
**Microbeam analysis — Scanning
electron microscopy — Qualification
of the scanning electron microscope
for quantitative measurements**

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Contents

	Page
Foreword.....	v
Introduction.....	vi
1 Scope.....	1
2 Normative references.....	1
3 Terms and definitions.....	1
4 Symbols and abbreviated terms.....	3
5 General principles.....	5
5.1 Condition setting.....	5
5.2 Contrast/brightness setting.....	5
5.3 Sample preparation.....	6
6 Measurement of image sharpness.....	7
7 Measurement of drift and drift-related distortions (imaging repeatability).....	8
7.1 Measurement of image drifts within specified time intervals.....	9
7.1.1 One-minute drift measurement.....	10
7.1.2 Ten-minute drift measurement.....	10
7.1.3 One-hour drift measurement.....	10
7.1.4 Long-term larger than one-hour drift measurement.....	10
7.2 Evaluation of the drift and the drift-related distortions by using image overlay.....	11
7.3 Evaluation of the drift and the drift-related distortions by using cross-correlation function (CCF).....	13
7.3.1 Measurement of the drifts by using the CCF.....	13
7.3.2 Measurement of the distortions by using the CCF.....	15
8 Measurement of electron-beam-induced contamination.....	15
8.1 Cleaning of the sample surface.....	16
8.2 Cleaning of the inner surfaces of the sample chamber.....	16
8.3 Measurement method of the contamination.....	17
8.3.1 Measurement of the height of the contamination growth.....	17
8.3.2 Measurement of relative carbon concentration of the contamination by the X-ray analysis.....	18
8.3.3 Measurement of the surface contamination by the change of SEM signal intensities.....	18
9 Measurement of the image magnification and linearity.....	19
9.1 Measurement of the image magnification.....	20
9.2 Measurement of the image linearity.....	21
10 Measurement of background noise.....	22
10.1 Evaluation methods by using noise profiles and processed images.....	22
10.2 Evaluation methods by calculating numerical image properties.....	28
11 Measurement of the primary electron beam current.....	30
11.1 Ten-minute primary electron beam current measurement.....	30
11.2 Long-term primary electron beam current measurement.....	30
12 Reporting Form.....	32
Annex A (informative) Measurement of image sharpness.....	34
Annex B (informative) Measurement of image drift and distortions caused by unintended motions.....	36
Annex C (informative) Measurement of electron beam-induced contamination.....	47
Annex D (informative) Measurement of the image magnification and linearity.....	53
Annex E (informative) Measurement of the primary electron beam current.....	56

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Foreword

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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 of the voluntary nature of standards, 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 www.iso.org/iso/foreword.html.

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The scanning electron microscope (SEM) is a very versatile instrument, which is widely used in production, development and scientific research across the world. While they are easy to operate and provide results quickly, there are a number of notorious problems, which hinder operating them at their best performance. These are the reasons for lack of excellent repeatability in SEM imaging and measurements. The most bothersome ones among these are unintended motions of the sample stage and the primary electron beam, geometry distortions, wrong image magnification, image blur (lack of sharp focus), noise and electron beam-induced contamination. Quantification of these essential performance parameters is very useful to ensure that all SEMs perform at manufacturers specifications and at users' own purpose. Quantified knowledge helps in the evaluation of measurement uncertainties, and necessary repairs.

This document pertains to measurement methods for the following SEM performance parameters:

- Image sharpness (spatial resolution, primary electron beam focusing ability).
- Drifts (the sample stage, the electron beam and the electron-optical column).
- Cleanliness (lack of beam-induced contamination).
- Image magnification and linearity (both in X and Y directions).
- Background noise.
- Primary electron beam current.

These parameters will also be influenced by the SEM conditions such as the lifetime of source (emitter conditions), lifetime of liner tube and apertures (contamination of the electron optical parts), time and intensity of last cleaning of vacuum chamber by the plasma cleaning or Ultra Violet irradiation, the sample preparation and final surface cleaning.

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Microbeam analysis — Scanning electron microscopy — Qualification of the scanning electron microscope for quantitative measurements

1 Scope

This document describes methods to qualify the scanning electron microscope with the digital imaging system for quantitative and qualitative SEM measurements by evaluating essential scanning electron microscope performance parameters to maintain the performance after installation of the instruments. The items and evaluating methods of the performance parameters are selected by users for their own purposes.

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 16700:2016, *Microbeam analysis — Scanning electron microscopy — Guidelines for calibrating image magnification*

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

ISO 22493, *Microbeam analysis — Scanning electron microscopy — Vocabulary*

ISO/TS 24597:2011, *Microbeam analysis — Scanning electron microscopy — Methods of evaluating image sharpness*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 22493 and the following apply.

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

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

— IEC Electropedia: available at <http://www.electropedia.org/>

3.1

scanning electron microscope SEM

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

[SOURCE: ISO 16700, 3.1]

3.2

image

two-dimensional representation of the specimen surface generated by SEM

[SOURCE: ISO 16700, 3.2]

3.3

image magnification

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

[SOURCE: ISO 16700, 3.3]

3.4

scale marker

line/generated line (intervals) on the *image* (3.2) representing a designated actual length in the specimen

[SOURCE: ISO 16700, 3.4]

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

[SOURCE: ISO Guide 30:2015, 2.1.1, modified — Note 1 to entry to Note 4 to entry are omitted.]

3.6

certified reference material

CRM

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

[SOURCE: ISO Guide 30:2015, 2.1.2, modified — Note 1 to entry to Note 4 to entry are omitted.]

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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* (3.5) or a CRM

[SOURCE: ISO 16700, 3.7]

3.8

accelerating voltage

absolute acceleration potential V_a [V] of the final anode to the electron emitter

Note 1 to entry: For the electron charge q_e [C], the accelerated electron will obtain the energy $|q_e|V_a$ [J] = V_a [eV] $\equiv E_L$ and enter the sample with this energy provided that the initial energy E_i from the emitter is negligible. The “landing energy” to the specimen means this energy E_L , typically expressed in the unit eV or keV.

Refer to [Clause 4](#) concerning eV.

4 Symbols and abbreviated terms

$A_{ACF,1}, A_{CCF,n}$	areas of the binarized pictures $F_{BAC}(I_1)$ and $F_{BCC}(I_1, I_n)$ respectively, typically expressed in pixel
ACF	auto-correlation function
CCF	cross-correlation function
D_{Hn}, D_{Vn}	drift quantities of the n-th image I_n ($n=1, 2, \dots$) for the horizontal (H) and vertical (V) directions respectively, typically expressed in pixel or in nm
D_X, D_Y	displacements for X and Y directions respectively from the origin, typically expressed in nm $\max D_X , \max D_Y $ mean the largest absolute values of displacements from the origin in X and Y directions respectively
D_0	distance $D_0 = (D_X^2 + D_Y^2)^{1/2}$ from the initial position (X_0, Y_0) which is regarded as the origin, typically expressed in nm $\max D_0$ - the largest value of the distance D_0
d	pitch length of the RM or the CRM, typically expressed in nm
d_S	measured mean pitch length, typically expressed in nm
d_{SA}	averaged value of measured d_S , typically expressed in nm
CG	contrast to gradient method for evaluating image sharpness
DR	derivative method for evaluating image sharpness
FT	Fourier transform method for evaluating image sharpness
eV	electronvolt, a unit of energy equal to approximately 1.6×10^{-19} joules (J). By definition, it is the amount of energy gained (or lost) by the charge of a single electron moving across an electric potential difference of 1 volt
$F_{AC}(I_1)$	auto-correlation function of the image I_1
$F_{CC}(I_1, I_n)$	cross-correlation function for the initial image I_1 and n-th image I_n ($n=2, 3, \dots$) in the measurements
$F_{BAC}(I_1)$	binarized picture of the auto-correlation function $F_{AC}(I_1)$ of the initial image I_1 by using a thresholding level T_B
$F_{BCC}(I_1, I_n)$	binarized picture of the cross-correlation function $F_{CC}(I_1, I_n)$ for the initial image I_1 and the n-th image I_n ($n=2, 3, \dots$) by using a thresholding level T_B
HFW	horizontal field width
H_{P1}, V_{P1}	horizontal (H) and vertical (V) peak positions of the auto-correlation function $F_{AC}(I_1)$ respectively
H_{Pn}, V_{Pn}	horizontal (H) and vertical (V) peak positions of the cross-correlation function $F_{CC}(I_1, I_n)$ respectively

I_{BG}	background noise image
I_{FB}	flat image whose signal intensity of the element (i, j) is the mean value S_{MEAN} of the background noise image I_{BG}
I_{PB}	processed images obtained from the background noise images I_{BG} , for example, by a method of contrast enhancement
I_{OS}	original secondary electron (SE) or backscattered electron (BE) scanning image
$I(i, j)$	signal intensity of the element (i, j) of the image I , where i and j mean horizontal and vertical numbers of the element respectively measured from the initial element $(1, 1)$
$I_{ref}(i, j)$	signal intensity of the element (i, j) of the reference image I_{ref} which is set to the flat image I_{FB} when calculating the peak signal-to-noise ratio S_{PSNR} $\max[I_{ref}(i, j)]$ means the maximum value of the given n_{IM} -bit imaging mode. If $n_{IM} = 8$ -bit imaging mode, then $\max[I_{ref}(i, j)] = 255$
$I_{test}(i, j)$	signal intensity of the element (i, j) of the test image I_{test} which is set to the background noise image I_{BG} when calculating the peak signal-to-noise ratio S_{PSNR}
$I_{refS}(i, j)$	signal intensity of the element (i, j) of the reference image I_{refS} which is obtained from the test image $I_{testS}(i, j)$ by applying a suitable image filter to reduce the image noise
$I_{testS}(i, j)$	signal intensity of the element (i, j) of the test image I_{testS} which is usually not the background noise image I_{BG} but actual SE or BE scanning image
I_p	primary electron beam current (probe current)
kV	kilovolt
$k_{A,n}$	ratio of the area $A_{CCF,n}$ to the area $A_{ACF,1}$ ($k_{A,n} = A_{CCF,n} / A_{ACF,1}$)
L	image size (total pixels of the image area), typically expressed in pixel such as $L_H \times L_V$
L_H, L_V	horizontal (H) and vertical (V) image sizes (lengths) respectively, typically expressed in pixel
L_p	pixel size, typically expressed in nm
L_{pHA}	horizontal (H) line profile which is obtained vertically averaged for specified band areas from the background noise image
L_{pVA}	vertical (V) line profile which is obtained horizontally averaged for specified band areas from the background noise image
l_S	the total measured length by a scaler on the screen or the photograph ($l_S = n_p \cdot d_S$)
M	image magnification for setting
N_M	number of measurements for beam current
N_{MD}	number of measurements for image drift
N_{MM}	number of measurements for image magnification

N_{MS}	number of measurements for image sharpness
n_p	total number of pitches for measurement
R_L	image sharpness, typically expressed in nm
R_{PX}	image sharpness, typically expressed in pixel
S_{MAX}	maximum value of intensities of pixels in an image
S_{MEAN}	mean value of intensities of pixels in an image
S_{MIN}	minimum value of intensities of pixels in an image
S_{PSNR}	peak signal-to-noise ratio
S_{STD}	standard deviation of intensities of pixels in an image
V_a	accelerating voltage
WD	working distance

5 General principles

The best performance of any SEM is at some optimized set of instrument settings; therefore, throughout this document for the various assessments those imaging parameters and instrument settings should be used if those are specified by the SEM's manufacturer for achieving the best performance. These basic principles are useful for users' own purpose in many cases.

5.1 Condition setting

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Some SEMs have only one accelerating voltage in these specifications; others may have more (e.g. 15 kV and 1 kV). All the assessments should be performed at all specified accelerating voltages and magnifications if those parameters and settings are applicable to user's purposes and evaluations. If optimization of SEM-based measurements requires different parameters (accelerating voltage, beam current, etc.) for users' own purpose, then for these all the assessments should be performed and the results recorded in the report.

Beyond setting the magnification, accelerating voltage, beam current, all pertinent parameters to the values specified by the instrument manufacturer for proving the best resolution performance, it is important to set the focus (astigmatism), contrast, and brightness to their SEM-specific, best settings for taking images.

5.2 Contrast/brightness setting

Only images with properly set contrast and brightness should be used for the various measurements and quantitative SEM assessments. The contrast and the brightness must be set so that all pixels have grey-scale levels that never reach the lowest (dark, under-saturation) or the highest (bright, over-saturation) level. This is important to make sure that no information is lost by setting contrast and brightness values incorrectly.

If the system has the signal monitor or can generate the histogram for the relative signal range [0,1] in the acquisition of SEM image, verify that the signals are within the range [0.2, 0.8] approximately.

In 8-bit imaging mode, in properly set images the intensities of the image pixels vary in between 0 and 255 grey levels. In 16-bit imaging mode the intensities of the image pixels vary in between 0 and 65535 grey levels.

Figure 1 shows examples of secondary electron (SE) images taken at accelerating voltage 5 keV, 19,5 µm HFW. Contrast and brightness are properly set a) and wrong b), c), d) and e). Figure 2 shows their corresponding histograms.

5.3 Sample preparation

Concerning the samples, there will not be the perfect or the almighty sample which are applicable to many evaluation items. In general, the best samples are different for the measurements of image sharpness, drift and drift-related distortions, electron beam induced contamination, image magnification and linearity. Select the ideal sample which is suitable for the required evaluation accordingly.

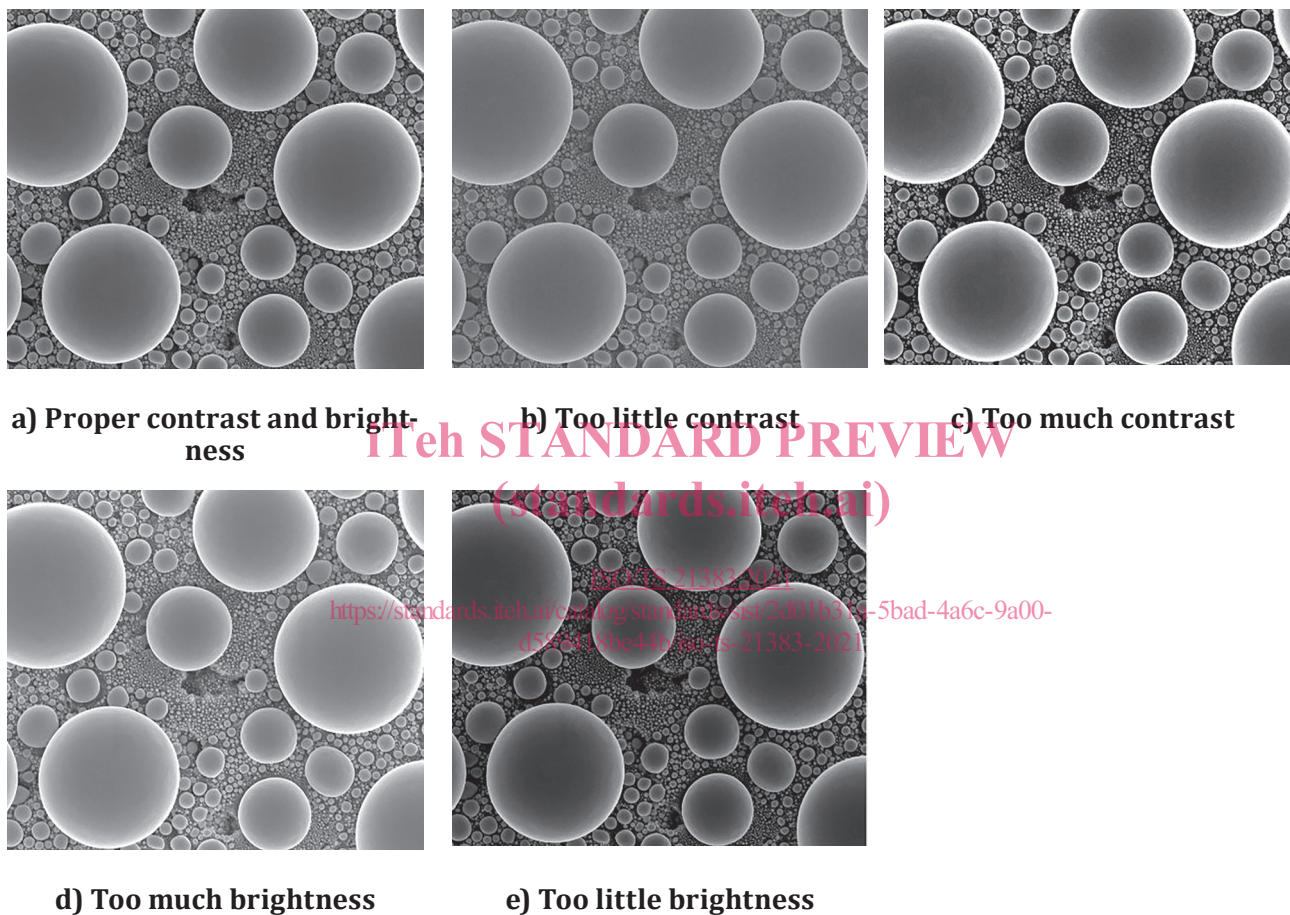
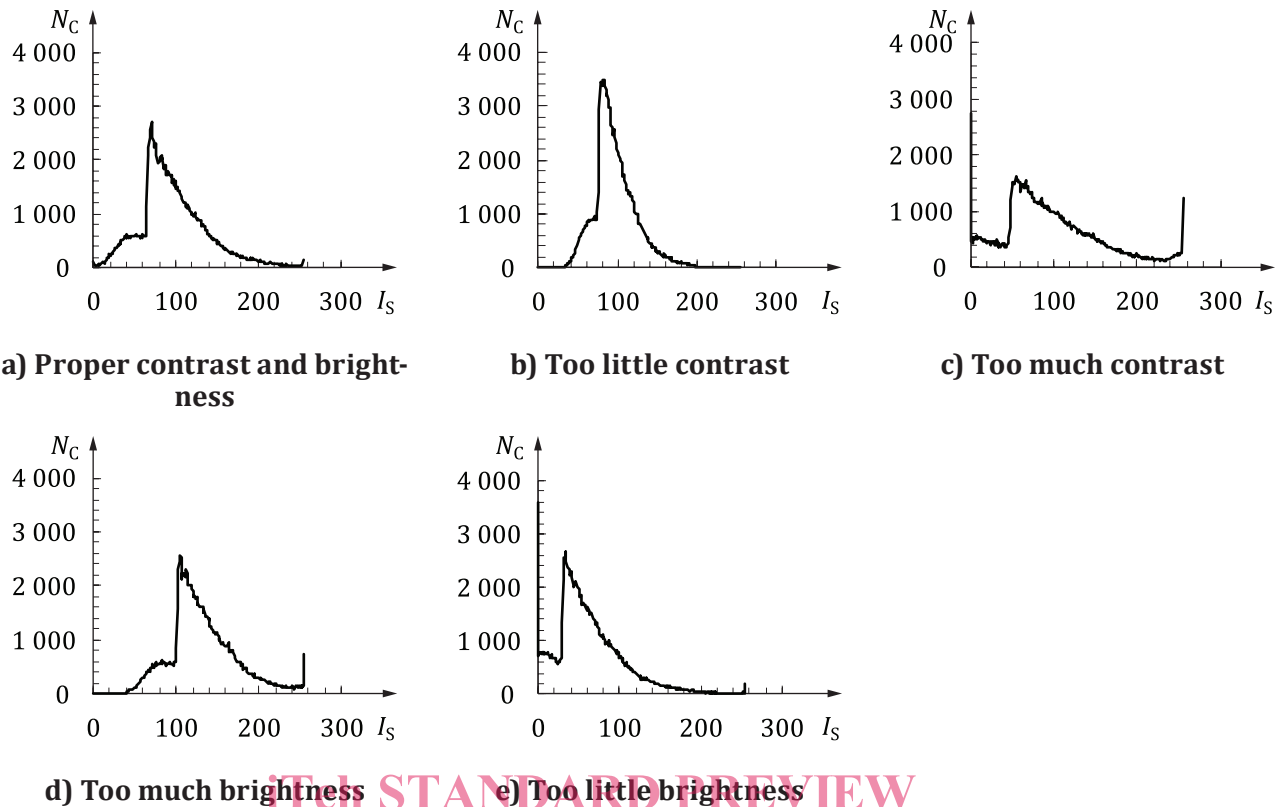


Figure 1 — Examples of SE images for various contrast and brightness setting

**Key** I_s signal intensity for 8-bit imaging mode N_c number of counts

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6 Measurement of image sharpness

The definition and the explanation of the term “image sharpness” are described in ISO TS 24597 for the SEM. Image sharpness is strongly related to the focusing ability of the SEM in forming the primary electron beam. It is one of most widely used, but certainly not sufficient performance parameter. However, it is very useful to know the image sharpness because the algorithms do not depend on the human sense and give quantitative results by using the procedures described below.

On the other hand, the term “Lateral resolution” or “Spatial resolution” are not defined strictly in SEM even though these terms are popular in the surface chemical analysis.

NOTE Refer to ISO 18115-1:2013 for terms used in spectroscopy^[1].

Even if various SEM manufacturers use these terms, the notion of resolution is not established scientifically, it is sample-and method-dependent, and there is no accurate way of measuring it today. The focusing ability is related to the size and shape of the primary electron beam at the surface of the sample. Its measurement is also very difficult, especially for sub-nanometre focuses, i.e., beam sizes. Furthermore, these values are not related to beam focusing only, but to interaction volume, stability of probe scanning and external disturbances as well.

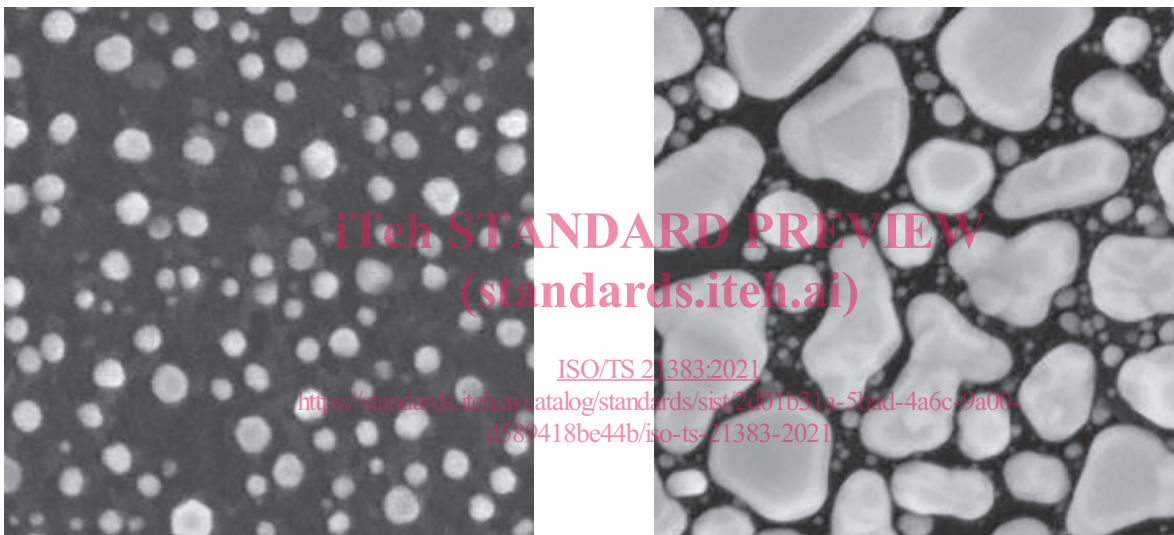
To acquire the images for the evaluation of the image sharpness, refer to the Clause 4 “Steps for acquisition of an SEM image” of ISO TS 24597. “4.1 General”, “4.2 Specimen”, “4.4 Selection of the field of view” and “4.7 Contrast-to-noise ratio of the image” will be useful information. The structure of the sample should not be “periodic mesh” or “line and space” because some specific features or frequencies are emphasized in the signal analysis. The samples as shown in [Figure 3 a\)](#) is typical and appropriate

because the particles with different diameters are randomly distributed, and their surface are flat and edges are sharp.

Set the focus and astigmatism to their best, the magnification, accelerating voltage, beam current, working distance to the values specified by the instrument manufacturer for proving the best image sharpness performance, and take several images.

To evaluate the SEM image sharpness performance, follow the procedure in ISO/TS 24597:2011. Select the evaluation method from the 3-methods DR (Derivative) method, FT (Fourier transform) method and CG (Contrast-to-gradient) method. For one image, plural methods can be used if necessary. Valuate that the obtained results are allowable or not for the quantitative measurements. Report evaluated results with the evaluation methods and the valuation.

Figure 3 shows the examples of the selected SEM images with the image size $L=512\times 512$ for the evaluation of the image sharpness R_{pX} [pixel] or R_L [nm]. The obtained values of image sharpness R_L are 1,9 nm for Figure 3 a) and 3,3 – 4,2 nm for Figure 3 b). The example of the evaluation process for these images is shown in Table A.1.



a) Accelerating Voltage $V_a = 15$ kV, beam current $I_p = 43$ pA, 265 nm HFW.

b) Accelerating Voltage $V_a = 1$ kV, beam current $I_p = 43$ pA, 657 nm HFW.

Figure 3 — Selected SEM images for the evaluation of image sharpness.
Sample: Evaporated gold on carbon.

See Clause 12 and Annex A for further pertinent information.

7 Measurement of drift and drift-related distortions (imaging repeatability)

Unintended motions make the primary electron beam land on wrong, unintended locations on the sample, which results in poor repeatability and/or distorted, blurry images, especially at high magnifications. These typically arise from mechanical and acoustical impacts, temperature variation of the room and the cooling water, hysteresis, adverse external electromagnetic fields, and from the noise in various circuits of the SEM. In the SEM with high working rate for many years, charging of contaminated apertures and inner walls of the liner tube for electron beam could be possible reason for unintended disturbances.

So, it is very useful to know the drifts and the drift related distortions quantitatively and qualitatively by applying the procedures described below because these properties tell us the upper limit of the significant measurements.

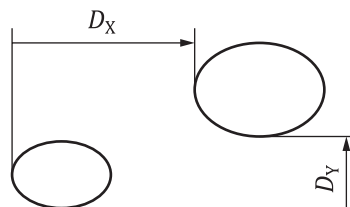
Modern SEMs can have close to 1 nanometre image sharpness, so even very small unintended motions can interfere or easily ruin the imaging and measurement performance of the SEM. Both the sample stage with the sample and the primary electron beam make unintended motions and they result in a combined error in the actual location where the electron beam-sample interaction takes place.

It is important to make sure that the geometry distortions of the SEM are sufficiently small for acquiring images and for carrying out measurements within requirements. These, depending on the task at hand, may be diverse, e.g. for high-quality, fine image sharpness imaging are a lot more stringent than for simple microanalysis. Depending on the intended use, the drift-related performance should be evaluated for one-minute (typical secondary electron image acquisition time), ten-minute (analytical acquisition), and for one and many hours-long time periods.

7.1 Measurement of image drifts within specified time intervals.

To determine the drift performance, set the focus to its best, magnification, accelerating voltage, beam current, to the values specified by the instrument manufacturer for providing the best image sharpness performance, and without intentionally changing the sample location, perform at least the 1- and 10-minute assessments, and if it is relevant for the task at hand, add further, longer ones. For longer time drift measurements, the magnification shall be lowered to keep the reference pattern always in the central 80 % of the image area (refer to ISO 16700). The guiding principle is to measure the drift for as long periods of time as the time of the measurement or procedure carried out by the SEM. Evaluate that the measured drifts are allowable or not in the quantitative SEM measurements. Report all results as image drift performance values and the valuation.

When drift performance is measured by watching the displacements (movements) D_X and D_Y of a marked object (or a particle) in the recorded image, use the determined left end or the right end of the object for the x - direction, and use the determined bottom end or the top end of the object for the y-direction as shown in [Figure 4](#). This is the reason why the definition of the displacement become unclear under the drift-related distortions. For the drift measurement, the initial position (X_0, Y_0) of the object should be near the centre of the field (the display). The initial position (X_0, Y_0) is regarded as the origin, and the distance D_0 from the origin is defined as $D_0 = (D_X^2 + D_Y^2)^{1/2}$.



Key

- D_X displacement for X direction, expressed in nm
- D_Y displacement for Y direction, expressed in nm

Figure 4 — Measurement method of the displacement

After the installation of the SEM, the drifts may be enlarged owing to the increased movements of the floor or the increased fluctuation of the external electromagnetic fields. Then, the obtained results do not reflect original SEM performances but the condition of the environments, or the ability of the isolation and the shielding. Measure the movements of the floor or the fluctuation of the external field by the methods SEM manufacturer have performed if possible. Consider the source of the change, and report the estimated results. Improper sample preparation, such as the sample surface with non-conductor and the insufficiently grounded samples, will also cause the severe drifts.

The acquired images in the drift measurement are also utilized for measurement of drift-related distortions (imaging repeatability). Refer to [7.2](#) and [7.3](#).