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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.

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.

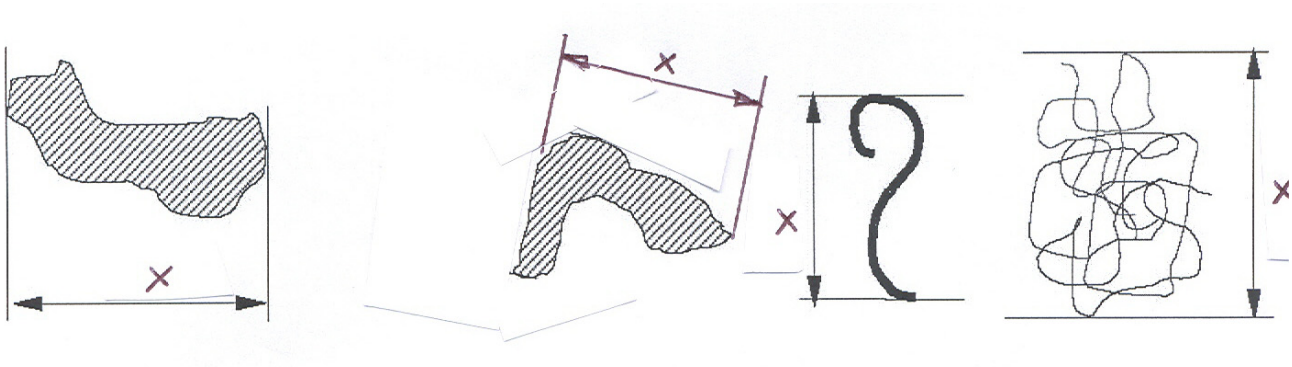

Figure 1 — Examples of longest dimension of a particle, X

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.

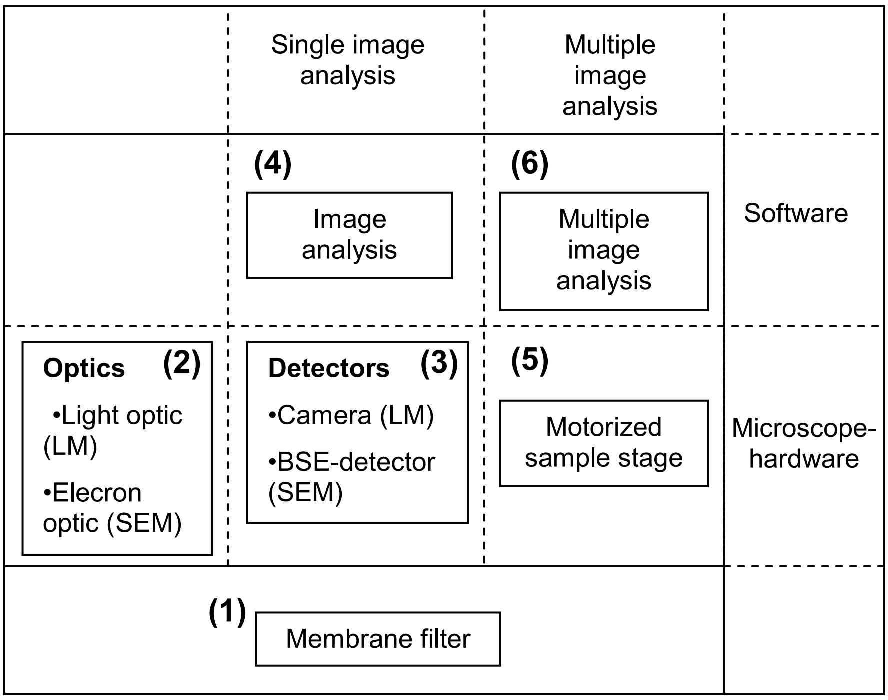

Figure 2 — Diagrammatic representation of the microscopic analysis of membrane filters

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 ⩽ 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.