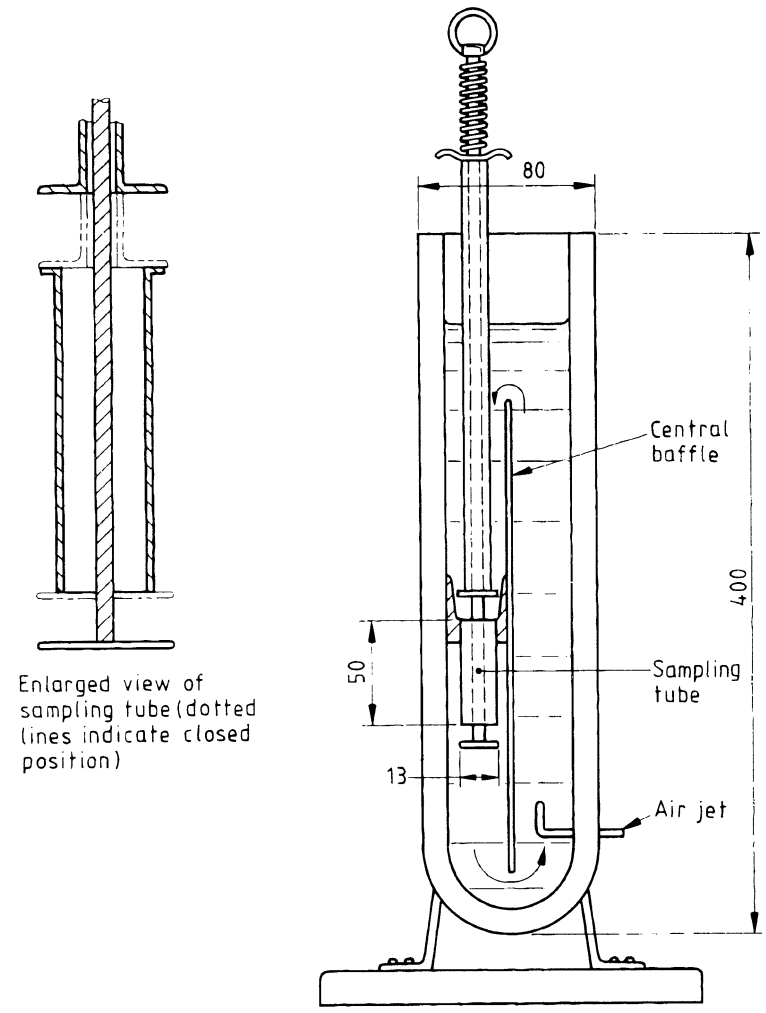
NOTE 3 This method is suitable for light microscopy with image analysis.

The refractive index of the mounting liquid should be very different from that of the particles. The drop should be centrally covered with a circular coverslip. This technique will always give a radial distribution of particle numbers and particle size. If it is ever used, it is important to perform the analysis of sectors to help compensate for this, and several preparations should be examined to ensure reproducibility, rather than accepting the results of one or two test portions. The technique can work quite well for automatic particle sizing when it is possible to assess large areas of the test portions rapidly.

d) *Mounting in a paste*

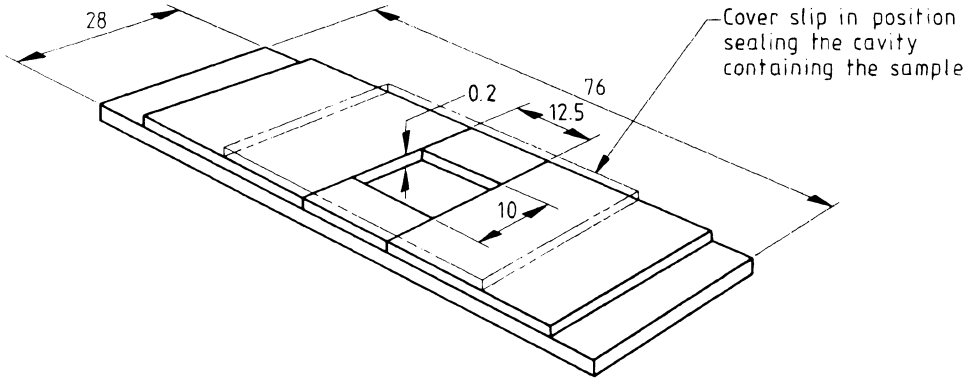
NOTE 4 This method is suitable for light microscopy.

The refractive indices of the liquid and the particles should be very different to ensure good optical contrast. When the paste is placed on the slide, it should be covered with a centrally placed circular coverslip and sectors should be surveyed to compensate for the radial distribution.



All dimensions are in millimetres.

Figure 1 — Liquid sample divider



All dimensions are in millimetres.

Figure 2 — Typical cell for examination of particles dispersed in liquid of low viscosity

For analysis by light microscopy, sampling from pastes does work very well, but can lead to a false sense of security with respect to the randomness mixing and reproducibility. This is particularly true for irregularly shaped particles, the more irregular, the greater the error. Before trying such work, a control sample should be made by dispersing carbon black in the viscous matrix so that there is no illusion about the amount of mechanical work required to form a random distribution.

7 Limits of size resolution of various microscope systems

7.1 General

The three commonly used microscope systems for particle sizing are the light microscope, the scanning electron microscope and the transmission electron microscope. Information on the three systems is given in Annex A.

In all forms of microscopy, image degradation can occur from a number of factors. These include poor sample preparation, flare, astigmatism, aberrations, type and intensity of illumination and the numerical apertures of the condenser and objective lens. The system manufacturer's setting up instruction should always be followed for the best performance.

NOTE Further information on microscopy is given in Royal Microscopical Society *Microscopy handbooks*.

7.2 Light microscope

For bright field images in the light microscope, which are commonly used for particle sizing, the minimum distance d (in micrometres) distinguishable in monochromatic light is given by:

$$d = \frac{0.6\lambda}{\mu \sin \theta}$$

where

λ is the wavelength (in μm);

θ is the half angle subtended by the particle at the objective lens (in degrees);

μ is the refractive index of the surrounding medium.

The theoretical lower limit is approximately 0.2 μm, but the diffraction halo around the particle gives a gross overestimate of size, and in practice, sizing should be carried out only for particles at least 10 times larger than the resolution limit of the objective lens used. Table 1 gives the resolutions and smallest particles that should be measured using some typical objectives. Methods of contrast enhancement are briefly referred to in **A.1**.

7.3 Scanning electron microscope (SEM)

In the SEM a fine beam of electrons scans the specimen. The signal arising from the secondary or backscattered electrons leaving the particle as the result of the impact of the primary electrons in the beam is displayed on a viewing screen synchronously as image intensity. The dimensions of the probe determine the ultimate resolving power of the instrument, and diffraction at the final aperture controls this, giving a theoretical minimum resolution d_{SEM} (in nanometres) of:

$$d_{\mathrm{SEM}} = \frac{0.6\lambda}{\alpha}$$

where

λ is the electron wavelength (in nm);

α is the angular aperture of the probe (in radians).

Resolution of better than 5 nm is possible with modern instruments. The angle of presentation is critical to avoid distortions through perspective effects (see **8.3.1.1**).

Imaging with backscattered electrons is recommended in preference to secondary electrons, as boundaries are more accurately defined. A substrate of a similar atomic number to the specimen should be avoided as image contrast will be too low.

7.4 Transmission electron microscope (TEM)

The limiting resolution of the modern TEM is of the order of 0.2 nm, which is well below the size of particles covered by this standard.

The resolution of a TEM is normally defined as the performance obtainable with an "ideal" specimen, i.e. one thin enough to avoid imposing a further limit on the performance due to chromatic effects. The energy loss suffered by electrons in transit through a specimen will normally be large compared to the energy spread in the electron beam due to thermal emission velocities, and large also compared to the instability of the high voltage supply to the gun and the current supplies to the electron lenses.

In general the specimen itself causes loss of definition in the image due to chromatic aberration of the electrons which have lost energy in transit through it. A "thick" specimen could easily reduce the attainable resolution to 1.5 nm 2 nm. This condition could occur if a particle preparation was very dense; a good preparation of a well dispersed particle array on a thin support film would not in general cause a serious loss in resolution.

Table 1 — Resolution and sizing limits for some typical objective lenses
(blue light λ = 400 nm)

Focal length mm	Nominal magnification	Numerical aperture	Resolution µm	Minimum particle size class µm
2	× 100	1.25	0.2	2
4	× 40[a]	0.85	0.3	3
6	× 40	0.65	0.4	4
8	× 20	0.50	0.5	5
16	× 10	0.25	1.0	10
32	× 5	0.10	2.5	25

[a] Apochromatic.

8 Calibration

8.1 General

Calibration of magnification is the process by which the measurement made on the image of a particle is related to the true size of the particle. The calibration factor is thus the ratio of the measured dimension in the image plane and the particle dimension in the object plane. Calibration should therefore include the whole system. This means that a calibrant of known size in the object plane is related to a calibrant of known size in the image plane. For example, the circles on an eyepiece graticule, the ruler used to measure photographs, the number of detected pixels in the image analyser are related to an artefact of known size in the object plane.

The process of calibration is illustrated in Figure 3. The image of the measuring scale is shown alongside that of the calibration artefact in the image plane. By comparing the two scales it is clear that each division on the measuring scale represents 2.74 in the object plane. This value is the calibration factor. Measurements can then be made on particles and the lengths multilpled by the calibration factor to give the true size of the particles. Area measurements should be multiplied by the square of the calibration factor and volume measurements by its cube.

8.2 Calibration of the light microscope

8.2.1 *General*

The measurement of particle size with the light microscope may be done automatically, semi-automatically or manually. The calibration of the IA for automatic measurement is covered in **8.4**. Semi-automatic measurement covers a wide variety of techniques and the appropriate calibration method should be chosen from **8.2.2** and **8.4**. Most automatic and semi-automatic instruments have manufacturer's instructions concerning calibration which should also be followed.

No matter which measuring method is being used, an artefact of known length should be put in the object plane, typically this is a stage micrometer, which is a microscope slide with ruled lines drawn on a central clear area. A typical scale is shown in Figure 3. A stage micrometer should be purchased from a reputable manufacturer who should be able to arrange for a certificate of measurement if required. The certificate should show that the scale has been checked by a method traceable to the standard metre.

An objective should be chosen of high enough NA to resolve the smallest particles to be sized (see **7.2**), the stage micrometer should be brought into focus and the microscope should be set for köhler illumination in accordance with Annex B. The scale on the micrometer should cover the portion of the field of view that is to be used to make measurements but should also be fine enough to determine the required length accurately. The scale is then compared with the measuring scale using the full width of the particle measurement area. If the microscope is fitted with an adjustable tube length or a zoom magnification, this should not be altered after calibration.

NOTE For some binocular microscopes, the tube length alters with inter-eyepiece distance adjustments. This should be checked before contemplating particle size analysis, and if such variations occur, the distance is not to be altered following calibration.

8.2.2 *Calibration of the light microscope for use with the eyepiece graticule for manual measurement*

The commonly used method of manually sizing particles necessitates the use of an eyepiece graticule of the design shown in Figure 4. The use of this graticule is described in **9.1** and guidance on the purchase of one of appropriate size is given in Annex C. The circles on the graticule have diameter sizes in a $\sqrt{2}$ geometric progression.

Oculars and any tube magnification factor can then be chosen so that the smallest circle on the graticule is clearly resolvable and distinguishable by size from the next circle. The longest dimension of the eyepiece graticule should not exceed five-eighths of the diameter of the field of view. The size of the grid can then be compared with a stage micrometer in accordance with **8.1**. The full width of the grid can be used for comparison, or the distance between the calibration marks which are 60.4 times the diameter of the smallest circle can be used.

8.3 Calibration of electron microscope

8.3.1 *Scanning electron microscope*

8.3.1.1 *Mode of operation*

The magnification of the system is determined by the relative sizes of the scan on the display tube and of the probe on the specimen surface. It is therefore dependent upon the excitation of the scan coils, as modified by any residual magnetic or stray fields. It also depends sensitively on the working distance between the lens and the specimen. It is not easy to measure this physically but it can be reproduced with fair accuracy by measuring the current required to focus the probe on the specimen surface.

The display tube itself may not have a completely linear scan, so distortions off the magnification can occur here also.

In considering the fidelity of the image, it is assumed that the specimen itself does not influence the linear response of the beam; in other words that charging effects on the specimen surface are negligible. If calibration measurements of any accuracy are to be made, any metal coating employed to make the surface conducting should be very thin compared to the structure to be measured, and is best avoided altogether if possible.

Since charging is much more serious for low energy secondary electrons than for the higher energy back scattered electrons, it is preferable to use the back scattered signal for any calibration work, if the instrument is equipped to operate in this mode. For similar reasons, if the specimen is prone to charging, the use of a low voltage primary beam rather than an applied conductive coating is much to be preferred.

The indicated magnification shown on the instrument is a useful guide but should not be relied upon for an accuracy better than ± 10 %.

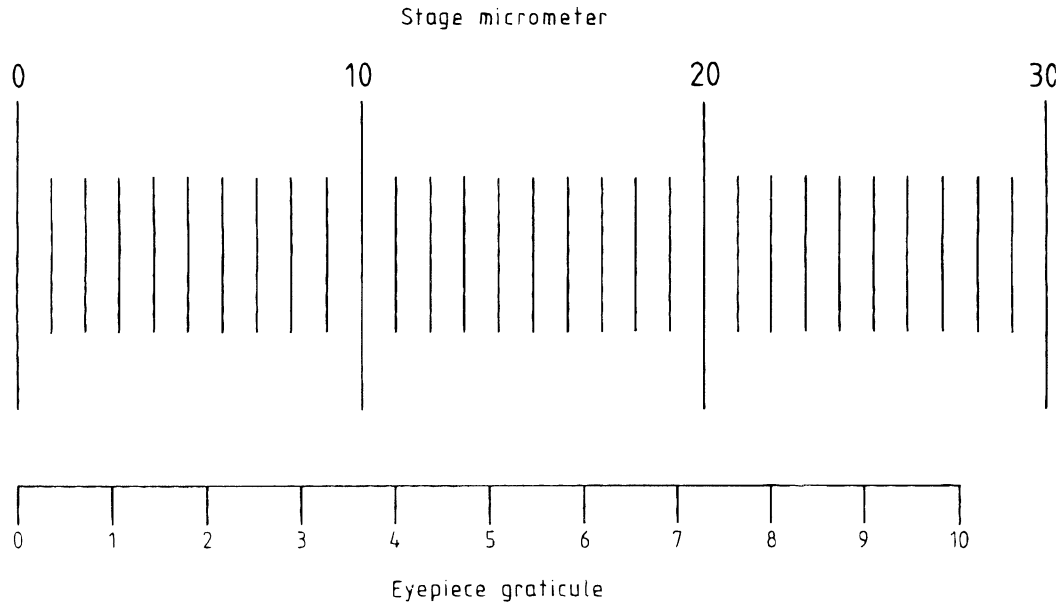


Figure 3 — Representation of stage micrometer and eyepiece graticule

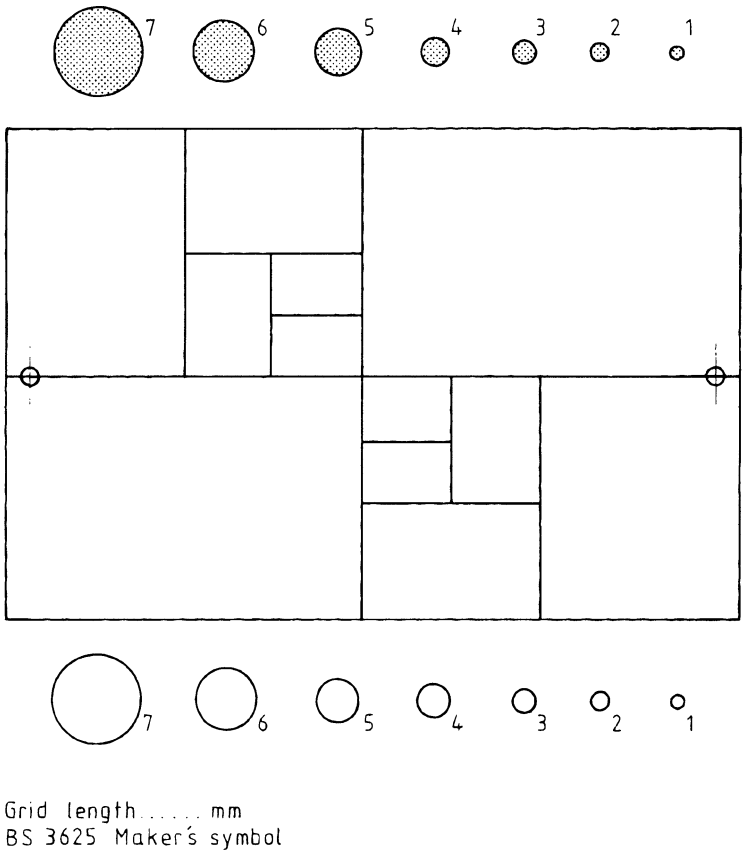


Figure 4 — British Standard graticule (see BS 3625:1963)

8.3.1.2 *Choice of calibration specimen*

Since there are various potential sources of image distortion in an SEM, it is a great convenience if the calibration specimen yields measurements over the whole extent of the screen and in two orthogonal directions. Thus a cross-ruled diffraction grating, or a square mesh of etched or electron beam written lines on a silicon substrate is an ideal specimen. The wide range of magnification covered by a scanning microscope requires that meshes of different dimension are available to cover the full magnification range.

There is a choice of a number of such meshes. The most coarse mesh, which is easily available, is a grid which has a period of about 16 μm and which yields an accuracy of better than 1 % if averaged over about 10 bar spaces (a procedure to be recommended with all the specimens described in this clause).

At progressively higher magnifications, copper foil grids, cross-ruled silicon substrates and metal replica diffraction gratings are available.

All of these specimens should be mounted flat on a specimen stub suitable for the SEM in use, and the stage tilt should be set at zero. This can be checked by traversing it in x and y directions to check that there is no change in beam focus and therefore no residual tilt. The beam tilt control should be set at zero.

It is most important that the working distance is not changed during the examination of the specimen or when changing to a calibration specimen. The indications of working distance given on the instrument are not sensitive enough to detect changes which could affect measurement accuracy in quantitative work of this nature. It is better to reset the exchange specimen stub against a physical reference surface which has already been matched to the stub carrying the specimen.

There is a considerable convenience in being able to include a magnification standard on the same specimen stub as the sample to be measured, since there is then no ambiguity in the operating conditions (working distance, accelerating voltage, etc.). This can be ensured by using a grid, as suggested above, or, even more integrally, by dispersing a preparation of polystyrene latex spheres on the specimen so that each field of view contains some of the calibrating spheres. It has to be emphasized that, although the various "uniform" latex suspensions do indeed have a well-defined mean size, the deviation from the mean allows a significant number of particles of different size to be present. It is essential therefore to include a statistically significant number of latex spheres in the measurement if the calibration is to be valid.

NOTE If latex spheres are used for reference purposes they should be calibrated under conditions identical to these used in measurement.

8.3.2 ***Transmission electron microscope***

8.3.2.1 *Mode of operation*

The final image magnification is made up of the magnifications of two to four electron lenses, and it is not feasible to measure the individual stages of magnification. Since the lenses are electromagnetic, the lens strength is not only dependent on the exciting currents, but also on the previous magnetic history of each iron circuit. It is essential therefore to cycle each lens in a reproducible manner if consistent results are to be obtained. Suitable circuitry is now included in many instruments, otherwise, each lens current should be increased to its maximum value before being returned to the operating value in order to ensure that the magnetic circuits are standardized. This should be done before each image is recorded.

The indicated magnification shown on the instrument is a useful guide but should not be relied upon for an accuracy better than ± 10 %.

8.3.2.2 *Choice of calibration specimen*

It is possible to calibrate the lower part of the magnification range using a specimen which has been calibrated optically, though this loses accuracy as the resolution limit of optical instruments is approached. At the top end of the scale, it is possible to image crystal planes in suitable single crystals of known orientation. These spacings are known to a high degree of accuracy by X-ray measurements. Unfortunately, there is at present no easy way of checking the accuracy of calibration in the middle of the magnification range. The specimen most often used is a plastic/carbon replica of a cross-ruled diffraction grating. While it is believed that these may usually be accurate to about 2 %, it has not so far proved possible to certify them. The very useful polystyrene latex suspensions of uniform diameter spheres can be used, as described for scanning microscopy in **8.3.1.2**. These can be obtained in many sizes ranging from 0.02 μm to 10 μm. It is emphasized that a statistically significant number of such spheres should be included in any field of measurement.

8.4 Calibration of the image analyser

8.4.1 *Linear calibration*

This is the measurement of the physical distances in the object plane represented by a distance in the image plane. The image plane is the digital image inside the computer and so the calibration is expressed in length per pixel or pixels per unit length. The procedure for the linear calibration of image analysers varies from machine to machine but usually involves indicating on the screen both ends of an imaged artefact of known dimensions in the object plane. This artefact may be a grid, grating, micrometer, ruler or other scale appropriate to the viewing system, and should be arranged to fill the field of view, as far as possible. The calibration should be measured both parallel to, and orthogonal to, the scan direction, and should not differ by more than 1 %. Some image analysers can be calibrated in both directions and use both these values.

Linear calibration can be altered by such things as drift in a tube camera, the sagging of zoom lenses and the refocusing of an electron microscope.

8.4.2 *Localized calibration*

The linear calibration may vary over the field of view. There may be image distortions in the optics or inadequately compensated distortions from a tilted target in an electron microscope. These distortions can be seen by comparing an image of a square grid with an overlaid software generated pattern, for example the software pattern might be a measurement frame.

Tube cameras are a source of localized distortion, especially at the edge of the screen near the start of the scan lines. The size of these distortions can be determined by measuring a graticule with an array of spots all the same size that fill the screen, or by measuring one spot or reference particle at different points in the field of view. Some image analysers allow localized calibrations to be made.

8.4.3 *Threshold calibration*

The threshold grey level at which particles and background are differentiated affects the measured size of the particles. The correct level will depend on the particles, background and illumination (see **10.2.2**).

8.5 Reference graticule

Many of the calibrations outlined in **8.3** can be performed easily with a calibrated graticule containing arrays of calibrated spots and a square grid. Such a graticule is the reference stage graticule for image analyser calibration which is obtainable from the National Physical Laboratory.

9 Manual methods of measurement

9.1 Light microscopy

9.1.1 *General*

NOTE This clause describes the measurement of praticle size by observation of the particles with the light microscope. The use of image analysers is covered in clause **10** and the measurement of particle images from photomicrographs is outlined in **9.2**.

Manual measurement of particle size by light microscope necessitates the use of an eyepiece graticule of the design described in **8.2**. This method is very tiring for the operator and the task should be shared between two people if possible. These operators should alternate approximately every 20 min.

9.1.2 *Microscope configuration*

The objective and eyepiece (and tube length if appropriate) should be selected to produce the required magnification as described in **8.2.2**. The corresponding eyepiece graticule should be inserted in the focusing eyepiece and the microscope should be set up as described in Annex B. If the microscope is fitted with a zoom facility, this should be locked in the appropriate position for the eyepiece graticule or, alternatively, the eyepiece graticule should be calibrated by comparison with a stage micrometer. Monochromatic illumination produces a better image if small particles are to be measured.

9.1.3 *Measurement areas*

It is usually not practical to measure all the particles on a slide. A few trial measurements should be made in accordance with clause **11** to determine the number of fields that should be measured to achieve the required accuracy. The area of the slide occupied by the particles should be mentally subdivided into the appropriate number of equal areas. The measurement fields are then taken to be at the centre of these areas. This procedure is shown in Figure 5 a) and is used to keep the particles measured representative of the whole sample. The regions at the edge of the sample may well be unrepresentative of the whole and should be avoided. The locations of the measurement fields can be found with the aid of the vernier scales on the microscope stage if these are fitted.

If the particles to be classified are few and far between, over 100 field areas may need to be analysed. Under these circumstances the measurement areas are defined as strips of width equal to the apparent distance between the upper and lower edges of the eyepiece graticule grid. The operator scans continuously along the strips, stopping to register any particles in the appropriate size range. The strips should be located at approximately equal intervals across the sample area as shown in Figure 5 b). The lengths of the strips should be recorded if they define the areas examined.

If there are a great many more fine particles than large particles in the sample then the fine particles can be measured over a smaller area than the large particles. This is done by measuring the large particles over the whole of the eyepiece graticule grid and the finer particles over one of the grid's subareas. Figure 4 shows that the grid is divided into areas which are 2, 4, 8, 16, 32 and 64 times as small as the whole. The areas over which the sizes have been measured should be recorded and clause **11** explains how all these values are used to produce representative results.

9.1.4 *Particle classification*

The slide should be positioned so that the correct portion of the sample is visible through the grid. Particles that lie wholly or partly within the grid and do not touch the lower or right edges of the grid are eligible for counting. This is shown in Figure 6. Each particle is considered in turn and, following any necessary focusing, its area is compared with that of the circles at the top or bottom of the eyepiece graticule. The comparison should be made with the open or closed circles, depending on which most closely resembles the images of the particles. The particles are classified by deciding between which two circles their areas lie. For example, if the area of a particle is estimated to be greater than the area of circle 4 but less than that of circle 5, it is assigned to the size class bounded by the diameters of circles 4 and 5. The presence of a particle in this class is recorded on a bank of registers, tape recorder or by some other method that does not distract the operator from observing the particles. This procedure is repeated for all the other particles in the field before moving on to the next field. It is worthwhile making a count of particles of area greater than circle 7 and less than an imaginary circle 8. This will indicate whether further measurements at a lower magnification are required. When changing objectives there should be an overlap of two size classes to provide cross-validation.

9.2 Scanning and transmission electron microscopy

The images from both these forms of microscopy can be recorded photographically, the scanning image usually on conventional or instant film, and in the case of the transmission electron microscope on plates or cut film. The following methods can also be applied to photographs taken using a light microscope.

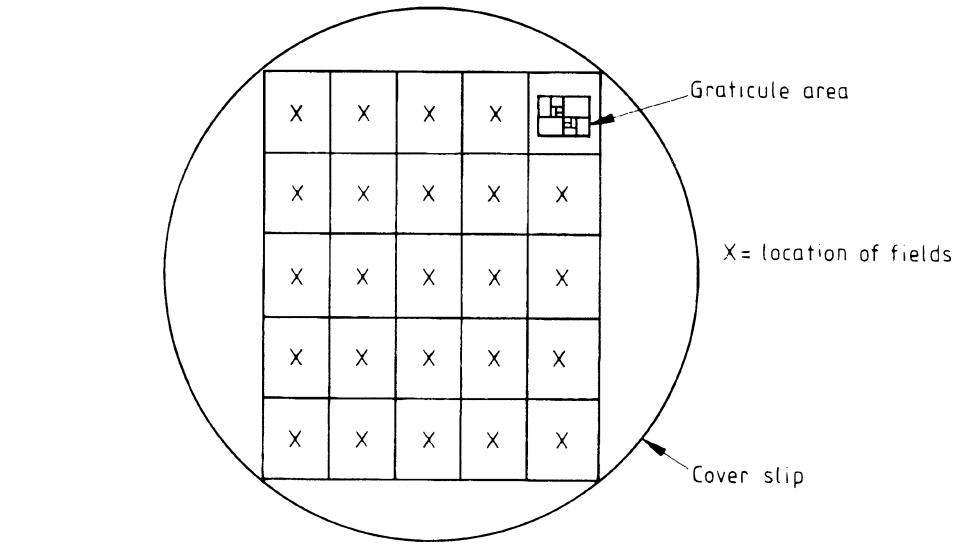
For the measurement of linear properties a travelling microscope or measuring eyepiece may be used. If however the particles are to be characterized by the diameter of circles of equivalent projected area, as described in this standard, one of the following methods should be used.

a) using an automatic image analyser, fitted with a macro viewing facility, as described in clause **10**.

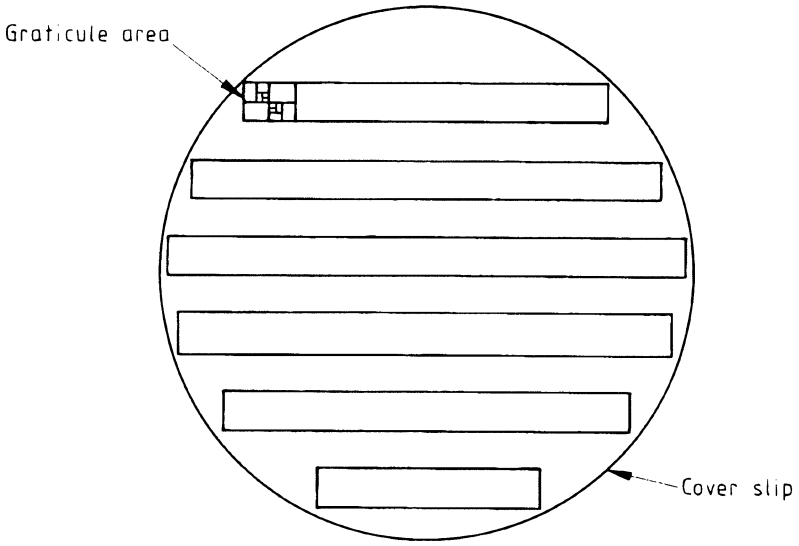
b) using semi-automatic image analyser as described in Annex D.

A calibration photograph, e.g. of a grating standard, is necessary in these methods to ensure accurate sizing; the use of photographic negatives is preferred. Care should be taken in using any photographic method that images are not distorted. From these photographic records, measurements of the particles under investigation can be made usually with a travelling microscope or a measuring eyepiece.

Some scanning microscopes may have micrometre bars superimposed on the image from which a crude set of sizes can be estimated from the screen, and in the case of the transmission microscope, reference scales, or circles of known diameter, may be scribed on the viewing screen for the same purpose.



a) Location of sample fields



b) Location of sample field for strip method

Figure 5 — Counting procedure

10 Measurement by image analyser

10.1 General

NOTE 1 This clause describes the use of video based image analysis (IA) techniques or the measurement of images of particles from optical or electron microscopes.

The main advantage of using IA for particle measurement is that it retains the relatively absolute nature of microscope methods while reducing the subjectivity and operator fatigue associated with these techniques. If the microscope is fitted with an automatic stepping stage and autofocus, under control of the IA system, it is possible to measure very large numbers of microscope fields so improving the statistics.

Because the IA does not measure area by comparison with the standard graticule (see BS 3625:1963), it is not confined to measuring particles of aspect ratio less than 3 : 1. If, however, comparison with manual methods is necessary, the IA should report separately on the number of particles with aspect ratio greater than or equal to 3 : 1.

Image analysis systems are less capable than human operators of discriminating against artifacts caused by sample preparation or microscopy. They are unable to adjust focus during measurements in the field of view which can cause depth of field problems when measuring distributions with a large dynamic range.

NOTE 2 Further information on image analysis is given in *Image analysis principles and practice*[2)].

10.2 Method

10.2.1 *General*

The IA system should be programmed to measure the individual areas of each particle image in a field and to accumulate the results as a size distribution using a geometric progression of size limits. A record should be kept of the area of measurement that has been taken. The measurements may be accumulated over many microscope fields but may be terminated before the full array of fields has been measured if a specified total particles count is reached first. If the range of the distribution is greater than can be measured at a single magnification and a representative part of the slide has been measured, an array of fields should be measured at a different magnification.

The same series of absolute size limits should be extended to the new range and a few size classes made common to both ranges to give an overlap. The final results are expressed on a scale of equivalent circle diameter (see **3.5**).

The method of programming the IA system to generate these measurements will depend on the particular system architecture. In some cases the system manufacturer or supplier will provide a compatible programme.

The main possible source of error in the IA system is in the detection process as the binary detected video signal may not correspond accurately to the geometry of the particles. There will also be serious errors if an attempt is made to measure particle images which are too small at any given magnification due to digitization effects and system resolution. (See **10.3**.)

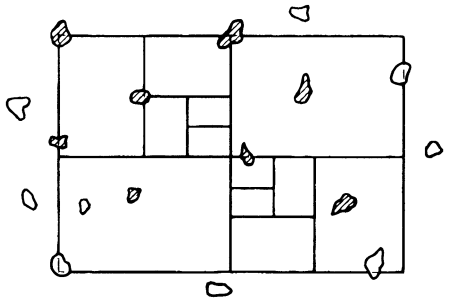
10.2.2 *Detection*

10.2.2.1 *Method of detection*

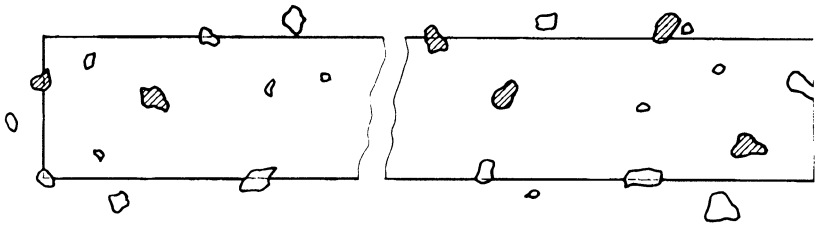
Detection is the separation of the image into regions representing particles and the rest. A particle is correctly detected when all the pixels within its geometric boundary are detected and no pixels outside particle boundaries are detected. Some systems detect particles on the basis of simple grey level thresholding while others use more sophisticated algorithms such as the so-called auto-delineation algorithm which attempts to predict the true edge of unsharp images of particles by reference to the local grey level values. This is used to improve the accuracy of measurements and to make them less sensitive to incorrect thresholding levels.

This British Standard does not recommend any particular method of detection but particles should appear to be correctly detected throughout the size range being measured and at any position in the measurement frame and at any time during the measurement.

[2)] Published by Joyce-Loebl, Marquisway, Team Valley, Gateshead NE11 0QW.



a) Counting isolated field



b) Counting strip

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

Figure 6 — Treatment of edge particles

10.2.2.2 *Finding the correct threshold level*

The methods of determining whether a particle is correctly detected involve taking a test particle, near the centre of the size range being measured at the magnification, and noting the detection settings at which it is best detected. A second test particle in the smaller size class being measured at the magnification is then measured, first at the threshold best for the small particle and then at the threshold and detector settings chosen for the medium sized particle. The last measurement should be repeated with the particle at positions near the four corners of the measurement frame. The detection of the small particle is satisfactory if the spread of its measurements made at the threshold chosen for the medium sized particle does not exceed one-half of a size class. The spread should be expressed as the ratio of the largest to the smallest area measurement and the square root of this ratio taken as the diameter ratio. This diameter ratio should not exceed one-half of the adjacent limit diameter ratio for the size distribution. Similarly the error due to measuring the small particle at the wrong detector setting as measured in the centre of the field, should not exceed one-quarter of a class.

10.2.2.3 *The half-amplitude method of threshold level setting*

The correct threshold level may be estimated using the half amplitude method. Some image analysers can find this level automatically but it can also be done by the following manual method. A small region of the background, which is a few pixels away from the boundary of a typical particle, is selected. The threshold level at which approximately half the pixels in the selected region are detected, is recorded. This process is repeated for an area a few pixels inside the particle boundary. The threshold level should be set at a value midway between these values. On some image analysers the assessment of the threshold levels for 50 % detection can be assisted by measuring the field area using a small measurement frame in the test regions. The true edge of the particle may not be precisely at the detected boundary but it will be a good approximation.

10.2.2.4 *Detection of phase images*

Phase images are not correctly detected at half amplitude points. A pure phase image would be detected at the zero amplitude contour but the practical images from optical microscopes of transparent particles are part amplitude, part phase in nature and vary slightly with focus. Transparent particles are generally detected as a ring which can be filled by the image processing algorithms available in some IA systems. No general method of choosing the best threshold is possible and it is best done by observation of typical test fields. The measurement is inevitably less precise than is possible with amplitude images.

NOTE In order for these tests to be performed, it should be possible to obtain individual feature area measurements of the chosen test particles. This may be possible by setting a small measurement frame to include one particle only or it may be possible by means of a light pen selector. For the actual measurements of size distributions the system need show only the size class allocated to each particle.

10.2.3 ***Counting logic***

If all the objects that appear in the image frame 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 lefthand lowermost pixel) as the feature count point. Objects are accepted only when their feature count point lies within the measurement frame. 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 particles are 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. 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 which would either be too large to be measured at the magnification or would be so acicular as to be unsuitable for classification by area anyway. IA systems which reject all particles intersecting a frame edge use an effective frame size which is different for each size class and also different for each particle shape.

10.3 Dynamic range

The smallest size limit, subject to the provisos in clause **7**, should be set so that measurements of an object of known size are confidently repeatable within 5 % in different orientations and positions on the screen. Smaller particles may be measured but smaller particles which do not meet the 5 % repeatability criterion should be sized at a higher magnification.

The largest size limit at any magnification should not exceed 10 % of the effective measurement frame area.

NOTE For systems rejecting all particles touching any side of the frame, the effective frame area for this large size limit will be considerably smaller than the actual frame.

11 Distribution determination, accuracy and precision

11.1 Choice of size classes

11.1.1 ***General***

When the particles have been measured they should be classified according to their equivalent circle diameters. The size distribution should be expressed as counts per unit area of the slide's surface and as the total number of particles counted in each size class. The size classes should be separated by a logarithmic series of limits with adjacent limit ratio of $1/(2c)$ for diameter, where c is an integer which may be chosen to give a sensible number of size classes over the expected distribution range. (See Table 2.)

When using manual methods, the classes are defined by the spots and circles on the eyepiece graticule.

Table 2 — Range of sizes

c	Adjacent limit ratio $[1/(2c)]$ for diameter limits	Range covered[a]		
		16	24	32
2	1.414	181	2896	46341
4	1.189	13.5	53.8	215
6	1.122	5.66	14.3	35.9
8	1.091	3.67	7.34	14.7
10	1.072	2.83	4.92	8.57
12	1.059	2.38	3.78	5.99
16	1.044	1.92	2.71	3.83
24	1.0293	1.54	1.94	2.45

[a] Range of sizes covered by 16, 24 or 32 size classes using a geometric series of size limits with various adjacent limit ratios. The range is expressed as the ratio of the last to first geometric mid-class diameter.

The absolute value of the diameter limits is optional but, when a distribution extends over more than one magnification range, the limit series should be continuous, in absolute units. It is possible to calculate the correct distribution if the classes for different objectives do not match and this may be the best option if analysing the results on a microprocessor.

11.1.2 ***Choice of size classes for pre-classified material***

Most powders that have been produced by, or have been subjected to, some comminution process have size distributions covering a very wide range of particle sizes with the median size value displaced markedly towards the lower size limit (i.e. there is a numerical predominance of the smaller particles). Such distributions can often be satisfactorily represented by a logarithmic form of the normal distribution. The main part of this standard is devoted to the determination of size distributions of materials of this type. However, where the laboratory sample has been drawn from pre-classified material (i.e. the bulk sample has been previously separated into bins covering a restricted range of particle sizes by a process such as sieving), the distribution characteristics are often more accurately represented by a standard normal distribution of sizes. In this case it will be more appropriate to use a linear progression of class limits for the distribution.

11.2 Calculation of size distribution

The size distribution should be calculated in the following manner.

Let there be j classes of particle of interest.

For the r^{th} class ($r = 1, \ldots, j$):

let m_r be the number of particles observed;

let d_r be the average equivalent circle diameter of the particles;

let A_r be the total area of observation so that

$A_r = n_r a_r$ when there are n_r sample fields each of area a_r

a) *The number size distribution.* For each class calculate m_r/A_r which is the average number of particles per unit area.

Then calculate:

$$p_r = (m_r/A_r) \Big/ \sum_{i=1}^{j} (m_i/A_i) \qquad (1)$$

Then, $100p_r$ is the number percentage of particles in the r^{th} class.

NOTE 1 A worked example is given in Annex E.

b) *The volume size distribution*. First calculate

$m_r d_r^3 / A_r$

Then calculate:

$$q_r = (m_r d_r^3 / A_r) \Big/ \sum_{i=1}^{j} (m_i d_i^3 / A_i) \qquad (2)$$

Then $100q_r$ is the volume percentage of particles in the r^{th} class.

NOTE 2 A worked example is given in **F.2**.

11.3 Calculation of the standard error (SE)

The calculations given in **11.2** are based upon the data obtained by sampling fields from a slide. The estimates of number and volume distributions are therefore subject to sampling variability; that is, different samples are likely to give different values of p_r and q_r for $r = 1, \ldots, j$. The standard method of expressing the extent of this variability is through the standard error of these estimates. The standard error is such that an estimate can be expected to be within 2 standard errors of the quantity it estimates, and two independent estimates can be expected to be within $2\sqrt{2}$ standard errors of each other. The assumptions made in the derivations of expressions for these standard errors are that the particles are scattered completely at random over the slide and that there are no experimental errors made in the size analysis of the sample.

The standard error for the estimator p_r is estimated using:

$$S(p_r) = \left[\frac{p_r^2}{m_r} \left(1 - 2p_r + m_r \left(\frac{p_1^2}{m_1} + \ldots + \frac{p_j^2}{m_j} \right) \right) \right]^{1/2} \qquad (3)$$

Similarly, the standard error for the estimator q_r is estimated using:

$$S(q_r) = \left[\frac{q_r^2}{m_r} \left(1 - 2q_r + m_r \left(\frac{q_1^2}{m_1} + \ldots + \frac{q_j^2}{m_j} \right) \right) \right]^{1/2} \qquad (4)$$

When the $\{A_i\}$ are equal, these equations simplify to:

$$S(p_r) = p_r \left(\frac{1 - p_r}{m_r} \right)^{1/2} \qquad (5)$$

11.4 Confidence intervals

An alternative method of expressing the accuracy of an estimator is through the confidence interval and the standard error is used in the computation of this. For the percentage of particles in a certain class, for example, the estimate cannot be expected to be equal to the true value. An interval, computed by taking an upper limit at 2 standard errors greater than the estimate and a lower limit at 2 standard errors (SEs) less than the estimate, can be expected (with 95 % confidence) to include the true value. Diagrammatically this has the form:

$$\left(\frac{2 \text{ SEs}}{\text{Estimate}} : \frac{2 \text{ SEs}}{} \right)$$

Note that the estimate is at the centre of the interval. A short interval implies greater accuracy as the estimated proportion is then known to be near the true proportion. For a wide interval, however, the estimate may be inaccurate.

Using the formulae given in Annex F, it follows that the 95 % confidence intervals are as follows:

a) for the true number size proportion for a class:

$p_r - 2S(p_r)$ to $p_r + 2S(p_r)$

b) for the true volume size proportion for a class:

$q_r - 2S(q_r)$ to $q_r + 2S(q_r)$

11.5 Amount of sampling necessary for specific accuracy

The accuracy with which the number proportions, volume proportions, etc. are estimated is represented by the widths of the confidence intervals (see **11.4**). These depend on the number of particles observed and, therefore, on the number of slides sampled. If the degree of accuracy required can be specified in terms of a desired width of confidence interval, equations (3) to (5) can be used to determine approximately the number of slides that need to be sampled.

Alternatively the desired accuracy may be expressed in terms of a percentage accuracy, e.g. 2 % accuracy implies that the estimate is within 2 % of the true value.

A difficulty arises that is common to sampling problems of this kind, namely that the sample size required depends on the values of the proportions to be estimated. The usual way around this is to conduct a pilot experiment from which rough estimates of the proportions can be calculated, and these can then be used to determine the complete sample sizes.

Equations for the total slide area (and, therefore, numbers of slides) to be sampled are given, on the assumption that a pilot experiment has already been conducted and the following rough estimates of the proportions are available; for a total slide area of A, for the pilot sample the number size distribution is estimated as $p_1, p_2, \ldots, p_j$. Equations (6) and (7) assume the total area for counting to be the same for all classes of particles so that the simplified form of the equation for the standard error, i.e. equation (5), applies. Even when this is not so, the equations should provide good approximations. Two cases are presented for slightly different accuracy specifications.

Case 1. If the desired width of interval for the i^{th} particle is w the total area to be sampled to give this accuracy, for the i^{th} particle is:

$$A_i = 16p_iA(1 - p_i)/Nw_i^2 \qquad (6)$$

where

N is the total number (all types) of particles observed.

Case 2. If the desired percentage accuracy for the i^{th} particle is ψ_i, i.e. the estimator is to be within ψ_i % of the true value, then the total area to be sampled to give this accuracy is given by:

$$A_i = A(1 - p_i)(200/\psi_i)^2/(Np_i) \qquad (7)$$

If all classes of particle are observed in the same total area then the total area to be sampled is the maximum value of A_i. Note this will always correspond to the class for which p is closest to 0.5. The number of slides to be sampled can be deduced from the total area to be sampled.

For sample size determinations for the volume size accuracy, the same equations apply but with p replaced by q.

NOTE A worked example is given in Annex F.

11.6 Reporting of results

In the reporting of results all details of the analytical conditions used in obtaining them should be included to allow the analysis to be reproduced under identical conditions. Full details including instrument calibrations should be included.

Annex A (informative)
Equipment

A.1 Light microscopy

The light microscope normally used is a standard biological microscope with bright field köhler or köhler-type illumination. It consists of a stand, suitable range of objectives and oculars, biological stage with *XY* translation and an Abbé condenser with numerical aperture greater than or equal to the numerical aperture of the objectives.

The above equipment is suitable for test samples with good optical contrast.

If good optical contrast cannot be obtained between the specimen and the mounting medium, visibility of the specimens can sometimes be enhanced by using other light microscope techniques, as in the following examples.

a) *Dark field microscopy*. A condenser stop excludes direct light from the objective and only the diffracted rays from the particles are collected. Particles appear bright against a dark background. Size measurements tend to be overestimates because of the bright diffraction halo.

b) *Polarized light microscopy*. Crossed polars can be used in some instances to discriminate against interfering particles during the size analysis. The addition of the first order red compensator can be useful if isotropic particles need to be discriminated from anisotropic particles. The microscope has to have a rotating stage and this has to be used to ensure particles are not in the extinction position. Low first order interference colours can lead to an underestimation in particle size, particularly when the particle has little relief against the mounting medium and has tapering edges. The technique is not suitable for measurements by image analysis.

c) *Phase and interference contrast microscopy*. These are forms of image enhancement for particles of low visibility. They should be used only when it is not possible to increase optical contrast by changing the mounting medium. The particle edges may be ill-defined and hence the technique is generally suitable only for comparing data from similar samples rather than for obtaining accurate values. The ray path length differences between the direct and diffracted rays are critical in providing a crisp amplitude contrast image. Edge haloes are produced when the particles are too thick or the refractive index difference between the particles and mounting medium is too great. If such effects are observed using one type of interference contrast, alternatives have to be investigated. Comparison of results obtained using different interference contrast techniques can be subject to large errors caused by the ill-defined edges. The phase contrast light microscope may also be used in particle sizing. Using this technique the ray traversing the transparent object is made to interfere with the ray traversing the surrounding medium, so that the microscope converts the resultant phase difference into a contrast difference. The phase shift φ (in degrees) is given by:

$$\varphi = \frac{(\mu_1 \times \mu_2)T \times 360}{\lambda}$$

where

T	is the thickness of the particles (in μm);
μ_1 and μ_2	are the refractive indices;
λ	is the wavelength of light (in μm).

For particles to be clearly visible φ should be 5°, and this gives a lower limit on the resolution of approximately 0.5 μm.

A.2 Scanning electron microscopy

The main features of SEM are as follows.

a) A high spatial resolution can be obtained (about 10 nm) under good conditions in the secondary electron imaging mode.

b) It is associated with an excellent depth of focus which need not be magnification dependent.

c) Very large samples can be put on stages (up to 150 mm in diameter) and thus highly dispersed particles can be studied, obviating at least some of the problems of overlapping particles.

d) The specimen is introduced into the high vacuum of the specimen chamber and the material should therefore be able to withstand these conditions. Furthermore the specimen area under examination is scanned with an intense electron beam which could cause heating, charging or radiation damage.

e) Many SEMs incorporate a wide array of detectors (secondary electron, back-scattered electron, energy dispersive and wavelength dispersive X-ray) together with significant amounts of signal processing. This means that it is usually possible to establish good contrast between the particulate matter and any background. Imaging modes other than secondary electron detection have a significantly poor resolution.

f) There are a number of "bolt-on" attachments allowing image analysis to be performed on one or more of the signals [see item e)]. These may include computer image processing with edge enhancement.

The details of the operation of a particular SEM are usually described adequately in the manufacturer's manual.

Since the image is formed by rastering a high energy beam (1 kV to 50 kV) over the surface of the specimen, charging may occur if the specimen is non-conducting. The usual method of avoiding this problem is to coat the specimen with a thin layer of conducting film such as gold or platinum in a sputter coater. This can sometimes be avoided by using back-scattered electron detection, coupled with a low energy imaging beam, provided that the requisite resolution can be obtained.

A.3 Transmission electron microscopy

The TEM should be used when the specimen to be examined is known, or suspected, to contain small particles near the lower size limit covered by this standard. No other method can reliably give information about such particles.

The TEM operates in a magnification range from about × 600 to × 1 000 000, so that it overlaps the magnification range of both the light and scanning microscopes.

Because the electron beam traverses the specimen, the support should be electron transparent and therefore very thin (preferably less than 10 nm). The specimen has to be examined in a high vacuum enclosure and should be capable of withstanding the vacuum environment without loss of shape and without evaporating.

The incident electron beam, although carrying a few microamps of beam current, is concentrated on a very small area. This can cause problems when examining poor conductors, especially if the particles are fairly large (1 μm diameter is large, in this context). Such particles may vapourize or may heat up enough to break the support film and so be lost.

Where the range of particle sizes includes some large particles (e.g. 0.5 μm) as well as many small ones, it should be regarded as essential to supplement the information from the transmission electron microscope with that from a light microscope or scanning electron microscope, to ensure that the largest particles are adequately represented.

The contrast of even small particles tends to be good, against the background of the thin carbon support film. There is a large depth of focus so there is no problem associated with loss of sharpness with different particle sizes.

Many TEMs have a closed circuit television system fitted which allows the extraction of the image directly into one of the many automatic image analysis systems available.

All TEMs have electron diffraction facilities as a normal feature, and many are also equipped for X-ray analysis which together provide a powerful additional source of chemical information.

A.4 Automatic image analysis (IA) system hardware

IA system hardware should be chosen in accordance with **A.5**.

All automatic IA systems operate on a video representation of an image. This may be generated by a video camera mounted on a microscope, a video camera focused on a photomicrograph of particles to be measured, or the video signal from a scanning electron microscope.

The video representation is digitized into an array of points, and each point is assigned a number according to its shade. This digitized image is then processed by a combination of computer hardware and software which derives the particle measurements. Some IA systems store the image in computer memory and take measurements from the stored image. Other faster systems take measurements directly from the incoming signal. Systems which store the image in memory before processing are often more suitable for handling problems of separating features by shape or resolving images of touching or overlapping particles.

The image analyser should have a monitor which can display the objects which the system is measuring. Operator control is provided via a keyboard or an interactive device such as a mouse.

Some image analysers automatically control the function of the microscope.

NOTE This annex does not apply to IA systems based on image scanning methods other than video cameras or scanning (transmission) electron microscopes. In principle, images may also be scanned by mechanical object plane scanning methods such as microdensitometers or by source plane scanning such as cathode ray tube flying spot systems and laser scanning systems. At the time of preparing this standard, these methods are not used generally so no standardization is yet possible, but many of the considerations covered here will be relevant to these systems also.

A.5 Choice of image analysis (IA) system

A.5.1 *General*

The IA system selected should depend on what is to be measured. For example, what amount of image distortion is acceptable? Image distortion, which is best expressed as the variation in measured size of particle when measured at different points in the image frame. Where the IA is to measure a particle size distribution with a wide dynamic range and consequently large size class widths, a 3 % variation may be acceptable; but where the distribution is a narrow one with small size classes, a 3 % distortion may be unacceptable.

A.5.2 *Optics and television*

Where the system is used with an optical microscope, the field curvature, distortion and shading should be measured for the combined microscope-television system with each magnification to be used for particle, analysis. Where the system is to be used for measuring photographs or electron micrographs of particles, the measurements of field curvature, distortion and shading should be made using the optics to be used for imaging the photographs over the full range of field sizes to be used.

The field curvature should be such that the smallest particles to be measured at that magnification are not noticeably defocused at the corners of the measurement frame, as seen on the television monitor display, when they are at the best focus at the centre of the field.

NOTE 1 The size of the measurement frame may be reduced provided that the system conforms to all the other recommendations with the reduced frame size.

The distortion should be such that a test particle, typical of the kinds to be measured, when measured by the system at the centre of a field and near the corners of the measurement frame, results in a spread of measured sizes covering not more than one-half of one size class. This test should be met using a test particle in the smallest size class to be covered at the magnification and one near the centre of the range.

The shading may be checked with any available shade correction device in use, provided it is also used for the measurements. The shading should be such that a test particle, typical of the kind to be measured, in the smallest size class to be measured at the magnification, can be detected in accordance with **10.2.2** at nine positions in the field, using the same settings on the detector. The nine positions are near the centre of the measurement frame, inside the frame near the four corners and inside the frame near the centres of the four sides.

NOTE 2 Shading is strongly affected by the alignment of the optics or illumination. It should be checked after any change has been made to the optical alignment, for example when the microscope lamp bulb has been replaced or when the optics have been dismantled for cleaning.

The magnification of the optical system forming an image onto the television camera tube should have a value within the useful range. The maximum useful magnification is that which just matches the resolving power of the television system to that of the image. The television will generally have a worse spatial resolution than the image forming optics and the intermediate magnification should not be increased to the point where the full television resolution cannot be used. The minimum useful magnification is that which just matches the microscope field size or the size of the photograph to the television frame size. Note that at low and medium magnifications of the optical microscope, say up to × 25 objective, a good quality microscope may resolve some 2.5 times more lines per field diameter than the television system so that there will be a useful range of intermediate magnification of some 2.5 : 1. At the highest magnifications of the optical microscope the apparent resolution is much less in the image so that there is a much smaller range of useful intermediate magnification. The measurement of the optical or system resolution will be beyond the scope of most users but the choice of intermediate magnification is likely to be satisfactory if the following method is chosen.

For optical microscopy, choose an eyepiece magnification which gives a sharp image with the chosen objective lens over the full field. Note the field diameter, either in terms of recognizable features in the image, or by using a stage micrometer. Then set up the intermediate magnification to the television camera so that the diagonal of the television field is from 40 % to 100 % of the visual field diameter for objectives with primary magnification below × 30 or from 70 % to 100 % of the visual field diameter with higher power objectives. The intermediate optics should be parfocal with the visual optics as any significant refocusing of the microscope for the television will indicate that the objective lens is not working at its designed tube length and this will cause loss of resolution because of spherical aberration.

The resolution and the grey level discrimination of the television and detection system will greatly affect system performance. The spatial resolution affecting the dynamic range of particle sizes which can be measured at one time and the grey level discrimination affects the image contrast required to separate particles from background and from other image features by brightness. For small particles, resolution and grey level discrimination are interdependent. The operator is not expected to measure resolution or grey level discrimination but should be aware that it is these properties of the system which will enable the detection and dynamic range specifications to be met.

Annex B (normative)
Setting the light microscope for Köhler illumination

Köhler illumination is a means of uniformly illuminating an object on a microscope stage even though the light source is of finite size and has non-even brightness over its area. The procedure described below refers to the field stop and the aperture stop. The field stop is an iris diaphragm, usually positioned near the light source, which can be imaged in the object plane of the condenser lens system. The aperture stop or condenser stop is located or imaged in the back focal plane of the condenser lens system. With the microscope set for Köhler illumination, the field stop controls the size of the illuminated field, while the aperture stop controls the NA of the illumination. To set the microscope for Köhler illumination, the following procedure should be followed.

a) Fully open the field and aperture stops.

b) Focus on an object using the lowest power objectives; it is good practice to bring the stage close to the objective and then achieve focus by moving the stage away from the objective using the coarse focus control.

c) Close the field stop and focus it in the field of view using the condenser focus control.

d) Centre the image of the field stop with the condenser centring controls if available.

e) Fully open the field stop. Insert an eyepiece telescope or Bertrand lens if available, otherwise remove an eyepiece and view the objective exit pupil directly down the eyepiece tube.

f) If the diffuser is removable and the lamp is fitted with controls, focus and centre the image of the source at the objective exit pupil.

g) Restore the normal viewing conditions, select the required objective and carefully focus on the object.

h) Close the field stop and check it is in focus, adjusting the condenser focus control if necessary.

i) Open the field stop to just beyond the field of view.

j) Inspect the objective exit plane as described in step e) but without opening the field stop. Close the field stop until it is approximately two-thirds the area of the unobstructed objective pupil.

k) Restore the normal viewing conditions.

NOTE Binocular microscopes often provide additional focus control for one of the eyepieces. The microscope should be focused using the non-adjustable eyepiece before the adjustable one is individually focused.

An eyepiece may contain a crosshair, filar or graticule. Such features should be focused, using a relaxed eye, with the eyepiece removed from the microscope.

Annex C (informative)
Eyepiece graticules for light microscopy

There are the following three different approaches to buying eyepiece graticules (see Figure 3 and Figure 4).

a) If the microscope is fitted with a zoom facility or draw tube, it is possible to purchase just one eyepiece graticule. With the aid of a stage micrometer, the magnification can be adjusted for each objective so that the size classes form a smooth progression. This means that the largest two classes for a high NA objective are the same as two of the smaller classes on a lower NA objective.

b) One eyepiece graticule can be bought and used for all objectives and the necessary calculations performed to reduce the results to a $\sqrt{2}$ geometric progression of size classes.

c) A different eyepiece graticule is chosen for each objective so that the size classes are all part of the same smooth progression as described in case a).

Whichever approach is used, the size classes should be all part of the same progression so that the statistical methods in clause **11** apply.

When ordering an eyepiece graticule, the microscope type, the outer diameter of the glass disc of the eyepiece graticule and the distance in millimetres between the reference points on the grid (Y_{ref}) should be considered. For cases a) and b), Y_{ref} can be found by inserting any eyepiece graticule, finding a dimension which is about five-eighths the diameter of the field of view, removing the eyepiece graticule and measuring the true length. For case c), the following procedure should be followed.

1) Place any eyepiece graticule in the eyepiece and set up the microscope for taking measurements.

2) Use a calibrated stage micrometer to measure in micrometres the apparent length (Y_{app}) in the object plane of the scale on the eyepiece graticule.

3) Remove the eyepiece graticule and measure the true length of its scale (Y_{true}) in millimetres.

4) From the size class limits given in **8.2**, and the relative dimensions in Table 1, calculate the required apparent reference length of the BS 3625 eyepiece graticule (Y_{req}) in micrometres.

5) Calculate the reference length to be specified (Y_{ref}), in millimetres using the equation:

$$Y_{ref} = \frac{Y_{req} \cdot Y_{true}}{Y_{app}}$$

Y_{ref} should be measured as accurately as possible or the BS 3625 graticule obtained for that objective will give slightly different size categories. This becomes crucial if there is a large size distribution in the particles to be assessed and more than one objective, and hence more than one graticule, should be used. The size classes obtained from different objectives will not overlap if the $\sqrt{2}$ geometric is not maintained.

On receipt of the graticules, they should be coded for each objective. They should then be inserted in the focusing eyepiece chosen for each assessment, and checked against the stage micrometer.

Annex D (normative)
Semi-automatic image analysis

All types of microscope images may be measured automatically. The image is often in the permanent form of a photographic negative or print, but may be equally well presented as a video image (or recording) which may be treated interactively using computer techniques.

The photographic negative is projected onto a digitizing tablet which allows the cartesian coordinates of a hand-held cross wire to be calculated by the associated computer. This process is achieved for example by generating a series of pulses which is fed to an array of fine wires embedded in the tablet; a pick-up in the hand-held device effectively transmits these signals to a processor and the coordinates are determined. Alternatively, a photographic print may be placed flat on the tablet surface and treated similarly. Transparent tablets are available for measuring images projected from underneath. Tablet areas up to 1 m^2 have been routinely used with photographs.

Using video techniques it is also possible to superimpose ("overlay") the cursor positions onto an image generated by a TV camera looking down the optical microscope, or from the signal of a scanning electron microscope. Details in the image processing techniques are controlled by the operator. Alternatively, a phototube may be used to superimpose the coordinates of the cursor onto the microscope image.

In either case, the cursor can be used to locate the required features in the image and the coordinates recorded in a "discrete-mode", or alternatively, a movement of the cursor over the surface of the tablet can give the length covered in a "continuous mode".

Interactive facilities allow a wide range of image editing techniques to be applied, for example, the adding or excluding of image structure regions, or the manipulation of brightness ("grey levels") during observation. Densitometric measurement on the whole or part of the field may also be made and displayed as a histogram of grey level distribution along an image section.

From the measurements, a whole series of derived values can be obtained, for example, the distance between two points or the angle between three points in the discrete mode, and the area enclosed, or the equivalent circle diameter (ECD) of that area traced in the continuous mode. Evaluation functions may be applied to the sets of data accumulated by such measurements, for example, the calculation of mean values and standard deviation. Statistical analysis by comparison to standard distribution functions (e.g. Gaussian) can be computed. Distributions may also be displayed as histograms, scatter-diagrams or as accumulated percentage measurements over a given size range. Indeed, the complete range of computer based statistical packages may be applied.

Assuming the best possible microscope image has been obtained, then the limitations of this form of image analysis are:

a) the limit of resolution of the photographic negative or print (photographic grain);

b) the quality of the projecting lens system for negative work;

c) the resolution of the digitizing tablet (which will be given by its manufacturer, but is typically 0.1 mm);

and for video images the limitations are:

1) the resolution of the TV camera or scanner;

2) the number of pixels defining the image (often an array of 512 × 512);

3) the contrast/brightness setting of the TV monitor (this is a subjective factor set by the operator, and reference should be made to the manufacturer's instructions).

When using projected images or prints, non-parallax errors should be anticipated between cursor and image. TV cameras (with their associated analogue to digital converters) have a time lag in going from one image to another, and this should be noted if rapid changes are being measured.

The errors in the measurements from such systems should be investigated systematically before routine use, and checked at regular intervals by the operator. The overall performance of the system should be established by placing a reference object in the microscope and measuring its size semi-automatically. This value should be checked at various points on the tablet in the discrete mode (the continuous mode of measurement is often in error by up to ± 10 %, due to approximations made in the calculation of length by the processor). The measuring tablets are susceptible to magnetic fields and the manufacturer's instruction for use should be carefully followed or non-systematic errors may be encountered.

As the computers associated with the semi-automatic analysers are often limited in speed, and the arithmetic scope for data handling and statistical analysis is often restrictive, it is valuable to be able to purchase additional memory when required. Ideally, the computer should be freely programmable in popular languages, and the data file should be readable by the high-level languages.

A different kind of semi-automatic particle sizer is the type where transparent or translucent photographs are placed on a viewing area and the image of a circular iris is projected onto the photograph. The size of the iris is adjusted with a handle until it is of the same area as the particle positioned in the centre. The different diameters of the iris are correlated with a series of counters, each counter corresponding to a certain diameter range. A footswitch is then depressed and the aperture of the iris is recorded either by a counter or by a computer. The action of depressing the footswitch also causes an arm to come down and punch a pinhole in the measured particle to avoid recounting. The method is slower than automatic image analysis and is operator dependent but has the advantages that overlapping particles can be discriminated and poor images with low contrast or uneven brightness can be measured.

Particle widths may be measured accurately using image shearing. This method requires the fitting of an image shearing eyepiece to the microscope. This eyepiece contains a number of prisms and mirrors which split the microscope image into two identical images. These two images are coloured red and green to provide contrast and they can be separated or sheared by a measurable amount. The eyepiece is calibrated against a stage micrometer by separating the image of the micrometer so that two selected divisions are superimposed and then recording the amount of travel required to shear the image the other way so that the second pair of images of the same two divisions are superimposed. This reading may be a vernier on the shearing control or, more conveniently, may be a digital readout. Images of particles can then be sheared so that the edges of the two images just touch without overlapping, the position recorded or the readout zeroed, and the images re-sheared so that the other two images of the particle just touch. This is a fairly slow method but accuracies of better than 0.2 μm are possible under ideal conditions. Another use for image shear is the fairly rapid counting of particles above and below a certain size. This is done by shearing the image by moving the eyepiece by the required distance and counting which particle images do not touch or overlap each other.

Annex E (informative)
Example of calculation of size distribution by number

An example of calculation of size distribution by number is given in Table E.1.

Table E.1 — Example of calculation of size distribution by number

Size class limits μm	Number concentration in given class per square millimetre	Number per cent in class per square millimetre
> 27	0	—
27 to 19	4	0.1
19 to 13	13	0.4
13 to 9.4	25	0.9
9.4 to 6.6	42	1.5
6.6 to 4.7	80	2.9
4.7 to 3.3	139	4.9
3.3 to 2.3	252	8.9
2.3 to 1.7	399	14.2
1.7 to 1.2	731	25.8
1.2 to 0.8	1 139	40.3
Totals	2 824	99.9

Annex F (normative)
Statistical errors of size determination by the microscope method

F.1 General

In a well prepared microscope slide the particles are spread out in a random manner. If a series of such slides is produced under precisely the same conditions, then however accurately these conditions have been maintained, the number of particles on any defined area will vary from slide to slide. The methods described in this annex allow for this random variation in counts. There will be additional errors due to manipulation and observation. It is assumed that these manipulative and observational errors are small compared with the natural random variation.

It is assumed that the material to be examined consists of particles of different size, but of similar shape and density and that it has been sampled and the slides prepared according to the recommended procedures. The number of particles belonging to each size class is counted within a defined area on each slide; this area need not be the same for the different size classes and will normally consist of a number of separate fields distributed over the slide. At the end of the count the total number of particles found in each size class is divided by the area of the slide examined for that size class to give the numbers of particles per unit area, and from these either the number or mass distribution is estimated.

F.2 Chance variations in the number of particles in a given area

If the process of sampling and making up of the slide has been carried out correctly, then every particle of the original bulk material has an equal chance of appearing in any defined area of the final slide and, providing that the concentration of the particles on the slide is low, this chance is independent of what might have happened to any of the other particles. The probability distribution arising from these assumptions is well known; the completely random distribution of points that has been described is known as the Poisson process. In particular, if β represents the expected average number of particles per unit area then the probability of observing m particles in a sampled area of size A is:

$$\frac{(A\beta)^m \exp\ (-A\beta)}{m!}$$

This is known as the Poisson probability distribution. The population mean β may be interpreted as the average number of particles per unit area that would be observed if an extremely large number of slides were sampled. It is, in a sense, the true average and will be different from the observed sample average.

This Poisson distribution has a mean equal to $A\beta$. If the experiment is repeated a number of times with slides of unit area, then the observed values of m will be distributed around a mean $A\beta$ with a standard deviation equal to $\sqrt{(A\beta)}$.

If there are several classes of particles, providing the volume concentration of particles in the dispersing medium is not too large, the probability distributions for the numbers of particles counted in the various classes will be independent and each will follow a Poisson distribution.

Variations that can be expected in two important statistics, that is the number proportion (of particles in a size class) and the mass proportion, due to the random variation in the basic count will be considered. In both cases these statistics are ratios of a single random variable to a sum of random variables. A standard approximation to the variance of such a statistic will now be established and the result applied to the number and mass proportions.

Let x_i take the values $i = 1, 2, \ldots, j$ and a set of independent random variables with means $\{u_i\}$ and variance $\{V_i\}$ will follow.

Let $u = u_1 + \ldots + u_j$ and $V = V_1 + \ldots + V_j$

Then if $z_i = x_i - u_i$ and $z = z_1 + \ldots + j$ it follows that:

z_i has a mean 0 and variance V_i

z has a mean 0 and a variance V

The proportion $t = x_i/\Sigma\, x_i$, which is of concern, is given by:

$$t = \frac{u_i}{u}\left\{\left(1 + \frac{z_i}{u_i}\right)\left(1 + \frac{z}{u}\right)^{-1}\right\}$$

$$= \frac{u_i}{u}\left\{\left(1 + \frac{z_i}{u_i}\right)\left(1 - \frac{z}{u} + \frac{z^2}{u^2} - \frac{z^3}{u^3} + \frac{z^4}{u^4} \ldots\right)\right\} \text{ by binomial expansion.}$$

Assuming z/u to be small:

$$t \approx \frac{u_i}{u}\left\{\left(1 + \frac{z_i}{u_i}\right)\left(1 - \frac{z}{u} + \frac{z^2}{u^2}\right)\right\} \approx \frac{u_i}{u}\left(1 + \frac{z_i}{u_i} - \frac{z}{u} - \frac{zz_i}{uu_i} + \frac{z^2}{u^2}\right)$$

Since:

$E(z_i) = E(z) = 0$; and $\text{var}(z_i) = E(zz_i) = V_i$; and $E(z^2) = V$;

then:

$$E(\mathrm{t}) = \frac{u_i}{u}\left(1 - \frac{V_i}{uu_i} + \frac{V}{u^2}\right); \text{ and}$$

$$E(t^2) = \left(\frac{u_i}{u}\right)^2 E\left(1 + \frac{z_i^2}{u_i^2} + \frac{z^2}{u^2} + \frac{2z_i}{u_i} - \frac{2z}{u} + \frac{2z^2}{u^2} - \frac{4zz_i}{uu_i} + \text{higher order terms}\right.$$

Applying $E(.)$ and ignoring higher order terms gives:

$$E(t^2) = \left(\frac{u_i}{u}\right)^2\left(1 + \frac{V_i}{u_i^2} + \frac{3V}{u^2} - \frac{4V_i}{uu_i}\right)$$

Substituting from $E(t^2)$ and $E(t)$ into $\mathrm{var}(t) = E(t^2)$ gives:

$$\mathrm{var}(t) = \left(\frac{u_i}{u}\right)^2\left\{\left(1 + \frac{V_i}{u_i^2} + \frac{3V}{u^2} - \frac{4V_i}{uu_i}\right) - \left(1 - \frac{2V_i}{uu_i} + \frac{2V}{u^2} + \text{higher order terms}\right)\right\}$$

Ignoring higher order terms, this gives:

$$\mathrm{var}(t) \approx \frac{u_1}{u}\left(1 + \frac{z_i}{u_i} - \frac{z}{u} - \frac{z_i z}{u_i/u} + \frac{z^2}{u^2}\right)$$

Then:

$$E(t) = \frac{u_i}{u}\left(1 - \frac{V_i}{uu_i} + \frac{V}{u^2}\right); \text{ and}$$

$$E(t^2) = \left(\frac{u_i}{u}\right)^2\left(1 + \frac{V_i}{u_i^2} + \frac{3V}{u^2} - \frac{4V_i}{uu_i}\right)$$

Hence:

$$\mathrm{var}(t) = E(t^2) - \{E(t)\}^2 = \left(\frac{u_i}{u}\right)^2\left(\frac{V_i}{u_i^2} + \frac{V}{u^2} - \frac{2V_i}{uu_i}\right) \qquad \text{(F.1)}$$

These general rules can now be applied to particular statistics.

NOTE The operator $E(.)$ is known as the "expectation" operator. It is the mean of the sampling distribution, e.g. $E(t)$ is the mean of the sampling distribution t and $E(t^2)$ is the mean of the sampling distribution of t^2. These expectations may also be interpreted as the limiting values of t and t^2 as the sample size becomes extremely large. var(t) represents the variance of the sampling distribution of t and is the square of its standard deviation.

F.3 Number distribution

Consider a size analysis carried out on a material containing j classes of particles. For class 1 (diameter d_1), n_1 fields, each corresponding to an area of a_1 of the slide, have been examined and a total of $m_1$have been found over the total area A_1 ($A_1 = a_1 n_1$). For class 2 (diameter d_2), n_2 fields of area a_2 have been examined and m_2 particles found, and similarly for the other classes up to j.

Then if p_1, p_2, etc. are the number proportions:

$$p_i = \frac{m_i/(A_i)}{m_1/(A_1) + m_2/(A_2) + \ldots + m_j/(A_j)}$$

If $x_i = m_i/A_i$ then p_i is a special case of t, and if it is written:

$$P_i\ \{\simeq E(p_i)\} = u_i/u,\ M_i = E(m_i) = A_i u_i,\ V_i = u_i^2/M_i$$

then on substituting into equation (F.1):

$$\mathrm{var}(p_i) = \frac{P_i^2}{M_i}\left(1 - 2P_i + M_i \Sigma_r \frac{P_r^2}{M_r}\right) \qquad \text{(F.2)}$$

for the special case in which all classes are counted over the same area, so that $A_1 = A_2 = ... = A_j$, equation (F.2) reduces to:

$$\text{var}(p_i) = \frac{P_i^2}{M_i}(1 - P_i) \qquad \text{(F.3)}$$

It should be noted that this result also applies to the case when all types of particles are counted in each area of the slide.

F.4 Volume distribution

Consider once more the results of size analysis carried out on a material containing j classes of particles. As before, for class 1 (diameter d_1), n_1 fields, each corresponding to an area of a_1 of slide, have been examined, and m_1 particles found in the total area A_1 ($A_1 = a_1 n_1$), and similarly for the other classes. From these results the volume distribution of particle size in the material is calculated.

The first step in this calculation is to express the results for each class as a volume U per unit area of slide.

$$U_1 = \frac{k_1 d_1^3 m_1}{A_1}, \text{ etc.} \qquad \text{(F.4)}$$

where

k_1 is a constant dependent on particle shape (and density).

The true value of k for each class is not usually known, and it is usual to assume that its value is the same for all classes. For the present purpose, the truth or falsity of this assumption is irrelevant since the effects of random fluctuations in the values of m on the calculated volume or mass proportions will depend merely on the method of calculation used and not on the truth of its underlying assumptions.

Then the volume or mass proportion, $q_i = u_i/\Sigma u_j$ so that q_i is also a special case of t, and if:

$$Q_i \{\simeq E(q_i)\} = u_i/u,\ M_i = E(m_i) = A_i u_i (k_i d_i^3)$$
$$V_i = k_i^2 d_i^6 M_i/(A_i^2),\ u_i^2/V_i = M_i$$

and on substituting into equation (F.1):

$$\text{var}(q_i) = (Q_i^2/M_i)\{1 - 2Q_i + M_i \Sigma_r (Q_r^2/M_r)\} \qquad \text{(F.5)}$$

F.5 Confidence intervals

The true number proportion P_i is estimated by the sample proportion p_i. The true volume or mass proportion Q_i is estimated by the sample proportion q_i. These estimators, however, cannot be expected to be equal to the true values but, together with the above expressions for the variances, they can be used to determine confidence intervals for the true values.

The variances in equations (F.2), (F.3) and (F.5) are themselves estimated by replacing M_i by m_i, P_i by p_i, and Q_i by q_i. Then, let $S(p_i) = \sqrt{[\text{var}(p_i)]}$ and $S(q_i) = \sqrt{[\text{var}(q_i)]}$ so that, in each case, S is the estimated standard error.

When m_i is large (e.g. greater than 30) but small compared with Σm_i (e.g. less than 174) the P_i and q_i will be approximately normally distributed and 95 % confidence intervals have the simple formulae:

a) A 95 % confidence interval for P_i is $p_i \pm 2S(pi)$

where

$$S(p_i) = \left\{ \frac{p_i^2}{m_i} \left(1 - 2p_i + m_i \Sigma_r \frac{p_r^2}{m_r} \right) \right\}^{1/2}$$

b) A 95 % confidence interval for Q_i is $q_i \pm 2S(q_i)$

where

$$S(q_i) = \left\{ \frac{q_i^2}{m_i} \left(1 - 2q_i + m_i \Sigma_r \frac{q_r^2}{m_r} \right) \right\}^{1/2}$$

NOTE If intervals are calculated using these formulae then, in the long run, the true value of P_i (or Q_i) will be within the interval on 19 occasions out of 20.

F.6 Worked examples (see Table F.1 and Table F.2)

Example 1. Computation of the standard error

Consider the estimation of the true number size for the size class (4.7 μm to 6.6 μm) given in Table F.1. It can be seen that the estimate of the true number size proportion is 0.258.

Using equation (3) given in **11.3** for the standard error (SE), the SE of the estimate is computed to be:

$$\left[\frac{(0.258)^2}{174}\left\{1 - (2 \times 0.258) + (174 \times \text{sum})\right\}\right]^{1/2} = 0.0168$$

where

$$\text{sum} = \frac{(0.006)^2}{4} + \frac{(0.043)^2}{29} + \ldots + \frac{(0.258)^2}{174} + \frac{(0.442)^2}{297} = 0.0015$$

NOTE In this instance, because the sample areas are equal, the standard error can be more easily computed using equation (5) given in **11.3** as:

$$\left(\frac{1 - 0.258}{174}\right)^{1/2} \times 0.258 = 0.0168$$

The 95 % confidence interval for the true number size proportion for the particles belonging to the 4.7 μm to 6.6 μm class is then:

0.258 – (2 × 0.0168) to 0.258 + (2 × 0.0168), i.e. 0.224 to 0.292

Hence the true proportion for this class size is between 22.4% and 29.2 %.

The true volume size for the same size class is estimated using the figures given in Table F.2. These show a true volume size proportion of 0.088. Using equation (4) in **11.3**, the standard error of this estimate is given by:

$$\left[\frac{(0.088)^2}{174}\left\{1 - (2 \times 0.088) + (174 \times \text{sum})\right\}\right]^{1/2} = 0.011$$

where

$$\text{sum} = \frac{(0.138)^2}{4} + \frac{(0.339)^2}{29} + \ldots + \frac{(0.054)^2}{297} = 0.0099$$

The 95 % confidence interval for the true volume size proportion for the particles belonging to the 4.7 μm to 6.6 μm class is then:

0.088 – (2 × 0.011) to 0.088 + (2 × 0.011), i.e. 0.066 to 0.110

Example 2. Computation of the total area necessary for the required accuracy

An experiment is to be conducted to estimate the number size distribution for four classes of particles and it is specified that estimates should be accurate to within 5 % of the true value.

A pilot experiment is conducted using a total slide area of 0.04 mm^2 for all classes of particle and the number of particles observed are:

10 16 29 45

so that the total number of particles observed is 100. The initial estimates of the number size distribution are:

$P_1 = 0.10$; $P_2 = 0.16$; $P_3 = 0.29$; $P_4 = 0.45$

Using equation (7) give in **11.5** the total area required for the specified accuracy is:

$A_1 = 0.040(1 - 0.10)(200/5)^2/(100 \times 0.1) = 5.76$

Similarly:

$A_2 = 3.36$; $A_3 = 1.57$; $A_4 = 0.78$

Then the total area of the slides that should be sampled is 5.76 mm^2. Since an area of 0.04 mm^2 has already been sampled, a further slide area of 5.72 mm^2 is required.

Table F.1 — Example of size distribution by number

Objective	Size class limits μm	Area of sample field A mm^2	Number of same fields n	Total sample area nA mm^2	Number of particles counted m	Number concentration $\beta = m/nA$ mm^{-2}	True number size proportion $\beta/\Sigma\beta$
	3.3 to 4.7				297	1 248	0.442
	4.7 to 6.6				174	731	0.258
	6.6 to 9.4				117	492	0.174
× 100	9.4 to 13	0.001986	120	0.238	53	223	0.079
	13 to 19				29	122	0.043
	19 to 27				4	17	0.006
	> 27				0	0	0
					Σm = 674	$\Sigma\beta$ = 2 833	

Table F.2 — Example of size distribution by volume (using data from Table F.1)

Size class limits µm	Mid-class volume diameter d_r[a] µm	Number of particles counted m	Volume of particles counted[b] $d_r^3 m$ 10^3 µm^3	Volume concentration $\beta_v = d_r^3 m/nA$ 10^3µm^3 ·mm^{-3}	True volume size proportion $\beta_v/\Sigma\beta_v$
3.3 to 4.7	4.04	297	19.6	82.4	0.054
4.7 to 6.6	5.70	174	32.2	135	0.088
6.6 to 9.4	8.08	117	61.7	259	0.170
9.4 to 13	11.3	53	76.5	321	0.211
13 to 19	16.2	29	123	517	0.339
19 to 27	23.2	4	49.9	210	0.138
> 27		0	0	0	0
				$\Sigma\beta_v$ = 1 524	

[a] This is calculated using the formula $\{(d_u^2 + d_1^2)(d_u + d_1)/4\}^{1/3}$ where d_u and d_1 represent the upper and lower class limits respectively.

[b] These volumes are only proportional. To calculate the true volumes, assumptions have to be made about the shapes of the particles, e.g. if the particles were spherical, the true volumes would be given by $\frac{\pi}{6} d_v^3 m$.

List of references (see clause 2)

Normative references

BSI standards publications

BRITISH STANDARDS INSTITUTION, London

BS 2955:1958, *Glossary of terms relating to powders*[3].

BS 3406, *Methods for determination of particle size distribution.*

BS 3406-1:1986, *Guide to powder sampling.*

Informative references

BSI standards publications

BRITISH STANDARDS INSTITUTION, London

BS 3406, *Methods for determination of particle size distribution.*

BS 3406-2:1984, *Recommendations for gravitational liquid sedimentation methods for powders and suspensions.*

BS 3406-3:1963, *Air elutriation methods (obsolescent).*

BS 3406-5:1983, *Recommendations for electrical sensing zone method (the Coulter principle).*

BS 3406-6:1985, *Recommendations for centrifugal liquid sedimentation methods for powders and suspensions.*

BS 3406-7:1988, *Recommendations for single particle light interaction methods.*

BS 3625:1963, *Specification for eyepiece and screen graticules for the determination of the particle size of powders.*

Other references

MDHS 39 *Asbestos fibres in air — Determination of personal exposure by the European reference version of the membrane filter method*[4].

Royal Microscopical Society *Microscopy handbooks*[5].

Image analysis principles and practice[6].

[3] Under revision.
[4] Available from the Health and Safety Executive.
[5] Available from the Royal Microscopical Society, 37/38 St Clements, Oxford OX4 1AJ.
[6] Published by Joyce-Loebl, Marquisway, Team Valley, Gateshead NE11 0QW.

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