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Microbeam analysis — Analytical electron microscopy — Methods for calibrating image magnification by using reference materials with periodic structures

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National foreword

This British Standard is the UK implementation of ISO 29301:2023. It supersedes BS ISO 29301:2017, which is withdrawn.

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A list of organizations represented on this committee can be obtained on request to its committee manager.

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ISO 29301

Third edition
2023-10

Microbeam analysis — Analytical electron microscopy — Methods for calibrating image magnification by using reference materials with periodic structures

Analyse par microfaisceaux — Microscopie électronique analytique — Méthodes d'étalonnage du grandissement d'image au moyen de matériaux de référence de structures périodiques

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Contents

Page

Foreword ... **iv**
Introduction ... **v**
1 Scope ... **1**
2 Normative references ... **1**
3 Terms and definitions ... **1**
4 Image magnification ... **5**
4.1 Definition of the image magnification ... 5
4.2 Expressing magnification ... 6
5 Reference materials ... **6**
5.1 General ... 6
5.2 Requirements for CRM/RM ... 7
5.3 Storage and handling ... 7
6 Calibration procedures ... **7**
6.1 General ... 7
6.2 Mounting CRM/RM ... 8
6.3 Setting TEM operating conditions for calibration ... 8
6.4 Capturing digitized image ... 9
6.5 Digitizing the image recorded on photographic film ... 10
6.5.1 General ... 10
6.5.2 How to decide the pixel-resolution for digitization ... 10
6.6 Measurement of the angle-corrected distance, D_t, from the digitized image ... 12
6.6.1 General ... 12
6.6.2 Measurement procedure ... 13
6.7 Digitization of reference scale for pixel size calibration ... 15
6.8 Calibration of image magnification ... 16
6.8.1 General ... 16
6.8.2 Calibration of scale unit (= pixel size), S ... 16
6.8.3 Calculating image magnification ... 18
6.9 Calibration of scale bar ... 18
6.9.1 General ... 18
6.9.2 Basic scale size corresponding to one pixel on the digitized image ... 19
6.9.3 Calibration of scale bar ... 19
6.10 Calibration procedure for length measurements using photographic film only ... 20
7 Accuracy of image magnification ... **20**
8 Uncertainty of measurement result ... **21**
9 Calibration report ... **23**
9.1 General ... 23
9.2 Contents of calibration report ... 23
Annex A (informative) **Parameters that influence the resultant magnification of a TEM** ... **25**
Annex B (informative) **Flowchart of image-magnification calibration procedure** ... **26**
Annex C (informative) **How to decide the number of lines for averaging** ... **27**
Annex D (informative) **Reference materials for magnification calibration** ... **29**
Annex E (informative) **Example of test report for calibration of TEM magnification** ... **33**
Bibliography ... **44**

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Foreword

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This document was prepared by Technical Committee ISO/TC 202, *Microbeam analysis*, Subcommittee SC 3, A*nalytical electron microscopy*.

This third edition cancels and replaces the second edition (ISO 29301:2017), of which it constitutes a minor revision. The changes are as follows:

— the element name of Silver in Table D.1 has been corrected to Silicon;

— normative references in Clause 2 have been updated.

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Introduction

The transmission electron microscope (TEM) is widely used to investigate the micro/nano-structure of a range of important materials such as semiconductors, metals, nano-particles, polymers, ceramics, glass, food and biological materials. The technique used involves the transmission of electrons through an ultra-thin specimen, interacting with the specimen as they pass through. This interaction results in a magnified image which is focused onto an imaging device, such as a photographic film, an imaging plate, or an image sensor built into a digital camera. A TEM is capable of imaging at significantly higher resolutions than ordinary (light) microscopes. It can be used to examine fine details as small as a single atomic column in a given specimen. This document addresses the need for magnification calibration of the images. It describes the requirements for calibration of the image magnification in the transmission electron microscope using a certified reference material or a reference material with periodic structures.

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Microbeam analysis — Analytical electron microscopy — Methods for calibrating image magnification by using reference materials with periodic structures

1 Scope

This document specifies a calibration procedure applicable to images recorded over a wide magnification range in a transmission electron microscope (TEM). The reference materials used for calibration possess a periodic structure, such as a diffraction grating replica, a super-lattice structure of semiconductor or an analysing crystal for X-ray analysis, and a crystal lattice image of carbon, gold or silicon.

This document is applicable to the magnification of the TEM image recorded on a photographic film, or an imaging plate, or detected by an image sensor built into a digital camera. This document also refers to the calibration of a scale bar.

This document does not apply to the dedicated critical dimension measurement TEM (CD-TEM) and the scanning transmission electron microscope (STEM).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 35, *Reference materials — Guidance for characterization and assessment of homogeneity and stability*

ISO/IEC 17025:2017, *General requirements for the competence of testing and calibration laboratories*

ISO 17034, *General requirements for the competence of reference material producers*

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
accuracy
closeness of agreement between a test result and the accepted reference value

Note 1 to entry: A “test result” is the calibrated magnification obtained by the procedure outlined in this document.

Note 2 to entry: The term “accepted reference value” is the magnification given by the TEM manufacturer.

[SOURCE: ISO 5725-1:1994[1)], 3.6, modified — new Notes 1 and 2 to entry have been added.]

1) Now withdrawn. Replaced by ISO 5725-1:2023.

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3.2
alignment
series of operations to align the incident direction of the electron beam to the *optical axis* (3.22) using deflectors and/or mechanical knobs

3.3
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 1 to entry: For the purposes of this document, a CRM possesses periodic structure(s), with the desired range of periodic interval and accuracy, to be used for the calibration of the *image magnification* (3.15).

3.4
contamination
formation of a deposited layer of any material due to the interaction of the electron beam with the sample and/or its immediate environment

3.5
crystal orientation
direction of crystal which is represented by crystal index

Note 1 to entry: During TEM imaging, it is often useful to have a crystalline specimen aligned such that a specific (low index) *zone axis* (3.36) is parallel, or nearly parallel, to the beam direction [*optical axis* (3.22)].

3.6
diffraction grating replica
shadow-casting carbon replica film constituting a grating which contains 500 to 2 000 parallel grooves per millimetre, or cross-line grating with a similar line spacing

Note 1 to entry: A diffraction grating replica can be used as a *reference material* (3.25) for calibration of the *image magnification* (3.15) in the low to medium-low magnification range.

3.7
digital camera
device that detects the *image* (3.13) using a chip-arrayed *image sensor* (3.18), such as a charge-coupled device (CCD) or complementary metal-oxide semiconductor (CMOS), that converts a visual image to an electric signal

3.8
dynamic range
range of detectable electron doses illuminated on the detector, in which the image signal can be detected properly

3.9
excitation current
electric current applied to the coil of the magnetic lens

3.10
focus
focusing condition in which the specimen height coincides with the object plane of the objective lens

3.11
glass scale
ruler on which a fine scale is drawn and utilized as the reference scale to measure the distance in the digitized image after digitizing it with an *image scanner* (3.17)

Note 1 to entry: The transparency and thermal stability of the glass scale are convenient to get the digitized reference image with a transmitted image scanner and to make the contact image on the *imaging plate* (3.24).

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3.12
horizontal field width
HFW
original length corresponding to full width in the horizontal direction on a magnified image

3.13
image
two-dimensional projection of the specimen structure generated by *TEM* (3.34)

Note 1 to entry: A *photographic film* (3.23), an *imaging plate* (3.16), and an *image sensor* (3.18) built into a digital camera are examples of devices for detecting the *image* (3.13).

[SOURCE: ISO 16700:2016, 3.2, modified — the term "SEM" has been replaced by the term "TEM".]

3.14
image file
computer file containing information relating to the digitized image

3.15
image magnification
ratio of the linear dimension of the specific structure/scaling on the image detector, such as a *photographic film* (3.23), an *imaging plate* (3.16), and an *image sensor* (3.18) built into a digital camera, to the corresponding linear dimension of the structure/scaling on the *specimen* (3.27)

3.16
imaging plate
IP
electron image detector consisting of a film with a thin active layer embedded with specifically designed phosphors

3.17
image scanner
device that converts an analogue image into a digitized image with the desired pixel-resolution

Note 1 to entry: There are mainly two different types of scanners: flatbed type and drum type.

3.18
image sensor
device, such as a charge-coupled device (CCD) array or complementary metal-oxide semiconductor (CMOS) sensor, that converts visual image information to an electric signal, built-in digital camera or other imaging devices

3.19
image wobbler
deflection coil used to change the direction of incident electron beam onto the *specimen* (3.27)

Note 1 to entry: This coil is activated in a periodic manner with the aim of identifying easily the place of *focus* (3.10).

3.20
lattice image
image (3.13) consisting of interference fringes formed by the interaction between the transmitted electron beam and diffracted electron beam from a specific crystal plane

Note 1 to entry: Lattice fringes can be used to calibrate *image magnification* (3.15) at the high end of the magnification range.

3.21
magnetic hysteresis
physical phenomenon related to the magnetizing loop in which the magnetic field strength depends on the direction of the adjustment of the exciting current for the magnetic lens

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3.22
optical axis
straight line passing through the symmetrical centre of the magnetic field of the electron lens

Note 1 to entry: The path of an electron beam along this axis goes through the lens without changing the direction.

3.23
photographic film
negative film
sheet or a roll of thin plastic coated by photographic emulsion for recording an *image* (3.13)

3.24
pixel-resolution
number of imaging pixels per unit distance of the detector

Note 1 to entry: The typical unit is sometimes expressed as dots per inch (dpi).

3.25
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

Note 1 to entry: For the purpose of this document, an RM possesses periodic pattern(s) with the desired range of periodic interval and accuracy, to be used for the calibration of the *image magnification* (3.15).

3.26
region of interest
ROI
region of the *image* (3.13) selected for a specific reason

3.27
specimen
small portion of a sample for observation

Note 1 to entry: For *TEM* (3.34), a specimen has to be thin enough to transmit the electron beam.

3.28
specimen cartridge
part of the *specimen holder* (3.31) which supports a *specimen* (3.27) and is attached to the tip of the specimen holder for use

3.29
specimen drift
unintentional movement of the *specimen* (3.27) due to any source (thermal, mechanical, electric, charging)

3.30
specimen height
specimen position along the *optical axis* (3.22) of the objective lens

Note 1 to entry: "Specimen height = 0" corresponds to the specimen position in correct focus under the *standard excitation condition* (3.32) of the objective lens.

Note 2 to entry: See Reference [6].

3.31
specimen holder
device that supports a *specimen* (3.27) in the right position in the pole-piece gap of the objective lens

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3.32
standard excitation condition
setting condition for excitation current to derive the highest performance of the objective lens

Note 1 to entry: Under this condition, *specimen height* (3.30) shall be set so that the *image* (3.13) is focused.

Note 2 to entry: This condition is provided by the TEM manufacturer for each instrument.

Note 3 to entry: *Image magnification* (3.15) is generally measured under this condition; however, as long as reproducible conditions are established, the magnification can be calibrated at any of the instrument settings.

3.33
super-lattice
stable periodic structure which is fabricated by alternating layers of at least two different kinds of materials

Note 1 to entry: The super-lattice can be used as a *reference material* (3.25) for calibration of *image magnification* (3.15) from a medium-high to high magnification range.

3.34
transmission electron microscope
TEM
instrument that produces magnified images or diffraction patterns of the *specimen* (3.27) by an electron beam which passes through the specimen and interacts with it

3.35
under focus
focusing condition in which the specimen height is further from the objective lens than its object plane

3.36
zone axis
crystallographic direction, designated [*uvw*], defined by the intersection of a number of crystal planes (h_1, k_1, l_1… …h_i, k_i, l_i) such that all of the planes satisfy the so-called Weiss zone law; $hu + kv + lw = 0$

4 Image magnification

4.1 Definition of the image magnification

The image magnification (or scaling factor) of the TEM is defined by the ratio of the linear dimension of the specific structure on the detected image to the corresponding linear dimension of the specific structure in the specimen. There are three main kinds of image detectors: photographic film, imaging plate, and image sensor, such as CCD array or CMOS sensor built in the digital camera.

In general, the value of image magnification detected on an image sensor is different from the value of image magnification detected on the photographic film or imaging plate under the same electron optical conditions for TEM imaging, because the image-detecting positions are different from each other (see Figure 1).

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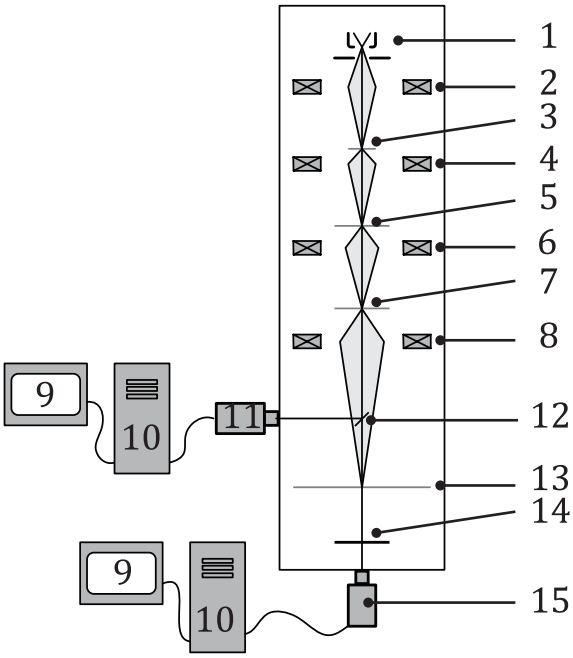

Key

1 electron gun
2 condenser lens
3 specimen
4 objective lens
5 first magnified image
6 intermediate lens
7 second magnified image
8 projector lens
9 monitor
10 computer
11 digital camera (image sensor) 1
12 screen/monitor
13 viewing screen
14 photographic film/imaging plate
15 digital camera (image sensor) 2

Figure 1 — Detector position in TEM system

4.2 Expressing magnification

The magnification of an image recorded on the photographic film or the imaging plate, or detected by the image sensor, is given by a number representing the number of times, and the number is accompanied by the symbol × (e.g. 10 000×, 10k×, 1 000 000×, 1M× or ×10 000, ×10k, ×1 000 000, ×1M, where 10 000, 10k, 1 000 000 and 1M are magnitude numbers). Alternatively, introducing a scale bar having a length corresponding to unit length on the specimen can be used to represent the magnification. The digitized image should also indicate a magnification by detailing the number of pixels per unit distance of the raw data file.

NOTE The horizontal field width (HFW) is another way to define the scaling on a magnified image.

5 Reference materials

5.1 General

For calibrating the magnification of an image, wherever possible, choose a CRM that is produced in accordance with ISO 17034 and certified in accordance with ISO Guide 35.

When a suitable CRM is not available, an RM produced in accordance with ISO 17034 may be used.

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5.2 Requirements for CRM/RM

Ensure that the chosen CRM/RM

— is stable with respect to vacuum and repeated electron-beam exposure,

— is aligned to a low-index zone axis along the electron optical axis, if the specimen region is a single crystal,

— provides a good contrast and clear interface for the periodic structure in the TEM image,

— can be cleaned to remove contamination without causing mechanical/electrical damage or distortion,

— has a smooth surface on both sides and identical thickness for a super-lattice structure, at least within the area used for the calibration process, and

— has an associated valid calibration certificate.

NOTE Single crystal specimens of pure elements used for calibration do not need a calibration reference certificate.

5.3 Storage and handling

The CRM/RM shall be stored in a desiccating cabinet or in a vacuum container.

To ensure minimal handling of the actual CRM/RM, it may be permanently mounted on a specimen holder or a specimen cartridge.

The CRM/RM should be carefully handled without causing damage during the handling.

Check the contamination and deterioration of the CRM/RM, as these may affect calibration. Do not use the CRM/RM if it is damaged or grossly contaminated.

Check the calibration of the CRM/RM at intervals by comparing its calibration values with those of other CRMs/RMs; record the results. The frequency of verification may depend on the nature and usage of the CRM/RM.

The CRM/RM shall be used for calibration purposes only.

6 Calibration procedures

6.1 General

Parameters that influence the magnification of a TEM may cause systematic errors. These are listed in Annex A for additional information.

A major factor that influences the reproducibility of the calibration is the magnetic hysteresis of the electromagnetic lens. It is necessary to minimize its influence by adopting the procedure described below in the same sequence each time, especially related to the direction of magnification setting (higher to lower, or lower to higher). Also, the specimen height and focus setting will influence the reproducibility of the calibration.

To obtain the value of the uncertainty within the laboratory, it is necessary to repeat the calibration procedure periodically.

The selection of the CRM/RM depends on the magnification range being used and the accuracy required. For the purpose of this document, ensure that the uncertainty and repeatability of the calibration is less than ±5 % and 98 %, respectively.

The flowchart of the calibration procedure is shown in Annex B for additional information.

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6.2 Mounting CRM/RM

At the time of mounting the specimen, ensure that the handling of the CRM/RM is carried out in accordance with 5.3.

Mount the CRM/RM in accordance with the instructions provided by the TEM and the CRM/RM manufacturers.

Check that the CRM/RM is securely fixed on the specimen holder or specimen cartridge so that it does not move from its mounting. This enables any image degradation caused by vibration to be minimized.

Check that the height of the specimen in the specimen holder is at the position recommended by the TEM manufacturer's instructions, in order to keep the eucentric condition.

It is desirable to use a double-tilt or tilt-rotate specimen holder for aligning the crystal orientation of the specimen to the optical axis.

6.3 Setting TEM operating conditions for calibration

Set the operating condition of the TEM according to the following procedures to ensure, as far as possible, use of the same conditions.

a) Check that the degree of vacuum in the TEM column is lower than 10^{-4} Pa and stable.

b) The high voltage shall be applied and an appropriate time be allowed for it to stabilize.

NOTE Oil-filled 100 kV tanks take about 2,5 h; gas-filled tanks take about 45 min. Higher voltage instruments are normally operated with the high voltage continually applied; therefore, a stabilization period is not usually required.

c) Use an anti-contamination device, if needed.

d) Select a specimen region of interest (region) for the calibration which is clean and free from damage, ensure the eucentric height of the region and adjust the height of the region, if necessary.

e) In order to minimize the effect of the magnetic hysteresis of the lenses, set the magnification of the TEM to the target value for calibration according to the same sequence; for example, adjust a higher magnification than the target magnification at first, then set the target magnification after that.

f) Set the excitation of the objective lens to the desired reproducible value; the standard condition is recommended.

g) Adjust the specimen height to focus the magnified image projected on the fluorescent screen, the TV monitor or the personal computer (PC) screen. If the TEM in question is not equipped with a specimen-height control function, this procedure can be omitted.

h) Correct astigmatism at a slightly higher magnification than the target value and adjust the accelerating voltage centre. For example, if the target calibration is ×100k, set the magnification in the range ×150k to ×200k for alignment.

i) Switch the observation mode of the TEM to the selected-area electron-diffraction (SAED) mode or the convergent-beam electron-diffraction (CBED) mode from the image mode. Also, make sure that the objective aperture is removed. For the SAED mode, it is necessary to insert a selected-area aperture over the area of interest of the specimen in order to project a selected-area electron-diffraction pattern on the viewing device (fluorescent screen, TV monitor, PC screen).

j) Adjust the condenser lens system to provide nearly parallel illumination conditions.

k) Align a low-index zone axis of the crystal parallel to the optical axis (i.e. zone-axis illumination), if the specimen is a single crystal (see Figure 2).

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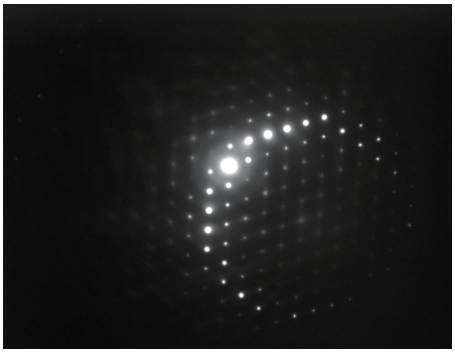
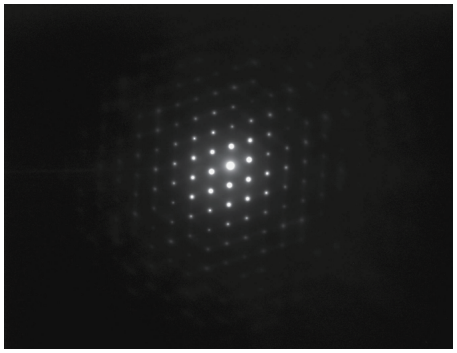


a) Off-axis condition



b) Zone-axis condition

Figure 2 — Difference of diffraction pattern by crystal orientation

l) Insert the objective aperture, centring it about the electron optical axis.

m) Switch the observation mode of the TEM back to the image mode.

n) Return the magnification to the target value of calibration, and set the excitation current of the objective lens to the standard exciting condition again.

o) Apply a relaxation function to relax the magnetic hysteresis of the objective lens, if the TEM has it.

p) Adjust the specimen height to focus the magnified image roughly. If the TEM in question is not equipped with a specimen-height control function, this procedure can be omitted.

q) Adjust the fine focus by varying the exciting current of the objective lens. If necessary, it is possible to use the image wobbler function for focusing the image. However, the function related to the optimum under-focus condition linked with the image wobbler function shall be turned off, if the TEM is equipped with this function.

r) Adjust the illumination condition of the condenser lens system (spot size and brightness) with reference to the dynamic range of each detector to obtain image contrast in the whole dynamic range.

The condenser lens system should be operated under conditions which approach parallel illumination. Alternatively, they should be done under a condition where it is documented that the beam convergence no longer affects the image focus. This can be done by recording multiple images under varying degrees of beam convergence.

6.4 Capturing digitized image

It is necessary to digitize the image in order to minimize readout error on the measurement of magnification. The bit depth of digitization of the image shall be larger than 8 bits. There are three ways of digitizing the magnified image corresponding to each image detector (see Table 1).

Table 1 — Comparison table for image detector

Image detection	Apparatus for digitization	Pixel size
Photographic film	Flatbed image scanner	Determined by pixel-resolution applied to image scanner
Imaging plate	Dedicated image digitizer	Determined by laser-beam diameter for readout
Image sensor (digital camera)	—	Same size as that of the image sensor

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