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

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

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

The committee responsible for this document is ISO/TC 202, *Microbeam analysis*, Subcommittee SC 4, *Scanning electron microscopy (SEM)*.

This second edition cancels and replaces the first edition (ISO 16700:2004), which has been technically revised.

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.

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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 documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/IEC 17025:2005, *General requirements for the competence of testing and calibration laboratories*

ISO Guide 30, *Reference materials — Selected terms and definitions*

ISO Guide 34, *General requirements for the competence of reference material producers*

ISO Guide 35, *Reference materials — General and statistical principles for certification*

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 ([3.1](#))

Note 1 to entry: 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/generated line (intervals) on the image ([3.2](#)) representing a designated actual length in the specimen

3.5

reference material

RM

material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

3.6
certified reference material
CRM

reference material (3.5) characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

Note 1 to entry: For the purposes of this International Standard, 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* (3.3).

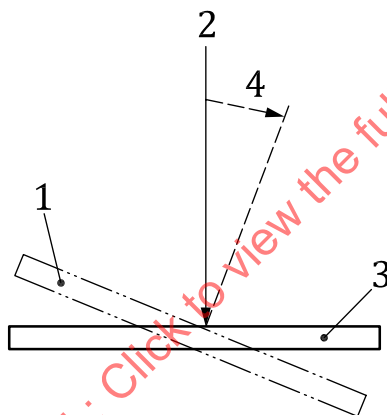
3.7
calibration

set of operations which establish, under specified conditions, the relationship between the magnification indicated by the SEM (3.1) and the corresponding magnification determined by examination of an RM (3.5) or a CRM (3.6)

3.8
tilt angle

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

Note 1 to entry: 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* (3.2)

Note 1 to entry: 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 (3.1)

3.11
pitch

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

3.12

accuracy

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

Note 1 to entry: A “test result” constitutes the observed values of a *pitch* (3.11) of a *CRM* (3.6) obtained by the procedure outlined in this International Standard.

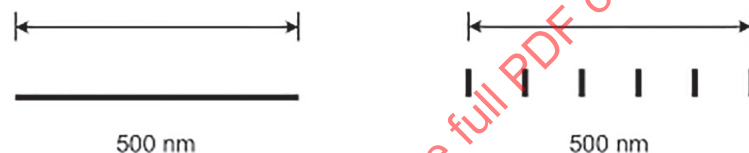
Note 2 to entry: The term “accepted reference value” is a value certified by a national or an international calibrating laboratory. There will be an uncertainty associated with this value which should also appear on the certificate.

Note 3 to entry: 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.



NOTE In Figure 2, the length indicated by the arrows corresponds to 500 nm after the calibration.

Figure 2 — Scale marker and its length

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 “ $\times$ ” (e.g. 100 $\times$, 10 000 $\times$, 10k $\times$ or $\times$ 100, $\times$ 10 000, $\times$ 10k, where 100, 10 000 and 10k are magnitude numbers).

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, and
- 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 There are cases where the CRM contains 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. In some cases, the CRM additionally contains other structures for testing image distortion and/or resolution.

NOTE 2 There are instances where the chosen CRM has different-sized pitch patterns to cover the whole range of magnifications for which calibration is needed. It can 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 can 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 1 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 2 It is practical that the actual number of pitches on the screen is about 10; e.g., for a 100 mm display screen, the values of typical pitch at the various magnifications are as follows.

× 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 useful 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 can 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 , using [Formula \(1\)](#):

$$M = \frac{D}{d} \quad (1)$$

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 using [Formula \(2\)](#):

$$L = f_{\text{ind}} \times M = f_{\text{ind}} \times \frac{D}{d} \quad (2)$$

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 the length of the scale marker is adjusted to correspond to the calibrated magnification, the discrepancy is minimized.

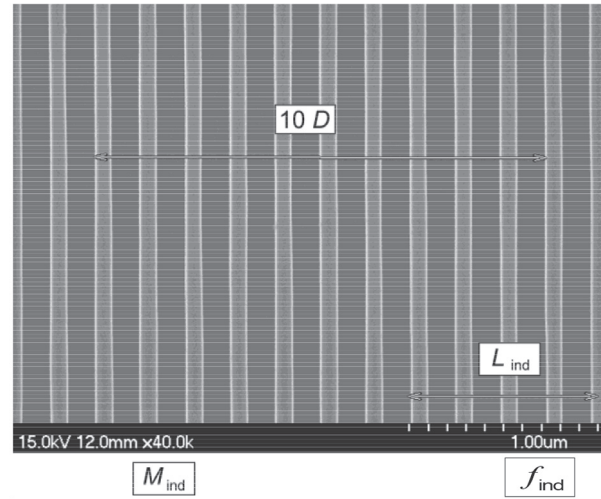


Figure 3 — Recorded image

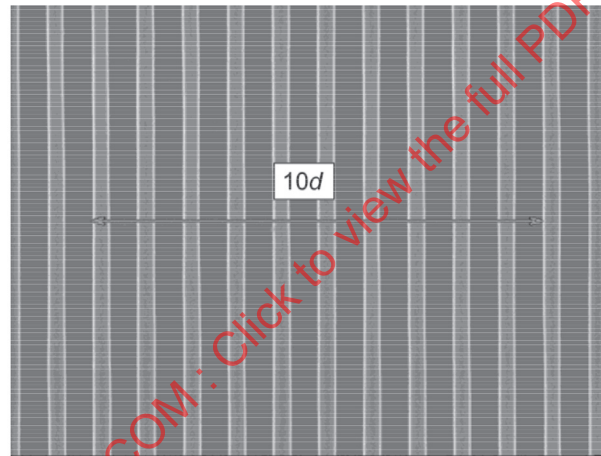


Figure 4 — Specimen (CRM)

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 [Formula \(3\)](#) and [Formula \(4\)](#):

$$\Delta M = M_{\text{ind}} - M = M_{\text{ind}} - \frac{D}{d} \quad (3)$$

$$A_m = \frac{\Delta M}{M} \times 100 \quad (4)$$

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 [Formula \(5\)](#) and [Formula \(6\)](#):

$$\Delta L = L_{\text{ind}} - L = L_{\text{ind}} - f_{\text{ind}} \frac{D}{d} \quad (5)$$

$$A_s = \frac{\Delta L}{L} \times 100 \quad (6)$$

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 can be determined by assuming that D and L_{ind} can be measured accurately to ± 1 pixel. D and L_{ind} can 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 customer, shall include all the information necessary for the interpretation of the calibration results and that required by ISO/IEC 17025:2005, 5.10.2.

In the case of calibrations performed for internal customers, or in the case of a written agreement with the customer, the results may be reported in a simplified way. The information listed in ISO/IEC 17025:2005, 5.10.2, which is not reported to the customer, 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) the calibration report title;
- b) the name and address of the laboratory;
- c) the number of the calibration report;
- d) the name and address of the customer, where relevant;
- e) the identification of the method used (i.e. ISO 16700);
- f) the manufacturer's name, model name and the serial number of the instrument used;
- g) the name and identification of the reference materials used;
- h) the specific operating values of accelerating voltage, in kilovolts; working distance, in millimetres; imaging mode; scan speed and magnification setting;
- i) the 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) the 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 If necessary, laboratories will include a statement specifying that the calibration report is not reproduced except in full, without written approval of the laboratory.

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Annex A (informative)

Reference materials for magnification

A.1 Overview

This annex gives examples of CRMs and RMs available at the time of drafting this International Standard for calibration of magnification scales for the SEM apparatus. In order to obtain some up-to-date information, several website links are provided in the Bibliography.

NOTE Other reference materials can be used.

A.2 Testing and calibration laboratories for certified reference materials (CRMs)

A.2.1 National Institute of Standards and Technology (NIST), USA

For more information, see Reference [3].

A.2.2 Physikalisch-Technische Bundesanstalt (PTB, The National Metrology Institute), Germany[4]

Calibration item: A pitch of 1D or 2D grating standards; Calibration method: Metrological Large-Range SPM; Pitch range: 50 nm to 50 µm; Expanded uncertainty with coverage factor $k=2$: some ten picometers depending on the number of periods measured and the quality of the gratings; Accreditation: CIPM-MRA.

Calibration item: Average pitch; Calibration method: Diffractometry; Pitch range: 150 nm to 4 µm; Accreditation: CIPM-MRA.

A.2.3 National Physical Laboratory (NPL), UK[5]

Calibration item: Average pitch of scale; Calibration method: Diffraction angle measurement; Pitch range, R_p : 350 nm to 50 µm; Expanded uncertainty, U , with coverage factor $k=2$: $U = \pm 1$ nm (for $R_p = 350$ nm to 400 nm), $U = \pm 10$ nm (for $R_p = 400$ nm to 10 µm), $U = \pm 100$ nm (for $R_p = 10$ µm to 50 µm); Traceable directly to National Length standards at NPL.

Calibration item: Selected structures; Calibration method: Metrological AFM with optical interferometry with traceability to National Standards of length; Structure size: Below 300 nm to about 100 nm; Expanded uncertainties, U , with coverage factor $k=2$: $U = \pm Q$ [2 nm, $2,03 \times 10^{-4} L$ nm] where $Q[A, B]$ represents the quadrature sum (square root of the sum of the squares) $(A^2 + B^2)^{1/2}$ and L in nm the total period measured; Traceable directly to National length standards at NPL.

A.2.4 Japan Quality Assurance Organization (JQA), JAPAN[6]

Calibration item: Average pitch of scale; Calibration method: Diffraction angle measurement; Range: 97 nm to 1 000 nm; Expanded uncertainty with coverage factor $k=2$: 0,04 nm; Accreditation: ILAC-MRA Mark.

Calibration item: A pitch length of scale; Calibration method: Laser interferometric optical microscope; Range: 1 µm to 10 mm; Expanded uncertainty: 20 nm to 40 nm with coverage factor 2.

A.3 Reference materials (RMs)

A.3.1 General

Examples of RMs are shown. Some of them may be supplied as CRMs after certification.

A.3.2 Square grid

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

Grid pitch 10 μm , 20 μm and 40 μm accompanied by X and Y line arrays with pitch 1 μm and 5 μm (S5000-S1000-CRM).^[7]

Si test specimen with pitch 10 μm .^[8]

Metal film with typical pitch 1 μm , 25 μm , 100 μm , 1 000 μm on Si substrate.^[9]

A.3.3 Nested square boxes

Pitch range from 0,08 μm to 500 μm nested square boxes accompanied by X and Y line arrays.^[10]

A.3.4 Line array

Line and space, typical pitch 70 nm, 100 nm, 292 nm, 500 nm.^[11]

Pitch range from 0,2 μm to 1 500 μm in the one chip (RM-8820).^[12]

One-dimensional line arrays with pitch range from 6,9 nm to 587 nm (BAM-L200).^[13]

A.3.5 Dot array

2-D dot array with typical pitch 144 nm and 300 nm, aluminium bumps on silicon.^[8]

2-D dot array with pitch 1,16 μm , 1,843 μm and 4,8 μm fabricated as 3-D standard.^[14]

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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