
**Microbeam analysis — Scanning electron
microscopy — Guidelines for calibrating
image magnification**

*Analyse par microfaisceaux — Microscopie électronique à balayage —
Lignes directrices pour l'étalonnage du grossissement d'image*



Reference number
ISO 16700:2004(E)

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Published in Switzerland

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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 16700 was prepared by Technical Committee ISO/TC 202, *Microbeam analysis*, Subcommittee SC 4, *Scanning electron microscopy*.

Introduction

The scanning electron microscope is widely used to investigate the surface structure of a range of important materials such as semiconductors, metals, polymers, glass, food and biological materials and this International Standard is relevant to the need for magnification calibration of the images. It describes the requirements for calibration of the image magnification in the scanning electron microscope using a reference material or a certified reference material.

Microbeam analysis — Scanning electron microscopy — Guidelines for calibrating image magnification

1 Scope

This International Standard specifies a method for calibrating the magnification of images generated by a scanning electron microscope (SEM) using an appropriate reference material. This method is limited to magnifications determined by the available size range of structures in the calibrating reference material. This International Standard does not apply to the dedicated critical dimension measurement SEM.

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 Guide 30:1992, *Terms and definitions used in connection with reference materials*

ISO Guide 34:1996, *Quality system guidelines for the production of reference materials*

ISO Guide 35:1989, *Natural rubber latex concentrate — Determination of mechanical stability*

ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

ISO/IEC 17025:1999, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

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

3.1

scanning electron microscope (SEM)

instrument that produces magnified images of a specimen by scanning its surface with an electron beam

3.2

image

two-dimensional representation of the specimen surface generated by SEM

NOTE A photograph of a specimen taken using an SEM is a good example of an image.

3.3

image magnification

ratio of the linear dimension of the scan display to the corresponding linear dimension of the specimen scan field

3.4
scale marker

line on the image representing a designated actual length in the specimen

3.5
reference material (RM)

material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials

3.6
certified reference material (CRM)

reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence

NOTE For the purposes of this document, an RM/CRM possesses pitch pattern(s) with the desired range of pitch size(s) and accuracy, to be used for the calibration of the image magnification.

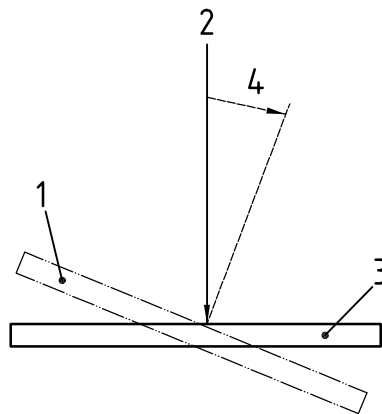
3.7
calibration

set of operations which establish, under specified conditions, the relationship between the magnification indicated by the SEM and the corresponding magnification determined by examination of an RM or a CRM

3.8
tilt angle

angle of the inclined specimen surface from the plane perpendicularly to the electron beam axis

See Figure 1.



- Key**
- 1 tilted specimen
 - 2 electron beam
 - 3 specimen
 - 4 tilt angle

Figure 1 — Tilt angle

3.9**display**

analog or digital device used for visualization of images

NOTE Examples of display are a cathode ray tube, plasma display panel, liquid crystal display, etc.

3.10**working distance**

distance between the specimen surface and the bottom plane of the objective lens of the SEM

3.11**pitch**

closest separation of two similar features on a specimen which are equivalent points on a repeat pattern

3.12**accuracy**

the closeness of agreement between a test result and the accepted reference value

[ISO 5725-1:1994]

NOTE 1 A “test result” constitutes the observed values of a pitch of a CRM obtained by the procedure outlined in this International Standard.

NOTE 2 The term “accepted reference value” is a value certified by a national or an international calibrating laboratory. There will be an uncertainty associated with this value which should also appear on the certificate.

NOTE 3 Accuracy and precision are different. Precision is defined as the closeness of agreement between independent test results obtained under stipulated conditions. See ISO 5725-1.

4 Image magnification**4.1 Scale marker**

To indicate magnification, superimpose on the image a scale marker and the corresponding length, in SI units, that it actually represents on the specimen. An example is shown in Figure 2.

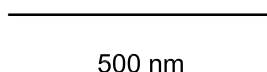


Figure 2 — Scale marker and its length

NOTE The value of the scale marker generated by the hardware and/or the software of the SEM may not correspond to the calibrated value.

4.2 Expressing magnification

Magnification of an image is given by a number representing the number of times the object has been magnified and it is accompanied by the symbol “x” (e.g. 100 x, 10 000 x, 10k x or x 100, x 10 000, x 10 k where 100, 10 000 and 10 k are magnitude numbers). See Annex A.

NOTE 1 It is not always necessary to show the magnification when the scale marker is shown on the image.

NOTE 2 The magnification shown on the image corresponds to a chosen output device, which can be a display monitor or a printer or a photographing device. The scale marker shown on the image is independent from the output device chosen by the operator of the SEM. The magnification shown corresponds to the scale marker only when the image is displayed or printed on the operator-chosen output device.

5 Reference material

5.1 General

See ISO Guide 30.

For calibrating the magnification of an image, wherever possible, choose a CRM that is produced in accordance with ISO Guide 34 and certified in accordance with ISO Guide 35.

When a suitable CRM is not available, an RM produced in accordance with ISO Guide 34 may be used.

5.2 Requirements for CRM

Ensure that the chosen CRM:

- is stable with respect to vacuum and repeated electron beam exposure;
- provides good contrast in the SEM image;
- is electrically conductive;
- can be cleaned to remove contamination occurring during normal use without causing mechanical damage or distortion;
- has an associated valid calibration certificate.

5.3 Pitch patterns on CRM

Pitch patterns on the CRM may be in any one or more of the following forms:

- an orthogonal cross grid;
- a line array;
- a dot array;
- an orthogonal dot array.

Ensure that the chosen CRM contains pitch patterns that allow for calibration in at least one direction, and that the uncertainty in the pitch patterns is consistent with the targeted accuracy.

NOTE 1 The CRM may contain pitch patterns both in X and Y directions so that the measurements can be performed in orthogonal directions without the necessity of mechanically rotating the CRM. The CRM may additionally contain other structures for testing image distortion and/or resolution.

NOTE 2 The chosen CRM may have different sized pitch patterns to cover the whole range of magnifications for which calibration is needed. It may also be necessary to have more than one CRM to cover the desired range of magnifications.

5.4 Storage and handling

Store the CRM in a desiccating cabinet or in a vacuum container.

NOTE To ensure minimal handling of the actual CRM, it may be permanently mounted on a stub.

Handle the CRM using fingerstalls, clean room gloves or tweezers.

Visually inspect the CRM surface for contamination and deterioration, as this may affect calibration. Do not use the CRM if it is damaged or grossly contaminated.

Remove any dust, loose debris or other contamination from the CRM using clean dry air or nitrogen gas, taking care not to damage the CRM.

Check the calibration of the CRM at intervals by comparison with other CRMs; record the results. The frequency of verification may depend on the nature and usage of the CRM.

Use the CRM for calibration purposes only.

6 Calibration procedures

6.1 General

Parameters that influence the resultant magnification of an SEM may cause systematic errors. These are listed in Annex B.

The stability of the SEM will be a major factor in determining the calibration interval. Initially it will be necessary to perform calibration at frequent intervals in order to verify that the SEM is stable.

The results obtained will provide an estimate of the reproducibility within the laboratory and the bias inherent in both the display and the data automatically superimposed on any output.

The selection of the CRM depends on the magnification being used and accuracy required. For the purposes of this International Standard, ensure that the accuracy of calibration is better than 10 %.

6.2 Mounting CRM

At the time of mounting the specimen, ensure that handling of the CRM is carried out in accordance with 5.4.

Mount the CRM in accordance with the SEM and the CRM manufacturer's instructions.

Ascertain that there is a good electrical contact between the CRM and the specimen stage of the SEM.

Check that the CRM is securely fixed on the specimen stage so that it does not move from its mounting. This enables one to minimize any image degradation caused by vibration.

6.3 Setting SEM operation conditions for calibration

Evacuate the specimen chamber to the working vacuum in accordance with the SEM manufacturer's instructions.

Optimize the electron beam brightness and alignment in accordance with the SEM manufacturer's instructions.

Set tilt angle to 0° following the SEM manufacturer's instructions so that CRM surface is perpendicular to the electron beam axis during operation.

Check the tilt of the CRM by the following procedures.

- a) Turn off the tilt angle correction, the scan rotation and the zoom control of the magnification.
- b) Select the imaging mode (secondary electron and/or back scattered electron).
- c) Bring the image into focus without visible stigmatic distortions in the image.

- d) Select the magnification at which the entire area of measurement is visible.
- e) Determine the tilt position where the measured value of pitch is maximum. If the difference of measured values is not found, assume that the tilt angle is 0°. Carry out subsequent recording of the image in this position.

NOTE If the image of the whole area cannot be brought into focus then it is necessary to remount the CRM, or readjust the mechanical alignment of the SEM.

- f) Select the accelerating voltage and the working distance for which the calibration is to be performed and bring the CRM into the correct position using the specimen stage controls.
- g) Wait until the instrument is fully stabilized at the desired operating conditions in accordance with the SEM manufacturer's instructions.
- h) Focus and adjust the image of the CRM on the display.
- i) If necessary, mechanically rotate the CRM so that the pattern to be measured is parallel to the X and/or Y directions of the display.
- j) Translate the CRM so that the end markings of the pitch pattern, on being measured, span about 80 % of the length and/or the width of the viewing display using the SEM specimen stage X and/or Y controls.
- k) If necessary, rotate again the CRM so the measurement patterns are parallel to the X and/or Y directions of the display and bring the image of the CRM pitch pattern back into sharp focus.

NOTE It is recommended that the actual number of pitches on the screen should be about 10; e.g. for a 100 mm display screen, the values of recommended pitch at the various magnifications are listed below.

× 50 000	0,2 μm
× 10 000	1 μm
× 1 000	10 μm

6.4 Image recording

Ensure that the pitch patterns are properly lined up in X and/or Y directions corresponding to those directions in the SEM.

Specify the photograph scanning speed.

Once the desired image is obtained, do not alter any other parameters of the SEM. Record the displayed image of the CRM with a scale marker on a photograph, or on a photographic film or in a digital form.

In the case of a photographic medium, allow sufficient time for it to stabilize prior to the measurement. This will minimize the effects of dimensional changes of the medium.

NOTE When, for the purpose of measurement, the recorded image in digital form is reproduced on paper or display, the length and the aspect ratio of the image could be different to that of the originally recorded image. In this case, it is recommended to record the length and the aspect ratio of the original image.

6.5 Measurement of image

For generating a calibration report, perform all measurements on a good reliable paper reproduction of the recorded image.

Use a traceable, calibrated ruler of a known accuracy, capable of measuring to less than 1 mm, to measure the length of the recorded image.

In order to minimize edge distortion effects, avoid measurements near the edges. Restrict measurements to the central 80 % of the image area.

Measure the pitch in the X and/or Y directions of the recorded image. This is achieved by measuring the distance between two of the markings, corresponding to an integral number of the pitch, within the area of the recorded image as specified above.

The measured distance should be approximately ten times larger than the pitch.

Repeat the measurement at least three times at separate locations at least 3 mm apart on the recorded image.

NOTE The pitch may be measured as the centre-to-centre or the edge-to-edge distance between the repeating features of the CRM.

6.6 Calibration of magnification and scale marker

6.6.1 General

Perform a calibration for magnification(s) commonly used in the laboratory.

6.6.2 Magnification

Determine the magnification, M , by use of the following equation:

$$M = \frac{D}{d}$$

where

D is the measured mean pitch (distance) on the recorded image (see Figure 3);

d is the actual pitch (distance) of the CRM corresponding to D (see Figure 4).

Obtain mean distances in both X and Y directions, then values of magnifications should be calculated for each direction.

If the difference of the magnification between X and Y directions exceeds an allowance set by the user, check the CRM mounting and possible misalignment in the SEM. Readjust and repeat the calibration.

6.6.3 Scale marker

Carry out the calibration of the scale marker for each calibration run.

Calculate the length, L , on the recorded image corresponding to the length of the value, f_{ind} , of the scale marker by the following equation:

$$L = f_{\text{ind}} \times M = f_{\text{ind}} \times \frac{D}{d}$$

where

L is the calculated length of the scale marker on the recorded image;

f_{ind} is the indicated value of the scale marker on the recorded image (see Figure 3).

NOTE If possible, the length of the scale marker should be adjusted to correspond to the calibrated magnification. If this is not possible the discrepancy should be noted.

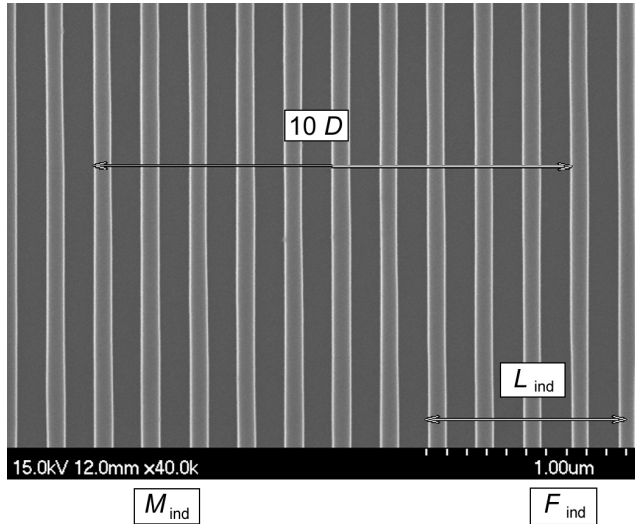


Figure 3 — Recorded image

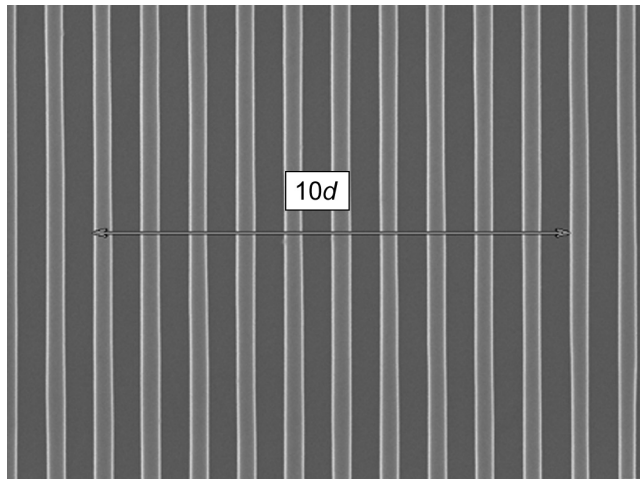


Figure 4 — Specimen (CRM)

7 Accuracy of image magnification and scale marker

The accuracy, A_m , in percent of the magnification can be determined by calculating the difference, ΔM using the following equations:

$$\Delta M = M_{ind} - M = M_{ind} - \frac{D}{d}$$

$$A_s = \frac{\Delta M}{M} \times 100$$

Where M_{ind} is the indicated magnification (see Figure 3).

The accuracy, A_s , in percent of the scale marker can be determined by calculating the difference, ΔL , using the following equations.

$$\Delta L = L_{ind} - L = L_{ind} - f_{ind} \frac{D}{d}$$

$$A_s = \frac{\Delta L}{L} \times 100$$

where L_{ind} and f_{ind} are the indicated length and the indicated value of scale marker on the recorded image individually (see Figure 3).

NOTE 1 Even if D and/or L_{ind} is measured with high accuracy (e.g. caliper), the minimum measurement error would be $\pm 0,2$ mm, because this is the resolution limit of the human eye.

NOTE 2 In digitally recorded images, accuracy may be determined by assuming that D and L_{ind} can be measured accurately to ± 1 pixel. D and L_{ind} may be expressed in terms of pixel length (in mm) to calculate A_m and A_s .

NOTE 3 It is noted that the uncertainty due to operating conditions of the SEM apparatus and statistical errors due to any unavoidable inhomogeneity of the CRM, are included in the result of the magnification calibration. (See Annex C.)

8 Calibration report

8.1 General

The calibration report carried out by the laboratory shall be accurate, clear, unambiguous and in accordance with the specific instructions in the calibration methods listed in this International Standard.

The results of the measurements shall be in a test report and, in addition to the information requested by the client, shall include all the information necessary for the interpretation of the calibration results and that required by 5.10.2 of ISO/IEC 17025:1999.

In the case of calibrations performed for internal clients, or in the case of a written agreement with the client, the results may be reported in a simplified way. The information listed in 5.10.2 of ISO/IEC 17025:1999, which is not reported to the client, shall be readily available in the laboratory that carried out the calibrations.

8.2 Contents of calibration report

In the calibration report include the following and any other relevant information which could affect the results of the calibration. An example is shown in Annex D.

- a) calibration report title;
- b) name and address of the laboratory;
- c) number of the calibration report;
- d) name and address of the client where relevant;
- e) identification of the method used (i.e. ISO 16700);
- f) manufacture's name, model name and the serial number of the instrument used;
- g) name and identification of the reference materials used;
- h) specific operating values of accelerating voltage, in kilovolts, working distance, in millimetres, imaging mode, scan speed and magnification setting;
- i) number of measurements taken, n , and results of calibration: length of the scale marker and/or magnifications in both X and/or Y directions with the accuracy expressed in percent;
- j) name of the person conducting the calibration;
- k) the date and time of the calibration;
- l) the name(s), function(s) and signature(s) of person(s) authorizing the calibration certificate;
- m) where relevant, a statement to the effect that the results relate only to the items tested or calibrated.

NOTE It is recommended that laboratories include a statement specifying that the calibration report shall not be reproduced except in full, without written approval of the laboratory.

Annex A (informative)

Reference materials for magnification

A.1 Scope

This annex gives examples of CRMs and RMs available for calibration of magnification scales for the SEM apparatus.

A.2 Certified reference materials (CRMs)

A.2.1 National Institute of Standards and Technology, USA

SRM 484g: SEM Magnification Standard; pitch range from 0,5 μm to 5 μm . Gold foil in nickel.

SRM 2069b: SEM Performance Standard; pitch range from 2 mm to 4 mm. Graphitized rayon fibers with smooth and uniform edges on a 12,5 mm diameter SEM specimen mount with a 3 mm peg.

SRM 2090: SEM Magnification Standard; pitch range from 0,2 μm to 3 000 μm . Silicon chip.

A.2.2 Physikalisch-Technische Bundesanstalt, Germany

IMS-HR 94 175-04: silicon oxide on a silicon wafer; 1 μm , 2 μm , 5 μm and 10 μm .

A.2.3 National Physical Laboratory, UK

Metal replicas with two orthogonal sets of ruled lines: one with a pitch nominally 50 μm and the other nominally 0,5 μm from originals ruled by SIRA and certified by the NPL.

A.2.4 GOST R, Russian Federation

CRM6261-91: it is manufactured in the form of a 15 mm \times 15 mm metal plate with periodical structural pattern in the form of strips. Certified characteristic: the distance between elements of periodical pattern on the surface, equal to 0,39 μm .

A.3 Reference materials (RMs)

A.3.1 Square grid

The 100 mesh in 254 μm pitch and 1 000 mesh in 25,4 μm pitch of gold, nickel or copper are recommended.

A.3.2 Nested square boxes

0,5 μm to 500 μm pitch patterns in nested square boxes.

A.3.3 Pitch pattern (National Institute of Standards and Technology, USA)

RM 8090: pitch range from 0,2 μm to 3 000 μm . Silicon chip.

NOTE This is the non-certified prototype of SRM 2090.

A.3.4 Silicon microscale certified by Japan Quality Assurance Organization (JQA)

Silicon microscale of 0,24 μm in pitch.

NOTE Other reference materials may be used.

Annex B (informative)

Parameters that influence the resultant magnification of an SEM

The parameters listed below may interact with each other, and are considered in the order of their location in the instrument.

- a) Electron gun high-voltage instability or drift can change the energy of the electrons, thereby changing the final focus that affects the working distance calibration which, in turn, determines the displayed magnification. In modern instruments the magnification compensation is determined by a computer algorithm, the input of which may be subject to error.
- b) Different condenser lens strength combinations change the focal point of the final lens.
- c) Uncorrected final lens astigmatism can give a false indication of exact focus.
- d) Residual magnetic hysteresis, particularly in the final lens, can change the focal conditions.
- e) Long depth of focus, particularly at low magnification and small beam divergence controlled by lens and aperture selection can lead to incorrect focus.
- f) Non-orthogonal deflection (X and Y axes) can be produced by the scan coils. Image distortion then appears.
- g) Scan generator circuits may be non-linear or change with aging of circuit components or both.
- h) Zoom control of magnification can be non-linear.
- i) Non-linearity of scan rotation accessory can distort magnification at different degrees of rotation.
- j) Distortion of the electron beam sweep may be produced by extraneous magnetic and electrostatic fields.
- k) The percentage error in magnification may be different for each magnification range.
- l) A tilted specimen surface (not perpendicular to the beam axis) will introduce foreshortening of the image and magnification variation.
- m) The tilt correction applied may not be at 90 ° (in the plane normal to the electron beam) to the tilt axis of the specimen or to a particular area on the specimen surface.
- n) Signal processing, particularly differentiation or homomorphic processing, can give a false impression of focus.
- o) For the same apparent magnification, different combinations of working distance, accelerating voltage and beam scan raster can produce different linear magnifications.
- p) Thermal and electronic drift of circuit components related to the above parameters can affect magnification with time in a random manner.
- q) Distortion of faceplate and non-orthogonal beam deflection of recording cathode ray tube (CRT) can produce non-uniform magnification.
- r) The image magnification on the recording CRT may not be the same as that on the viewing CRT.

- s) Recording camera lens distortion and change in ratio of the photographic image to CRT image can lead to magnification error.
- t) Expansion or contraction of photographic material, photographic enlarging, and control of contrast, can all have a significant effect on the final apparent image magnification.
- u) In digitally recorded images, magnification errors may occur due to the inaccuracies or distortion of the digital devices (e.g. printers, etc.). The aspect ratio (X and Y magnification) may be different than that of the original image.

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Annex C (informative)

Uncertainties in magnification measurements

The uncertainty is the range of values into which any measurement of the magnification can, with a specified level of confidence, be expected to fall. This uncertainty, u , can be determined from all the components of uncertainty, σ_i , using:

$$u = \sqrt{(\sum \sigma_i^2)}$$

Example: The components of uncertainty and typical values for them for a SIRA grid are:

A: that associated with the calibrant/certified reference material, $\pm 1\%$

B: repeatability, $\pm 4\%$

(The variation in magnification due to these components shows a normal distribution and the uncertainty, if determined as 95 % confidence limits, should be divided by 2, before combining them using the above equation.)

C: temperature effects, $< \pm 0,5\%$

D: resolution of the feature being measured, $\pm 1\%$

E: the uncertainty in the measuring device and the effects of temperature on this, $\pm 1\%$

(The effect of these factors results in a rectangular distribution and therefore magnitudes should be divided by $\sqrt{3}$ prior to combining them.)

The uncertainty expressed using the above approach will only be correct at the time that the calibration is performed. Repeat calibrations, over an extended period of time, lead to different results, due to uncertainty in operating conditions (specimen positioning, operator, instrument). For a given instrument configuration, a further component of uncertainty,

F: drift, $\pm 3\%$

needs to be considered.

After several recalibrations at a fixed (displayed) magnification M_d , a measured (true) magnification, M_t , together with some measure, σ_t , of the distribution about this value, can be determined. σ_t , by virtue of its method of determination, offers a practical method of combining the above components of uncertainty. With the exception of A, there will be two components of uncertainty which, when combined, will provide an uncertainty, u , the magnitude of which will tend towards that of u to be quoted.

$|M_t - M_d|$ will be a measure of the accuracy of the display on the instrument and output. For examination at a displayed magnification of M_d the results should be reported as using a magnification of $M_t \pm nu$. With $n = 2$ the operator is 95 % confident that the magnification at the time of examination was within the range expressed by these limits. The same argument applies to scale marker. Uncertainties in the magnification and scale marker length can be expressed by quoting respective standard deviations of these measurements.

Annex D (informative)

Example of a test report

D.1 Magnification calibration table

Example of a 'conditions' matrix for tracking calibrated magnifications as part of a quality control programme.

In the following table, insert the actual magnification measured against the calibration standard.

The given accelerating voltage, magnifications and working distances are for example only. These values should be adjusted to represent those settings that are used in practice. Different numbers of settings to those given in this example may be used.

The data should be plotted as a control chart to show variability over time.

Table 1 — Test report for calibration of scale maker

Name of the laboratory: _____ Model: _____ Serial Number: _____
 Address of the laboratory: _____ Operator: _____
 Number of the calibration report: _____ Date: _____
 ISO Standard reference: ISO 16700 Name and signature of person authorizing: _____
 Name of the manufacturer: _____

Magnification	WD (mm)	Measurement				Acc. Voltage				2kV			(C)RM		
		D_x	D_y	M_x	M_y	$L_{ind} \& f_{ind}$	L_x	L_y	ΔL_x	ΔL_y	A_{xs}	A_{ys}	d	Name of (C)RM	
(M_{ind})	5	1													
		2			$(= \frac{D_{xm}}{d})$										
		3			$(= \frac{D_{ym}}{d})$										
		mean value		D_{xm}	D_{ym}										
	10	1													
		2													
		3													
		mean value													
	15	1													
		2													
		3													
		mean value													
X1 000	5	1													
		2													
		3													
		mean value													
	10	1													
		2													
		3													
		mean value													
	15	1													
		2													
		3													
		mean value													

$$A_s = \frac{\Delta L}{L} \times 100$$

$\Delta L = L_{ind} - L$
 L the calculated length of the scale marker on the recorded image
 ΔL the difference
 L_{ind} the indicated length of the scale marker on the recorded image
 f_{ind} the accuracy of the scale marker

$L = f_{ind} \times M$
 L the calculated length of the scale marker on the recorded image
 f_{ind} the indicated value of the scale marker on the recorded image
 M the calibrated magnification

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