BRITISH STANDARD

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16232-7:2007

Road vehicles — Cleanliness of components of fluid circuits —

Part 7: Particle sizing and counting by microscopic analysis

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British Standards

National foreword

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Road vehicles — Cleanliness of components of fluid circuits —

Part 7: Particle sizing and counting by microscopic analysis

Véhicules routiers — Propreté des composants des circuits de fluide —

Partie 7: Détermination et comptage des particules par analyse microscopique

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Foreword

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

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

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

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

ISO 16232-7 was prepared by Technical Committee ISO/TC 22, *Road vehicles*, Subcommittee SC 5, *Engine tests*.

ISO 16232 consists of the following parts, under the general title *Road vehicles — Cleanliness of components of fluid circuits*:

— *Part 1: Vocabulary*

— *Part 2: Method of extraction of contaminants by agitation*

— *Part 3: Method of extraction of contaminants by pressure rinsing*

— *Part 4: Method of extraction of contaminants by ultrasonic techniques*

— *Part 5: Method of extraction of contaminants on functional test bench*

— *Part 6: Particle mass determination by gravimetric analysis*

— *Part 7: Particle sizing and counting by microscopic analysis*

— *Part 8: Particle nature determination by microscopic analysis*

— *Part 9: Particle sizing and counting by automatic light extinction particle counter*

— *Part 10: Expression of results*

Introduction

The presence of particulate contamination in a fluid system is acknowledged to be a major factor governing the life and reliability of that system. The presence of particles residual from the manufacturing and assembly processes will cause a substantial increase of the wear rates of the system during the initial run-up and early life, and may even cause catastrophic failures.

In order to achieve reliable performance of components and systems, control over the amount of particles introduced during the build phase is necessary, and measurement of particulate contamination is the basis of control.

The ISO 16232 series has been drafted to fulfil the requirements of the automotive industry, since the function and performance of modern automotive fluid components and systems are sensitive to the presence of a single or a few critically sized particles. Consequently, ISO 16232 requires the analysis of the total volume of extraction liquid and of all contaminants collected using an approved extraction method.

The ISO 16232 series has been based on existing ISO International Standards such as those developed by ISO/TC 131/SC 6. These International Standards have been extended, modified and new ones have been developed to produce a comprehensive suite of International Standards to measure and report the cleanliness levels of parts and components fitted to automotive fluid circuits.

This part of ISO 16232 defines methods of microscopic examination to determine the particle size distribution of contaminants which have been removed from the component under analysis and collected using an approved extraction method.

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Road vehicles — Cleanliness of components of fluid circuits —

Part 7:
Particle sizing and counting by microscopic analysis

1 Scope

This part of ISO 16232 defines methods for determining the size and number of contaminant particles, which have been extracted from components and deposited on the surface of a membrane filter, as determined by using either a light optical microscope (LM) or a scanning electron microscope (SEM). The result of this measurement is the particle size distribution on the membrane filter.

As the function of parts and components can be impaired by the presence of a single or a few critical particles, a complete analysis of the total membrane filter surface is essential.

These analyses can be performed either manually or automatically using Image Analysis (IA) techniques if the appropriate equipment is available.

NOTE 1 Manual full-surface counting is a difficult and tiring task associated with errors. For this reason, an automatic counting system is recommended if the membrane filter is prepared in a suitable way as described herein.

NOTE 2 The results of counting and sizing depend on many parameters, such as type and model of microscope, magnification, illumination, and other settings used.

2 Normative references

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

ISO 16232-1, *Road vehicles — Cleanliness of components of fluid circuits — Part 1: Vocabulary*

ISO 16232-2, *Road vehicles — Cleanliness of components of fluid circuits — Part 2: Method of extraction of contaminants by agitation*

ISO 16232-3, *Road vehicles — Cleanliness of components of fluid circuits — Part 3: Method of extraction of contaminants by pressure rinsing*

ISO 16232-4, *Road vehicles — Cleanliness of components of fluid circuits — Part 4: Method of extraction of contaminants by ultrasonic techniques*

ISO 16232-5, *Road vehicles — Cleanliness of components of fluid circuits — Part 5: Method of extraction of contaminants on functional test bench*

ISO 16232-10, *Road vehicles — Cleanliness of components of fluid circuits — Part 10: Expression of results*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16232-1 apply.

4 Principles

The entire volume of extraction liquid used to extract particles from the test component, as described in ISO 16232-2, ISO 16232-3, ISO 16232-4 and ISO 16232-5, is filtered on a membrane filter and the separated particles are counted and sized using microscopic techniques. The longest dimension of a particle is used to determine particle size.

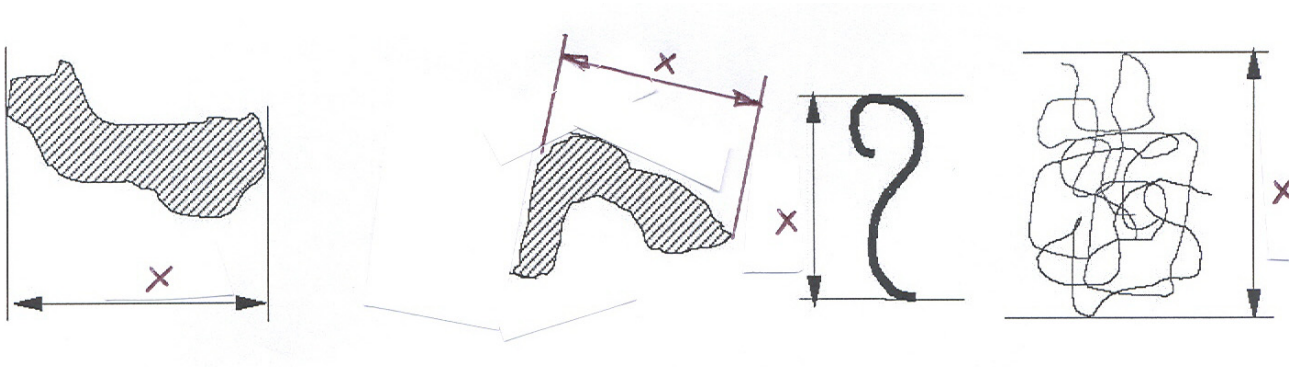


Figure 1 — Examples of longest dimension of a particle, X

To determine the particle size, a light microscope uses the optical contrast between the particle and the surface of the membrane filter. The contrast is mainly achieved by adjusting the intensity of illumination. The basis for counting particles using SEM is the material contrast which occurs as a result of the differing intensity of back-scattered electrons.

NOTE Because the mechanisms of detection are based on different types of contrast, the counting results obtained from optical and scanning electron microscopy cannot be compared with one another.

The filter and the analysis system are selected depending upon the amount of contamination expected and the relevant particle size range noted in the cleanliness specification.

5 Equipment

5.1 Equipment for the preparation of membrane filters

5.1.1 If necessary, a controllable non-ventilating oven capable of maintaining a temperature of 80 ± 5 °C.

5.1.2 The membrane filter shall be compatible with the extraction liquid and any rinsing liquid or chemicals used in the processes. The pore size of the membrane filter shall be suitable for the minimum size of particles to be collected. The diameter of the membrane filter shall be large enough to avoid the contact or overlapping of particles which causes errors through coincidence.

When using light microscopes, there should be a good optical contrast between the particles and the surface of the membrane filter.

For scanning electron microscopy, a smooth-surfaced filter should be chosen (e.g. polycarbonate, cellulose nitrate, cellulose acetate, polyamide).

NOTE 1 Gridded membrane filters for assisting in orientation when counting particles manually with an optical microscope cannot be utilized for automated counting using image analysis.

NOTE 2 To ease examination, it is recommended that the pore size of the membrane filter be less than 1/3 of the smallest particles to be analysed.

5.1.3 There are two methods for separating the particles from the extraction liquid and these are described below:

a) Membrane filter holder connected to the extraction equipment: The membrane filter holder device is directly fitted below the drain of the collection equipment. Several membrane filter holders may be mounted behind one another (cascade) to obtain a pre-selection of specific particle sizes during the filtration process. The equipment shall be designed so as to avoid the settlement or loss of particles in the tubing.

NOTE 1 A wider size range of pore sizes for pre-selection can be achieved using mesh type discs, either metallic or polymeric. If so then the filter disc holder should be carefully designed so that the discs can be easily extracted without losing particles.

b) The extraction liquid is collected in a suitable vessel and then filtered using separate filtration apparatus made up of the following components: membrane filter holder base with suitably-sized funnel fixed with a clamp, vacuum flask possessing a capacity compatible with the entire volume of the extraction liquid.

The cleanliness of the filtration equipment shall be consistent to the presumed cleanliness of the component being tested. This is validated when performing the blank test.

NOTE 2 If necessary the membrane filter holder should be earthed to avoid the build-up of electrostatic charge and subsequent discharge.

NOTE 3 In the ISO 16232 series, the words “earthing” and “grounding” are synonymous.

See Annex A for an example equipment diagram.

5.1.4 Use of a rinsing liquid as specified in the inspection document shall be compatible with all the equipment used in the process.

5.1.5 The source of rinsing liquid is specified in the inspection document.

5.1.6 The sputtering (coating by vacuum deposition) equipment is only necessary when using a SEM which requires a conducting film on the membrane filter.

NOTE 1 Coating with carbon should be preferred to sputtering with other elements, e.g. gold, silver. As the membrane filters are usually composed of organic materials, the carbon applied affects the measurement results much less than a layer of sputtered gold.

NOTE 2 In some types of SEM charging can be reduced by reducing the vacuum but this can affect the resolution in some designs.

5.1.7 Tweezers able to handle membrane filters without damaging them shall be used.

5.1.8 Vacuum device able to generate a vacuum of at least 65 kPa shall be used.

5.2 Analysis equipment

5.2.1 General

5.2.1.1 Figure 2 shows the equipment involved in microscopically counting particles on a membrane filter. In the process, differences occur between light-optical and scanning electron microscopes at the level of the lens and of the detectors. For both LM and SEM, computer-aided recording and counting image analysis techniques up to and including full-surface analysis are essentially identical.

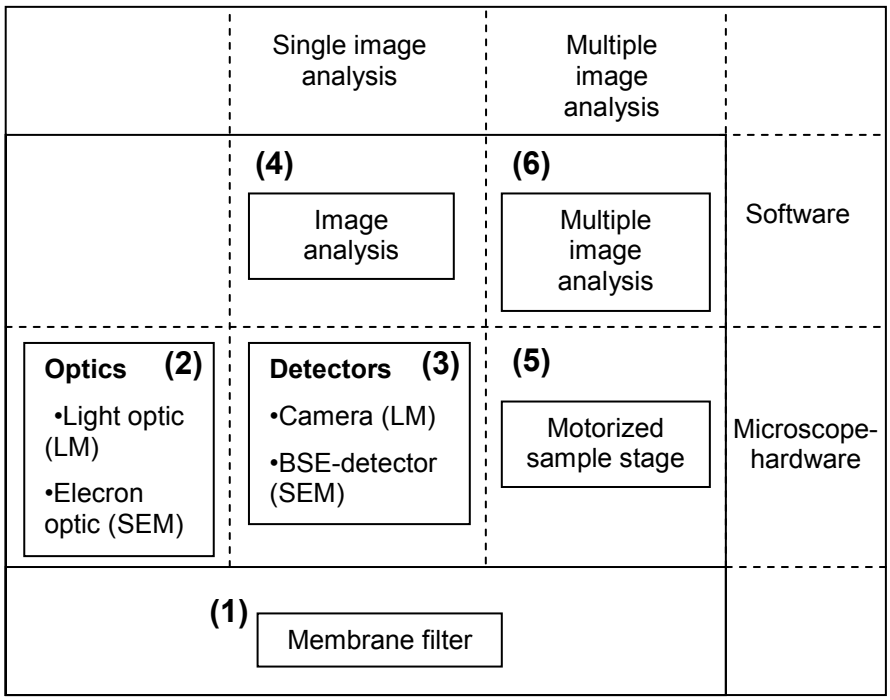


Figure 2 — Diagrammatic representation of the microscopic analysis of membrane filters

5.2.1.2 The membrane filter (1) containing the particles extracted from the test component is placed on the sample stage and is imaged in magnified form by an optical system (2). In the case of the light microscope, this is done using a suitable light source that homogenously illuminates the field of view and the optical segment containing one or several objective lenses and an eyepiece. This is also the minimum configuration required for manual/visual counting and sizing. In the case of a SEM, the sample is scanned by a focused high-energy electron beam in a vacuum chamber.

5.2.1.3 The optically-magnified information is gathered by a detector system (3), a video or digital camera in light microscopy and a detector which usually detects the back-scattered electrons with a high material contrast in SEM. The next step is performed by an image analyser (4) which separates the particles from the membrane filter background and measures and counts them using pre-given algorithms. Using the components 1-4 described, it is possible to perform single image analysis.

5.2.1.4 For automated analysis of areas which are greater than the field of view of the microscope, i.e. the full-surface counting of membrane filters, the two following points are required:

— Motorized sample stage (5) for advancing the membrane filter in steps beneath the optics. For this, the sample stage control shall be coupled with the image analysis software.

— The software shall then also be able to combine the data obtained from recording several images in order to perform a comprehensive particle analysis of the effective filtration area of the membrane filter (6). See Annex B for further information on field scanning

5.2.1.5 Table 1 summarises characteristics of the different types of microscopes used for counting and sizing particles.

Table 1 — Characteristics of the different types of microscope used for counting particles

Type of microscope	Light microscopy		SEM
	Standard microscope	Stereo microscope	
Particle measurement range	> 2 µm (dependent of the objective lens)	> 25 µm	> 20 nm
Detection principle	Brightness contrast	Brightness contrast	Material contrast
Depth of field	low	high	high

NOTE The maximum countable size is dependent on the type of the equipment.

5.2.2 Light microscopes

5.2.2.1 Standard microscope

5.2.2.1.1 In the case of a standard light microscope, the field of view is observed either through a single eyepiece (Monocular) or two parallel eyepieces (Binocular) possessing an identical beam path. For manual counting, the eyepiece is equipped with a micrometer scale. When counting is carried out automatically, the field of view is viewed using either a digital chip, a digital camera or a video camera mounted onto either the eye piece itself, or a special adaptor usually fitted to the trinocular head of a microscope. The degree of magnification is selected using interchangeable lenses.

5.2.2.1.2 The magnification, resolution and depth of field of the microscope are set using the lens selected. The decisive parameter for an accurate particle measurement is the optical resolution (not primarily the magnification) of the lens. It is determined according to the wavelength of the light used and the numerical aperture of the lens.

Lenses shall be selected for the particle-counting procedure so that their optical resolution is $\leqslant$ 1/10 of the size of the smallest particle to be measured. If it is necessary to count and size small particles ($<$ 20 µm), the rule of 1/10 would lead to long measuring times because of the small fields of view of high resolution lenses. In this case, lenses shall be selected so that their resolution is maximum 1/5 of the smallest particle size. Examples for both cases are given in the following table for common microscope lenses.

Table 2 — Examples of magnifications for optical microscopes and minimum observable particle sizes

Magnification with ocular lens (×10)	Objective lens	Numerical aperture	Resolution µm	Minimum particle size µm 10 × optical resolution	Minimum particle size µm 5 × optical resolution
× 50	× 5	0,10	2,5	25	12,5
× 100	× 10	0,25	1,0	10	5
× 200	× 20	0,50	0,5	5	2,5
× 500	× 50	0,7	0,35	3,5	1,7

5.2.2.1.3 The illumination equipment and the sample stage are usually integrated into the microscope.

5.2.2.2 Stereo microscope

With this instrument, the field of view is observed through two eyepieces (with a micrometer scale for manual counting) which view the field of view from slightly different angles through the lens. In this way, the image appears to the observer to be a three-dimensional object. Microscopes of this type may also be equipped with camera systems for image analysis. In general, these microscopes possess a zoom function for selecting the degree of magnification. Compared to standard microscopes, they are unable to give as high a degree of magnification or resolution. They possess a much larger field of view with a higher depth of field and are therefore suitable for the rapid counting of large particles. A minimum particle size of 25 µm can be used as a reference value. In order to be able to perform correct and reproducible measurements, the zoom function shall be fixed in defined positions.

Neither illumination equipment nor sample stage is usually integrated into the microscope and, subsequently, modifications are required.

5.2.2.3 Illumination

5.2.2.3.1 Selecting the type of illumination is dependent upon the combination of the membrane filter and the particles to be detected. Generally, both incident and transmitted light are suitable. Combinations of various illumination methods are also possible.

5.2.2.3.2 When carrying out measurements automatically using image analysis, the illumination of the imaging area of the microscope shall be homogenous and constant with regard to time:

— homogeneity shall be ensured for all the magnifications used during the particle-counting procedure;

— a diffuser filter may be used to homogenize the illumination;

— if necessary, the electrical current supplied to the light source shall be stabilized;

— the illumination equipment should be integrated into the microscope or at least be able to be fixed in one place to prevent unintentional alterations in the illumination from occurring and to ensure reproducible results.

NOTE The homogeneity of the illumination can usually be checked using the same image analysis software that is needed for the particle-counting procedure.

5.2.2.4 Camera

5.2.2.4.1 Generally, either a video or a digital camera is used. Both possess a camera chip which consists of an array of light-sensitive elements.

The number of pixels or size of the camera chip shall be adapted to the resolution of the microscope lens. Similarly to the case with the optical resolution, here the smallest particle dimension to be measured shall also be reproduced on 10 camera pixels or 5 pixels for small particles (see 5.2.2.1.2).

NOTE A further increase in the number of pixels does not improve the measurement result due to the fact that the resolution of the system is limited by the optical resolution of the lenses. On the other hand, if the number of pixels is reduced, the full resolution of the lens cannot be utilized with the result that loss of information and measurement inaccuracy of small particles occurs.

5.2.2.4.2 The camera's sensitivity to light has a similar influence on the analysis image as the intensity of the illumination. In order to obtain precise and reproducible measurement results, the camera shall be operated using defined sensitivity settings which can be fixed. Automatic functions regulating brightness shall be switched off.

5.2.3 Scanning electron microscope

5.2.3.1 Electron optical system

The sample to be imaged is scanned point-for-point in a vacuum using a finely-focused high-energy electron beam. In the process, electrons are emitted from the sample and are captured using appropriate detectors. The intensity of these signals, combined with the actual position of the electron beam, gives information about the image signal which the observer then receives in the form of a magnified image.

The magnification is determined by the dimension of the scanned area of the sample and can be selected over a wide range. Most of the systems available are limited as far as their maximum particle size range is concerned. This range is determined by the minimum magnification of the microscope.

The electrical charges applied to the sample by the electron beam shall be dissipated in order to prevent the particles from being electrically charged, which would have a negative effect on the quality of the image. The discharging works when electrically-conductive samples are used. Since membrane filters are generally not conductive, they shall be treated by one of the two following processes: with a high vacuum SEM, the sample is made conductive by depositing carbon on it; with a lower vacuum SEM, the charges are dissipated by the residual air molecules left in the vacuum chamber.

NOTE The current of the electron beam in a SEM is equivalent to the intensity of illumination in light microscopes. The current stability is crucial to the quality of the analysis. The electron gun is sufficiently warmed up to ensure that the emissions are stable before the analysis is started.

5.2.3.2 Detector

When determining the amount of particle contamination on a membrane filter, a back-scattered electron detector (BSE) shall be used. An image results from the signal of this detector with contrasts created mainly by the differences in materials in the sample.

Particles showing no material contrast to the surface of the membrane filter (usually organic substances) cannot be automatically detected.

The brightness contrast shall be adjusted for each sample type.

5.3 Image analysis

Image analysis software classifies certain areas of the image as being particles and the remaining areas as being the background of the membrane filter. The total range of brightness of the image is divided into grey value steps ranging from black to white. For particle analysis, 256 grey steps are generally used. By determining a grey value threshold (binarization threshold), all image points and the corresponding pixels below the threshold are classified as belonging to particles and all those above the threshold as belonging to the membrane filter background (or vice-versa depending upon the illumination, colour of the filter and type of particle).

NOTE 1 The pixel resolution of the analysis image (µm/pixel) shall be ⩽ 1/10 of the smallest particle size to be measured or 1/5 for small particles, as is the case for the resolution of the objective lens or the camera of the light microscope (see 5.2.2.1.2).

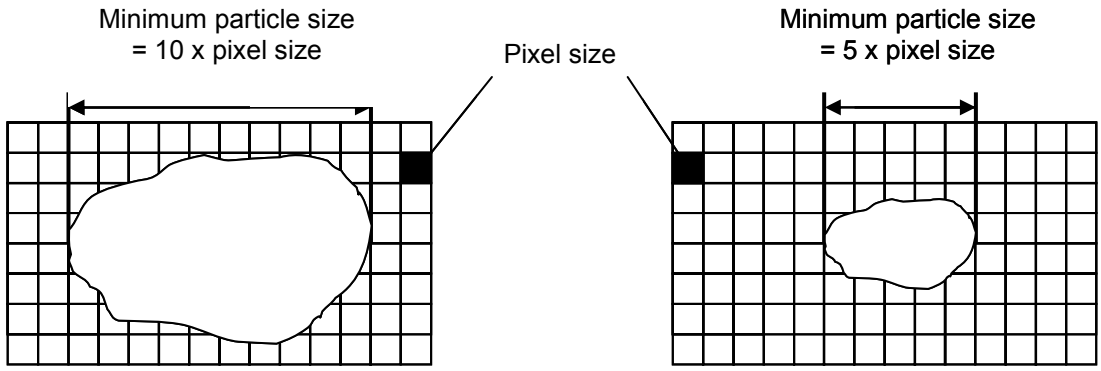


Figure 3 — Pixel resolution of the analysis image

The determination of the grey value threshold is crucial to particle measurements and is therefore carried out by the operator before each measurement (see Clause 7).

NOTE 2 In order to compensate for any non-homogeneities still present in the illumination of the field of view of an optical system, especially where degrees of magnification are low, a shading correction of the measurement image can be performed by the software. In this way, non-homogeneities in the illumination which are measured in a test pre-run are then excluded from the measurement image by the software.

NOTE 3 Additional software filters for increasing contrast or sharpening the edge of particle structures should not be used for automated measurements as they may often be the cause of inexplicable measurement errors.

5.4 Motorized sample stage

High demands shall be placed on the accuracy of the positioning axes because:

— If an automatic analysis is carried out, the membrane filter shall be completely analysed;

— Particles requiring later manual analysis shall be able to be reliably located.

The advancement accuracy shall lie within the range of the smallest particle detected, i.e. 5 µm in the case of the size classes of ISO 16232-10.

NOTE If the traversing path of the sample stage is adequate and if the vacuum chamber is large enough when using SEM, sample mountings can be realized for several membrane filters which are then automatically analysed in series. The working distance shall be maintained over this path length.

5.5 Multiple image analysis

The image analysis software shall allow particles lying partially over the frame of a field of view to be recorded in their full size.

Several methods exist (see Annex C).

The system shall be able to reassemble the image fields or measurement frames without gaps or overlaps.

When using a light optical microscope, the camera should be aligned with the movement of the sample stage. When using a SEM, it should be ensured that the scanning direction of the electron beam is aligned with the movement of the sample stage (see Annex D).

5.6 Environmental conditions

The cleanliness of the environment where the analysis is performed shall be adapted to the presumed cleanliness of the component to be tested. This is validated when performing the blank test.

The site for the microscope should be selected to avoid environmental factors such as vibration of the building, or external light from influencing the imaging quality and accuracy of the particle measurement. If these factors cannot be controlled, appropriate measures shall be taken (vibration absorbers, encapsulation, etc.).

5.7 Health and Safety

5.7.1 Local Health and Safety procedures shall be followed at all times, any equipment shall be operated in accordance with the manufacturer's instructions and personal protection equipment used where appropriate.

5.7.2 Chemicals used in the procedures can be harmful, toxic or flammable. Good laboratory practices shall be observed in the preparation and use of these chemicals. Care shall be taken to ensure compatibility of the chemicals with the materials used (refer to each Material Safety Data Sheet [MSDS]). Follow the precautions for safe handling and usage as described in the MSDS available from the supplier.

5.7.3 Volatile liquids: care shall be taken with flammable liquids to ensure that they are used in accordance with the MSDS, at temperatures below the stated flash point and away from potential sources of ignition. Appropriate precautions should be taken to avoid inhalation of fumes from these solvents. Always use suitable protective equipment.

5.7.4 Electrical: appropriate care should be applied in the use of electrical power.

5.7.5 Static: the build-up of electro-static charges (created by friction as fluid flow) shall be dissipated and not be allowed to build-up where it can discharge and create a spark. An earthing strap shall be provided where there is a risk, especially for the vacuum apparatus where often volatile liquids are involved.

5.7.6 Disposal: all liquids and substances shall be disposed of in accordance with local environmental procedures. In the event of spillage it shall be cleaned-up in the manner detailed in the MSDS.

6 Calibration

The measured size of the image of a particle is assigned to the actual particle size. The calibration factor is the ratio between the length measured in the image and the actual particle size of the object. Therefore, when calibrating length, all parts of the system involved in the image reproduction shall be included. This means that an object-standard/scale (e.g. a stage micrometer) is set in a ratio with (see Annex E for an example):

— The eyepiece scale used in manual measurements on the optical microscope;

— The ruler scale used in manual image measurements on a video screen;

— The pixel size of a digitalized image if image analysis is used for carrying out automated measurements. The calibration factor is filed in the image analysis software.

The object scale shall be certified.

Calibration shall be carried out for all the degrees of magnification used in the measurement of particles on the surface of a filter membrane. In the case of systems possessing zoom functions (e.g. stereo microscopes) the magnification shall be fixed in defined positions.

Calibration should be carried out once a year and each time the optical system is subject to major interventions (adjustment work, modification, etc.).

NOTE If the software of an EDX system is used in combination with a standard SEM for the counting and sizing of particles (with or without element analysis), care is taken with regard to the following point: the calibration of length is performed using images generated by the EDX system as this device possesses its own scan generator which takes over the control of electron beam during the measuring. As a result, in certain circumstances, there could be differences in the images compared to those obtained from the SEM alone.

7 Procedure

7.1 Cleaning and preparing of equipment

Prepare clean filtration apparatus, membrane filter holders, collecting and rinsing containers, tweezers and analysis equipment. The required cleanliness level of the apparatus should be such that contaminants are unable to significantly contribute to the overall result and shall be adapted to the presumed cleanliness of the component to be tested. This cleanliness level is validated when performing the blank test (See ISO 16232-2, ISO 16232-3, ISO 16232-4 and ISO 16232-5).

7.2 Preparation of the membrane filter

7.2.1 General

Depending on the type and equipment used for the extraction procedure, there are two possibilities for filtering the extraction liquid (See Annex A for an example):

a) the extraction liquid is filtered using a filtration apparatus directly connected to the extraction equipment;

b) the extraction liquid is collected in a suitable sample container and then filtered using separate filtration apparatus.

7.2.2 Sample preparation:

— Case a), no preparation of the extraction liquid is required as it is directly filtered while performing the extraction procedure.

— Case b), particularly when the extraction liquid is stocked or transported, the sample container shall be checked to see that it is reliably sealed to avoid environmental contaminants from affecting the cleanliness level of the liquid. Before filtration, the outside of the sample container shall be cleaned. If the sample is left to stand for a period of time, particle settlement and agglomeration will occur. The agglomerates shall be broken up and the particles re-dispersed evenly. This can be done either by manual or automatic shaking or by using an ultrasonic bath. The method chosen shall not alter the original particle size distribution.

7.2.3 Separation of particles by vacuum filtration

7.2.3.1 Using the tweezers, remove the selected membrane filter from its container. Then place it centrally in the membrane filter holder. Close the holder and connect it to the extraction equipment or, in case b) of 7.2.1, lower the funnel onto the membrane filter without sliding. Secure the clamping device. Connect the vacuum device to the flask or the holder. If necessary connect the earthing strap to the membrane filter clamp before filtering.

NOTE 1 If necessary rinse the two faces of the membrane.

NOTE 2 In the ISO 16232 series, the words “earthing” and “grounding” are synonymous.

7.2.3.2 Perform the extraction procedure as described in the inspection document or, in case b) of 7.2.1, pour the entire contents of the sample container into the funnel and carefully rinse the container into the funnel.

7.2.3.3 Apply vacuum to the filtration equipment. When the filtration process is almost complete, carefully rinse the extraction equipment or the funnel with test liquid or clean rinsing liquid. Do not direct the stream of the liquid onto the surface of the membrane filter as it will disturb the particle distribution.

NOTE If necessary, sufficient rinsing liquid is added to the filtration equipment to completely flush the sample liquid from the membrane filter.

7.2.3.4 Release the vacuum, open filter holder or lift funnel and remove membrane filter.

7.2.3.5 Dry the membrane filter by evaporating the rinsing liquid or, if water-based cleaning liquids are used for the extraction procedure, by drying in a non-ventilating oven.

7.2.3.6 If the analysis of the membrane filter is performed using an SEM which requires a conducting sample, the membrane filter shall be coated with a conducting element using a suitable sputtering device.

NOTE Great care is taken to avoid loss of particles during preparation, handling and analysing procedures.

7.2.4 Validation

Prepare a membrane filter in accordance with 7.2.2 and 7.2.3 by filtering the complete liquid volume used for the extraction of particles from the test component. View the membrane filter with a suitable magnification and illumination and check that particles are evenly distributed over the whole surface of the filter without any of them touching one another or overlapping.

If touching or overlapping of particles is observed, reject the membrane filter and prepare another one with extraction liquid from another identical component with one of the following differences:

— Choose a membrane filter with a larger diameter;

— Prepare more than one membrane filter with the volume of the extraction liquid.

When the filtration procedure has been optimized, prepare the membrane filter for counting and sizing.

NOTE If the membrane filter(s) retains a large amount of very small particles which are not relevant for the analysis result it is possible to choose a membrane filter with a larger pore size to achieve a better contrast.

7.3 Particle sizing and counting procedure

7.3.1 Classify the particles in the required size ranges. The relevant particle sizes are given in the inspection document. The size classes are defined in ISO 16232-10.

7.3.2 For both manual and automated measurements, the total effective filtration area of the membrane filter shall be analysed. Care shall be taken to ensure that the measuring fields adjoin completely without any gaps and without overlapping (see Annexes B and D) in order to avoid particles from being missed or from being counted a second time.

7.3.3 Particles lying over the margin of the measuring field shall always be counted and their entire size measured (see Annex C).

7.3.4 Select the type of microscope and the appropriate magnification in accordance with Table 1 and Table 2.

7.3.5 The membrane filter shall be mounted and fixed in the microscope's filter holder or, in the case of an SEM, into the vacuum chamber which has been evacuated to a stable level.

7.3.6 In the case of manual counting, the illumination of the light microscope shall be set so that the particles in the field of view or on the observation screen can be seen by the user with a maximum degree of contrast. For the same reason, the parameters such as acceleration voltage and beam current at the SEM shall be set.

The effective filtration area of the membrane filter shall then be counted and the specified particle sizes measured. The focus shall be checked after each movement of the sample stage when changing to the next measuring field and re-adjusted where necessary.

7.3.7 In the case of automated counting:

a) The illumination of the light microscope is switched on and allowed to warm up. If an SEM is used, the beam parameters are adjusted and the cathode is warmed up until the beam current is stable. Especially for light microscopy, an exactly-focused image is essential to ensure a correct measurement. Therefore, the levelness of the membrane filter shall be checked by advancing at least three sites of the membrane filter which are as far away as possible from one another and then by controlling their focal point. The image shall be well-focused on the whole membrane filter either by using an autofocus camera or by fixing the membrane filter with a transparent non reflecting cover, e.g. by setting it between two glass slides. The thickness of the upper cover slide shall be selected to ensure that the particles are in focus at the magnification used. The depth of field of the stereo microscope or the SEM is much higher so that this aspect is not as crucial as for the standard microscope with a high-magnification objective lens.

b) Adjust the microscopes and image analysis systems to attain maximum reproducible image information.

c) So far as the light microscope is concerned, the camera settings for sensitivity, intensity, etc. shall be identical to those used in the measurement mode. Automatic functions for correcting brightness shall be switched off. Beginning with a low degree of intensity, the brightness of the illumination shall then be slowly increased whilst observing the grey value distribution of the field of view. This process shall be continued until the first pixels of the image attain the “white level” of the image analysis (as a rule, 255). In this way, it is ensured that the total dynamic range of the image analysis is utilized without the image being over-exposed. The measurement is then carried out using this illumination setting. The equivalent procedure for the SEM is to adjust the sensitivity of the BSE-detector to use the total dynamic range of the image analyzer.

d) Select the grey value threshold for the particle measurement: The grey value threshold shall be selected by the operator so that the relevant particle size after binarization appears to be the same size as when visualized in the unprocessed image. This means that the grey value threshold shall be selected so that neither the background of the membrane filter around the particle is classified as being part of the particle nor that only parts of the particle are recorded.

NOTE When performing a full-surface automated measurement, the determination of the grey value threshold should be carried out at several points on the effective filter area in order to obtain a value which gives correct values during the entire analysis.

e) Start the automated measurement.

NOTE 1 To verify the results of the automatic measurement, a few of the larger particles (which are usually the crucial ones which may impair the function of a component) should be checked manually to confirm sizing.

NOTE 2 The measurement of characteristics of the detected particles other than their dimension should be performed or controlled manually on completion of an automatic measurement. For example, the ratio of the particle length and width of 1:10 is easily measured using image analysis but there is no certainty that the object concerned is a fibre or a metal chip. The type of particle may have a negative or no effect on the function of a component. One exception is the analysis of the particle material. This can be performed automatically using an EDX system as described in ISO 16232-8.

8 Results

8.1 Test report

The test report shall include all information useful for interpretation of results such as the microscopic examination conditions. An example of a test report is given in Annex F.

8.2 Report the results

Report the results of the component cleanliness examination in accordance with ISO 16232-10.

Annex A
(informative)

Filtration

A.1 Filtration connected to the extraction equipment

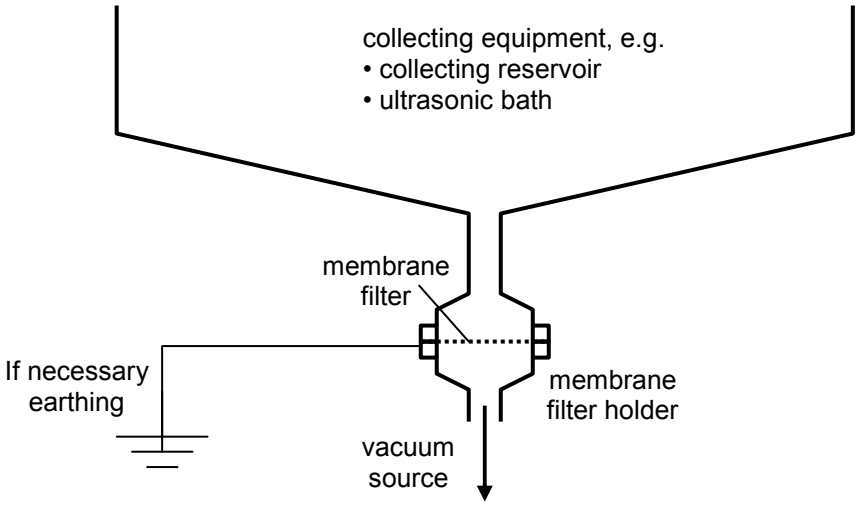


Figure A.1 — Filtration connected to the extraction equipment

A.2 Separate filtration

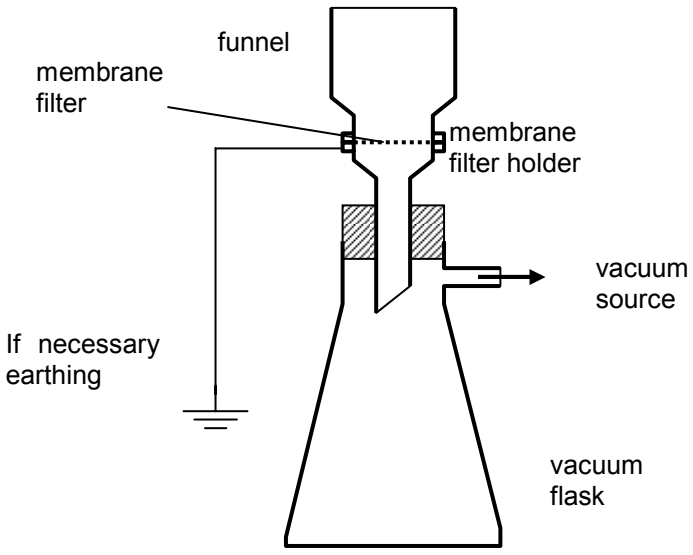


Figure A.2 — Separate filtration

Annex B
(informative)

Field scanning

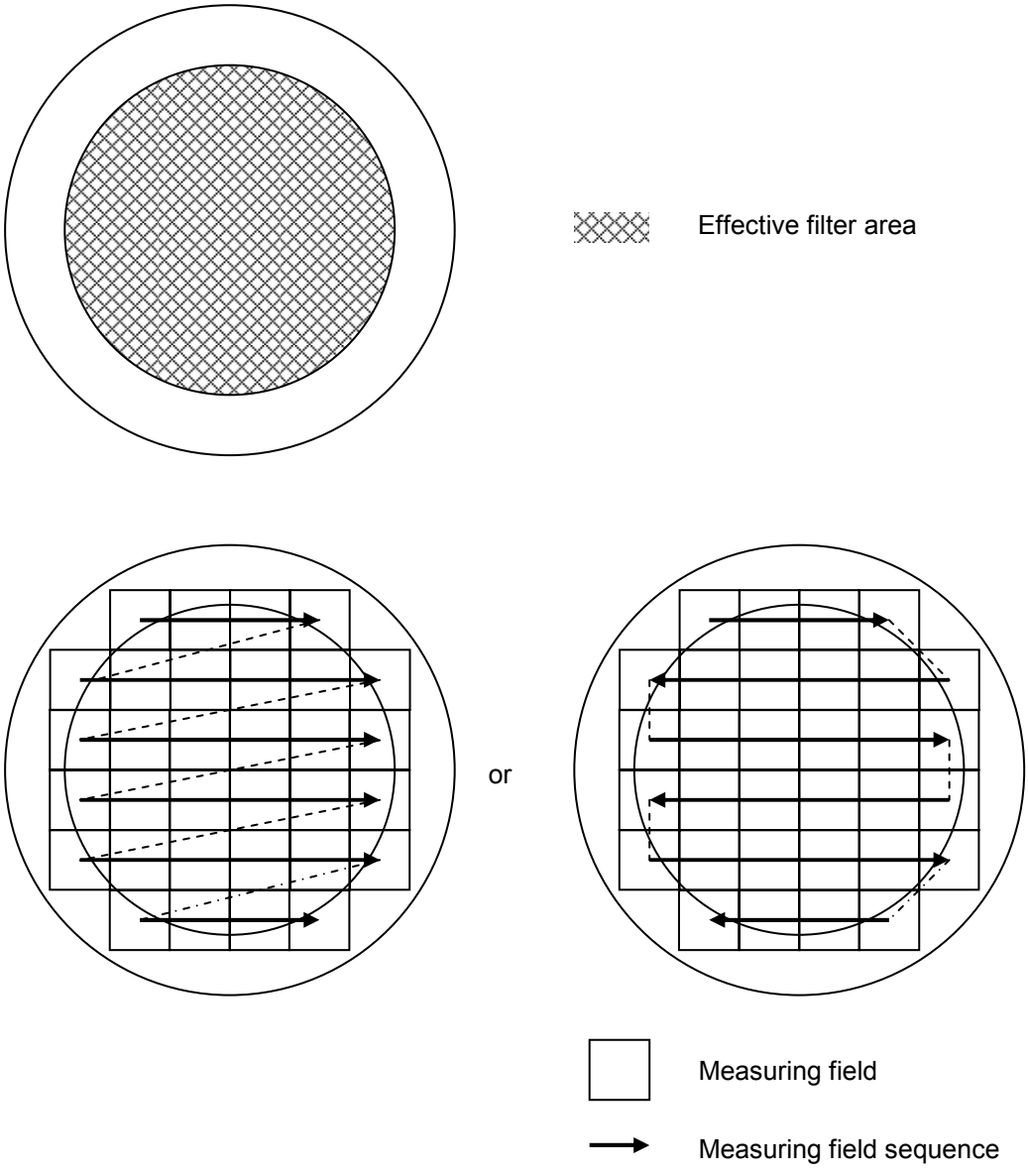


Figure B.1 — Field scanning

Annex C
(informative)

Particle counting on the margin

In order to achieve a correct particle counting and sizing result, care should be taken to avoid particles lying at the edge of the field of view from being counted and measured either twice, only partially or even not at all.

C.1 One way of recording particles on the margin during automated measurement is to reassemble the individual image to form one large image (mosaic or montage) which is then analysed with regard to the number and size of particles contained within it.

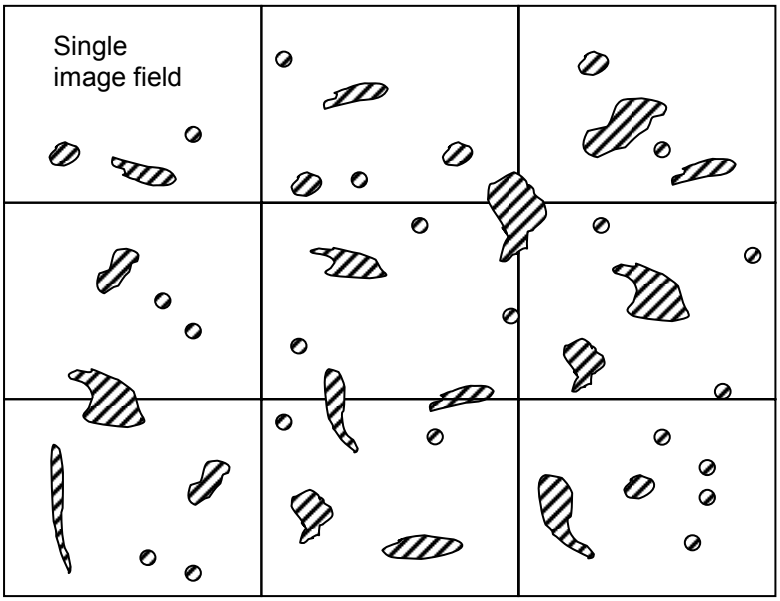


Figure C.1 — Example of a reassembled mosaic image

C.2 Another possible method is using a measuring frame smaller than the field of view. During the measurement, only particles which lie within the measuring frame or which intersect the left-hand side or upper edge should be recorded.

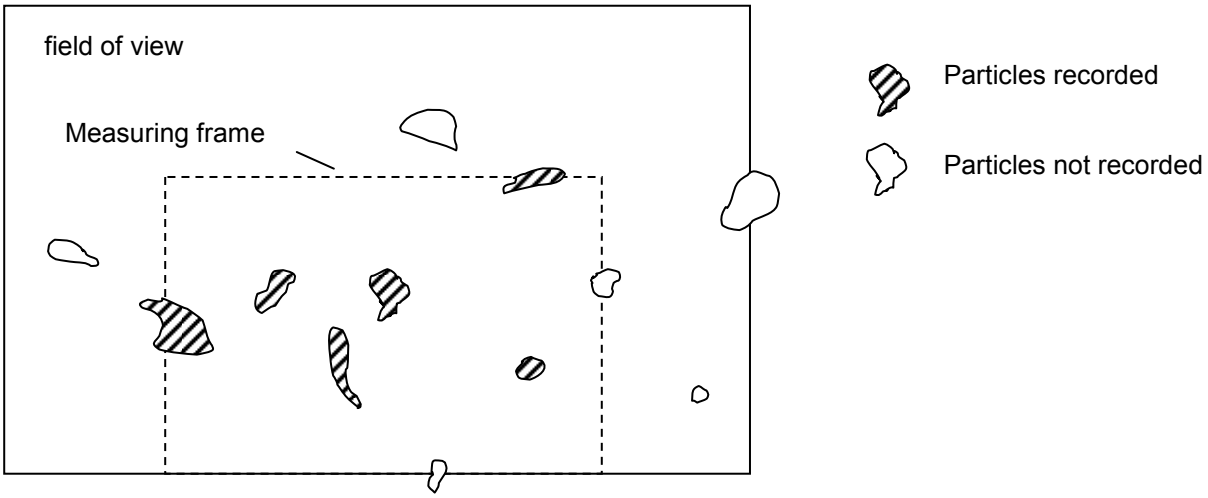


Figure C.2 — Example of measuring frame smaller than the field of view

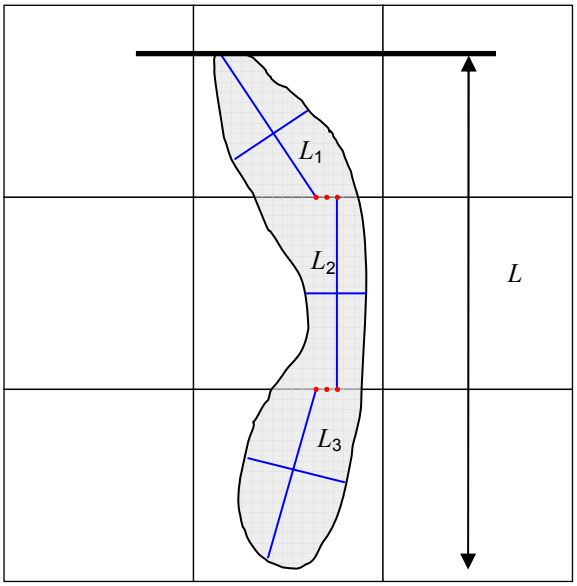
For the following measurement, the field of view should then be shifted to the right by the size of the measuring frame and the measurement repeated using the same procedure described above. In this way, the particles are now recorded at the left-hand margin of the measuring frame which were not recorded during the previous measurement when they were on the right-hand margin.

NOTE 1 When the analysis of one row has been completed, the field of view should be shifted lower by the size of the measuring frame and the next row should be measured in the same way until the total effective filter area has been counted (see diagram in Annex B).

NOTE 2 This procedure can be used both for manual counting and for a program sequence for automated counting.

In both cases, the mosaic and the measurement frame method, high requirements should be placed on the accuracy of the motorized axes of the sample stage to enable the image information to be combined without any gaps or particle loss.

C.3 Another possible method is the reconstitution of several particles in one particle



Length of the particle = L

Figure C.3 — Example of measuring frame smaller than the field of view

In all cases, high requirements should be placed on the accuracy of the motorised axes of the sample stage to enable the image information to be combined without any gap or particle miscounting.

Annex D
(informative)

Multiple image analysis

When carrying out a full-surface analysis on large surfaces, as is the case when counting particles on a membrane filter, the camera of a light optical microscope should be aligned with the movement of the sample stage for scanning the membrane filter. If the camera is not aligned with the movement of the sample stage, gaps in the analysis could occur when reconstructing the measuring fields. This situation should be avoided.

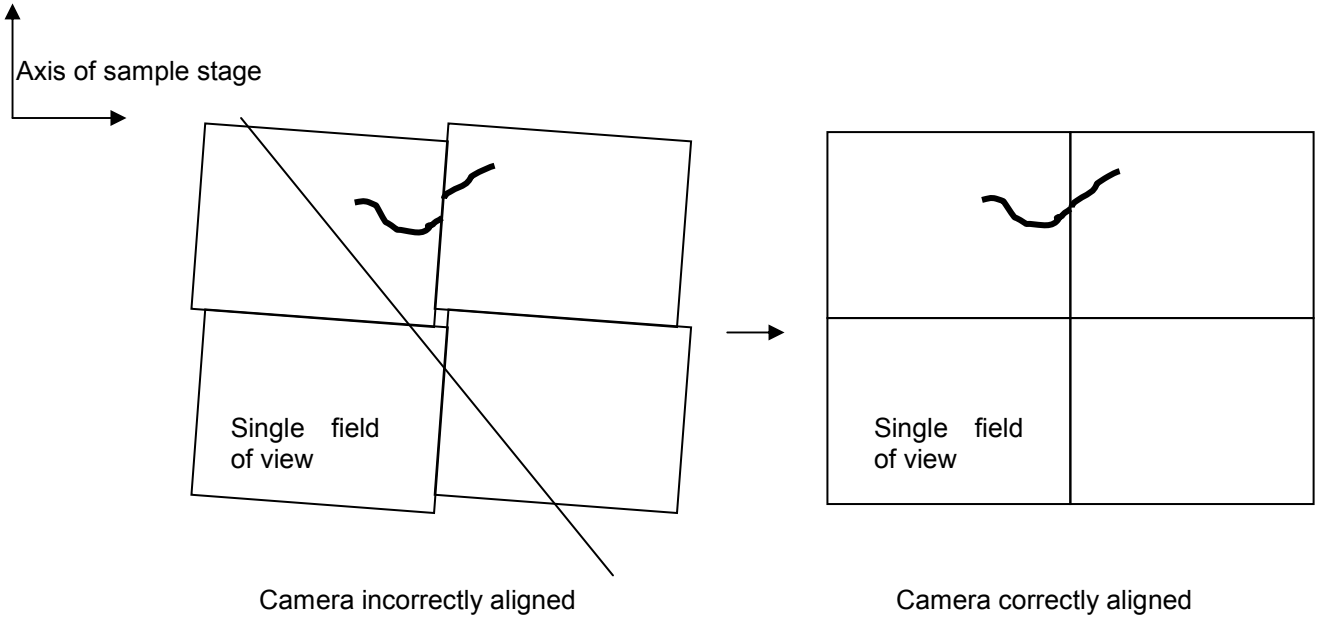


Figure D.1 — Multiple image analysis with an optical microscope

In the case of a scanning electron microscope it should be checked that the scanning direction of the single images is aligned with the movement of the sample stage.

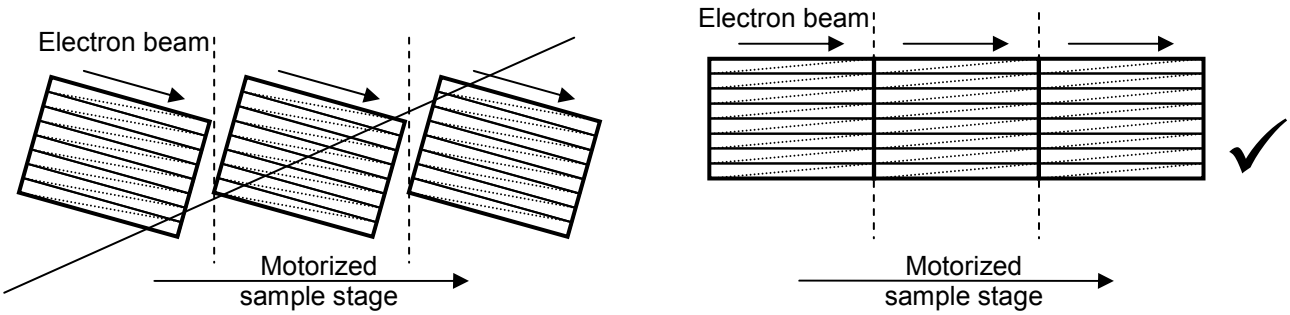


Figure D.2 — Multiple image analysis with a SEM

Annex E
(informative)

Resolution and calibration of an image analysis system

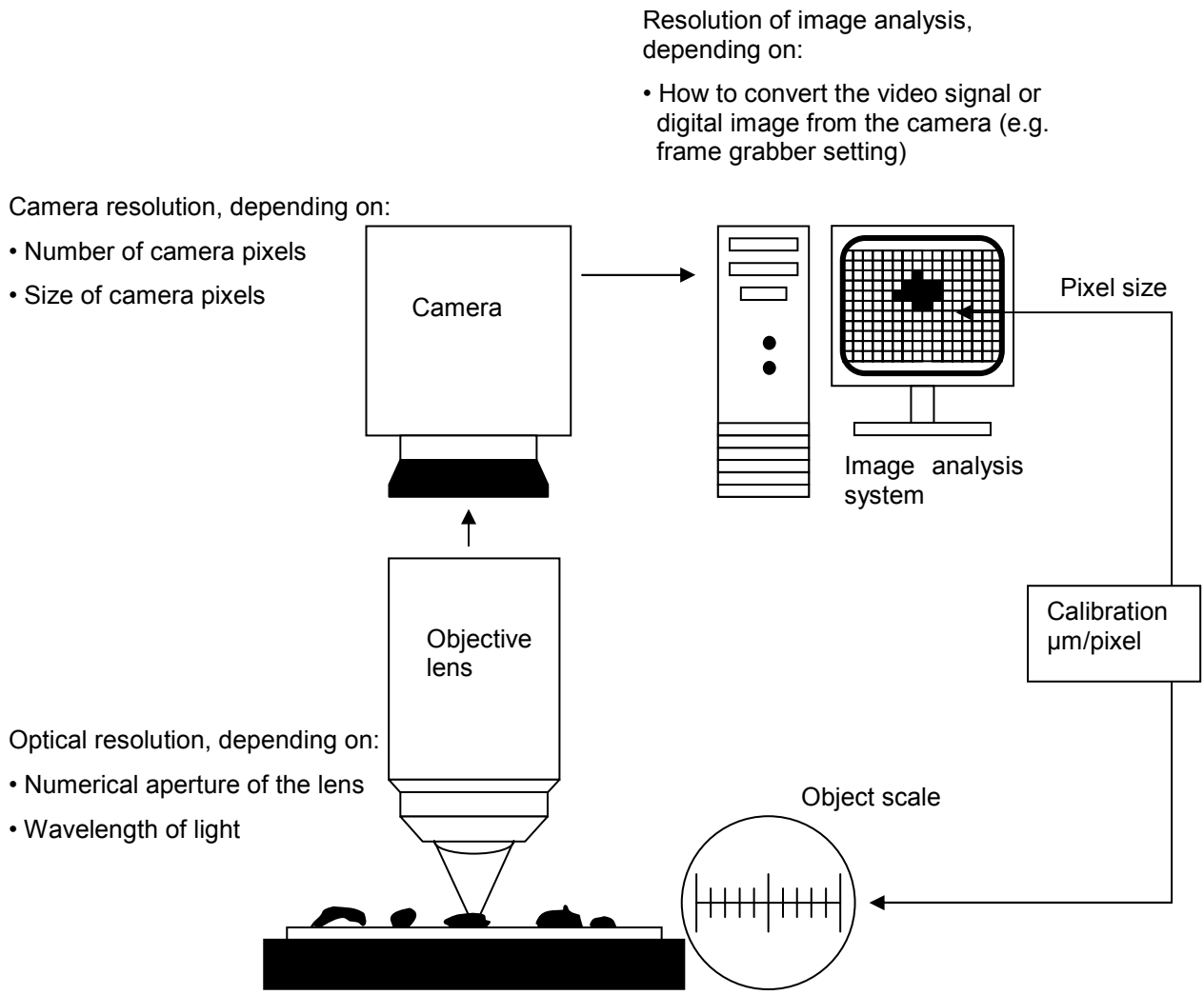


Figure E.1 — Resolution and calibration of an image analysis system

Annex F
(informative)

Example of a test report

F.1 Customer identification

Company: _____ Order: _____

Contact: _____ Date: _____

Address: _____

_____

F.2 Report and analysis identification

Laboratory identification _____ Report Number: _____ Project No. _____

Date of analysis: _____ Operator: _____

F.3 Identification of test component

Type: _____ Reference number: _____

Initial conditioning: _____ Serial Number: _____

Number of parts analysed: _____

Wetted surface area: _____ cm^2 Wetted volume: _____ cm^3

Controlled surface area: _____ cm^2 Controlled volume: _____ cm^3

Material: _____

F.4 Extraction procedure

Principle: _____ Reference number: _____

Fluid: Type: _____ Volume: _____

Filtered on _____ membranes

F.5 Microscopic Analysis Conditions

Environment ☐ Industrial ☐ Laboratory ☐ Controlled (ISO 14644-1 Class:)
Membrane filter: Material: _____ Mean pore size: _____ µm Diameter: _____ mm
Reference: _____ Camera ref.: _____ Software: _____
Optic calibration: Date: _____ Certificate Number: _____
Magnification: _____ X Pixel size: _____ µm/pixel
Type of microscope: ☐ Optical ☐ SEM ☐ Stereoscope Illumination: ☐ transmitted ☐ incident
Counting : ☐ manual ☐ automated

F.6 Analysis results

Particle size (µm)	$5 \leqslant x$ <15	$15 \leqslant x$ <25	$25 \leqslant x$ <50	$50 \leqslant x$ <100	$100 \leqslant x$ <150	$150 \leqslant x$ <200	$200 \leqslant x$ <400	$400 \leqslant x$ <600	$600 \leqslant x$ 1 000	$x \geqslant 1\,000$	Fibres [a]
Size class	B	C	D	E	F	G	H	I	J	K	
Blank counts											
Membrane number 1											
Membrane number 2											
Total counts (N)											

[a] Fibres are defined as particles greater than 100 µm with a length/width ratio $\geqslant$ 30.

F.7 Observations / comments (magnification, illumination, grey level, camera settings, etc.)

Date: Name:

Signature:

Bibliography

[1] ISO 4407:2002, *Hydraulic fluid power — Fluid contamination — Determination of particulate contamination by the counting method using an optical microscope*

[2] ISO 14644-1:1999, *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness*

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