
**Nanotechnologies — 3D image
reconstruction of rod-supported nano-
objects using transmission electron
microscopy**

*Nanotechnologies — Reconstruction d'images 3D de nano-objets
soutenus par des tiges à l'aide de la microscopie électronique à
transmission*

iTeH STANDARD
PREVIEW
(standards.iteh.ai)

[ISO TS 22292:2021](https://standards.iteh.ai/catalog/standards/sist/4e6ab78a-3d5b-4380-8241-db576a34420c/iso-ts-22292-2021)

[https://standards.iteh.ai/catalog/standards/sist/4e6ab78a-
3d5b-4380-8241-db576a34420c/iso-ts-22292-2021](https://standards.iteh.ai/catalog/standards/sist/4e6ab78a-3d5b-4380-8241-db576a34420c/iso-ts-22292-2021)



**iTeh STANDARD
PREVIEW
(standards.iteh.ai)**

ISO TS 22292:2021

<https://standards.iteh.ai/catalog/standards/sist/4e6ab78a-3d5b-4380-8241-db576a34420c/iso-ts-22292-2021>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2021

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

	Page
Foreword	v
Introduction	vi
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
3.1 Nanotechnology-related terms.....	2
3.2 Instrument-related terms.....	2
3.3 Measurement-related terms.....	3
4 Sample considerations	4
4.1 General.....	4
4.2 Choice of sample rod diameter.....	4
5 Instrument factors	4
5.1 Microscope set up.....	4
5.1.1 General.....	4
5.1.2 Acceleration voltage.....	5
5.1.3 Convergence semi-angle.....	5
5.1.4 Collection angle.....	5
5.1.5 Microscope magnification.....	5
5.1.6 Number of pixels of the detector.....	5
5.1.7 Image acquisition time.....	6
5.2 Microscope calibration.....	6
6 Image capture (data acquisition)	7
6.1 General.....	7
6.2 Procedure.....	7
7 Data alignment and volume reconstruction	10
7.1 General.....	10
7.2 Procedure.....	10
8 Reconstructed volume evaluation and data analysis	10
8.1 General.....	10
8.2 Identification of nanoparticles and 3D volume.....	10
8.3 Thresholding for measurand extraction.....	11
9 Expression of results	13
9.1 Extracting parameters for each well-separated nano-object.....	13
9.2 Measurement uncertainty.....	14
9.3 Sources of errors.....	14
9.3.1 Error arising from sample that is not representative of the object of interest.....	14
9.3.2 Acquisition of 2D projected images.....	14
9.3.3 Instrument calibration.....	16
9.3.4 Alignment of the projected images.....	16
9.3.5 Reconstruction of the 3D volume.....	16
9.3.6 Discrete representation of the objects (nanoparticles) in 3D.....	16
9.3.7 Interpretation of the obtained measurands.....	17
9.3.8 Limited number of observed objects (nanoparticles).....	17
10 Test report	19
Annex A (informative) Sample preparation	21
Annex B (informative) STEM set up	28
Annex C (normative) Tomography reconstruction and visualization software packages	30
Annex D (informative) Microscope data collection parameters	32

Annex E (informative) Case study: Metal nanoparticle, ILC results	33
Annex F (informative) Case study — Organic nanoparticles — Sample preparation and use	38
Annex G (informative) Particle distortions arising from FBP and SIRT reconstruction methods	39
Annex H (informative) Uncertainty budget	41
Bibliography	43

**iTeh STANDARD
PREVIEW
(standards.iteh.ai)**

[ISO TS 22292:2021](https://standards.iteh.ai/catalog/standards/sist/4e6ab78a-3d5b-4380-8241-db576a34420c/iso-ts-22292-2021)

<https://standards.iteh.ai/catalog/standards/sist/4e6ab78a-3d5b-4380-8241-db576a34420c/iso-ts-22292-2021>

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 jointly by Technical Committee ISO/TC 229, *Nanotechnologies*, and Technical Committee IEC/TC 113, *Nanotechnology for electrotechnical products and systems*.

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.

ISO/TS 22292:2021
<https://www.iso.org/standard/68342.html>
 3d5b-4380-8241-db576a34420c/iso-ts-22292-2021

Introduction

Electron tomography, in transmission electron microscope (TEM), has impact on nanotechnology and nanomaterial metrology like that of computer tomography in medicine. For example, industries using nanotechnologies have requirements to verify materials, processes and products. Quantitative measurement at the nanoscale, including three-dimensional (3D) image reconstruction of nano-objects using TEM, responds to this need.

TEM, a two-dimensional (2D) imaging instrument, can provide 2D projection images of materials at the nanoscale, in the length range from below 1 nm to above 100 nm. From multiple 2D TEM images collected at suitable tilt increments, the 3D shape, size and volume parameters can be determined. This document describes sample preparation, instrumentation setup, data acquisition and processing for 3D image reconstruction of nano-objects using TEM, from which dimensional parameter values can be determined and interpreted. Variation in methodology for use with scanning transmission electron microscopy (STEM) is included in an informative annex.

The method described herein is limited to samples dispersed on or within an electron-transparent rod-shaped support. This method is particularly useful when the detailed shape of a limited number of objects, such as nanoparticles, is sought. For example, when 2D measurements yield a non-uniform distribution of objects, 3D image reconstruction can be used applied to study a small number of the objects in more detail. A variant of sample preparation is described that allows 3D reconstruction to be used in conjunction with 2D TEM analysis of a sample area of interest, such as an area containing outliers.

Potential applications for 3D image reconstruction of nano-objects using TEM are broad and might include validation of metrological artefacts, such as polystyrene latex nanoparticles, and site-specific analysis of interfaces buried within devices, and measurement of individual objects such as nanoparticles. The method might also be utilized to obtain detailed shape of non-symmetric nano-objects such as nanorods and nanocrystals.

Other applications include calibration for a variety of nanoscale characterization tools, particularly nanoscale characterization instruments and artefacts, to ensure that they are applied in a consistent way.

Case studies are provided in informative annexes, including variations of sample preparation, data acquisition, alignment and reconstruction methods. It is noted that placing of alternative data acquisition, alignment and reconstruction methods in annexes does not imply that a method is inferior to the one described in the main body of the document. Conversely, such might be the subject of future revisions of this document. However, the process, from sample preparation on a rod-shaped support to extraction of measurands, has been tested in accordance with the steps described in this document and tested on samples described in the annexes.

[Figure 1](#) summarizes the procedure steps in this document. Normative aspects are highlighted in red. Informative aspects are highlighted in blue and appear in annexes. Additional annexes not listed in this figure are also included.

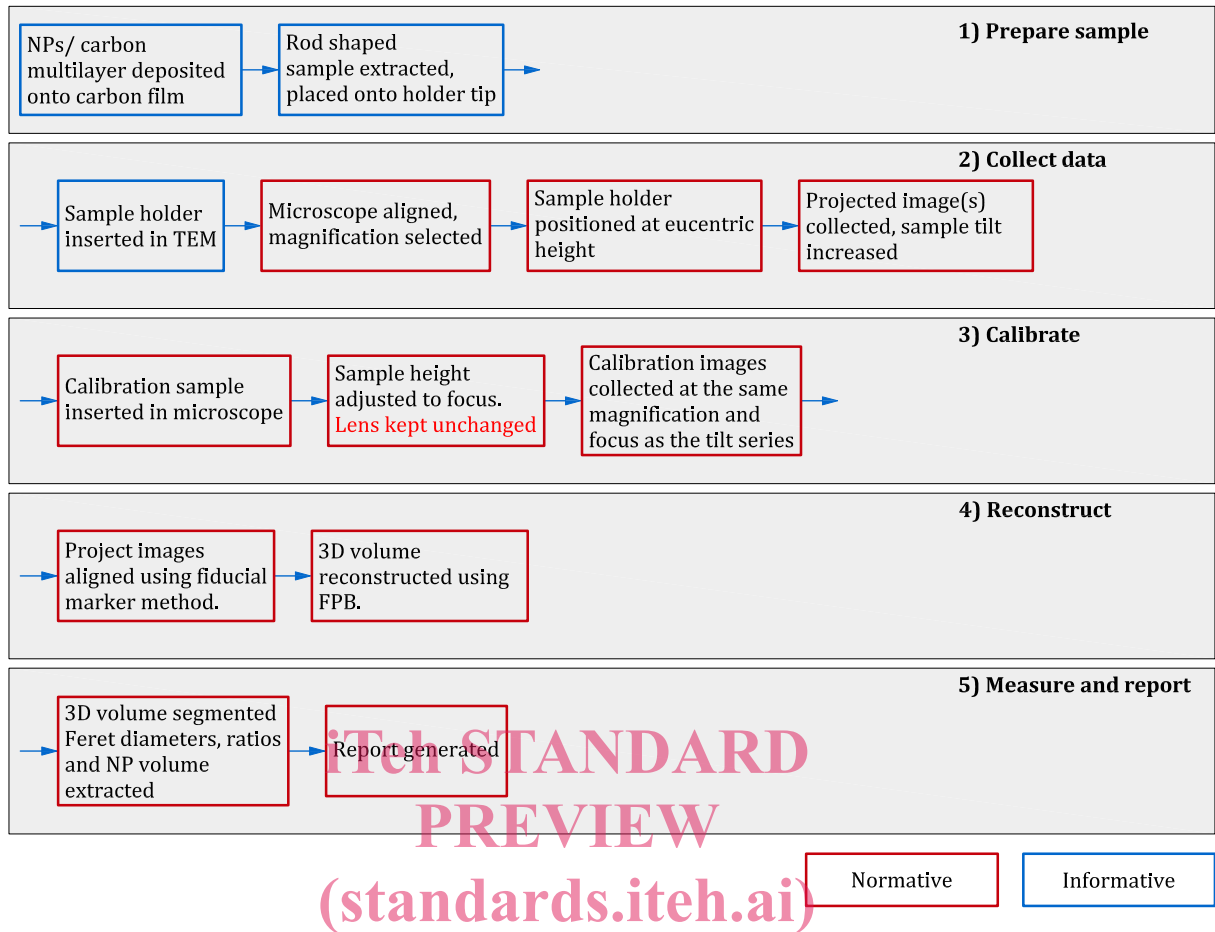


Figure 1 — Procedure steps

ISO TS 22292:2021

<https://standards.iteh.ai/catalog/standards/sist/4e6ab78a-3d5b-4380-8241-db576a34420c/iso-ts-22292-2021>

**iTeh STANDARD
PREVIEW
(standards.iteh.ai)**

[ISO TS 22292:2021](#)

<https://standards.iteh.ai/catalog/standards/sist/4e6ab78a-3d5b-4380-8241-db576a34420c/iso-ts-22292-2021>

Nanotechnologies — 3D image reconstruction of rod-supported nano-objects using transmission electron microscopy

1 Scope

This document provides guidance for sample preparation, data acquisition by transmission electron microscopy, data processing, and three-dimensional image reconstruction to measure size and shape parameters of nano-objects on rod-shaped supports. The method is applicable to samples dispersed on or within an electron-transparent rod-shaped support.

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/IEC Guide 99:2007, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

ISO/TR 945-2:2011, *Microstructure of cast irons — Part 2: Graphite classification by image analysis*

ISO/TS 10797:2012, *Nanotechnologies — Characterization of single-wall carbon nanotubes using transmission electron microscopy*

ISO 21363, *Nanotechnologies — Measurements of particle size and shape distributions by transmission electron microscopy*

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

ISO 26824:2013, *Particle characterization of particulate systems — Vocabulary*

ISO/TS 80004-1:2015, *Nanotechnologies — Vocabulary — Part 1: Core terms*

ISO/TS 80004-2:2015, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

ISO/TS 80004-6:2021, *Nanotechnologies — Vocabulary — Part 6: Nano-object characterization*

ISO/TS 80004-8:2020, *Nanotechnologies — Vocabulary — Part 8: Nanomanufacturing processes*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/IEC Guide 99:2007, ISO/TR 945-2:2011, ISO/TS 10797:2012, ISO/TS 24597:2011, ISO 26824:2013, ISO/TS 80004-1:2015, ISO/TS 80004-2:2015, ISO/TS 80004-6:2021, ISO/TS 80004-8:2020 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 Nanotechnology-related terms

3.1.1

nanoscale

length range approximately from 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from larger sizes are predominantly exhibited in this length range.

[SOURCE: ISO/TS 80004-1:2015, 2.1]

3.1.2

nanomaterial

material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale

Note 1 to entry: This generic term is inclusive of nano-object and nanostructured material.

[SOURCE: ISO/TS 80004-1:2015, 2.4]

3.1.3

nano-object

discrete piece of material with one, two or three external dimensions in the nanoscale

Note 1 to entry: The second and third external dimensions are orthogonal to the first dimension and to each other.

[SOURCE: ISO/TS 80004-1:2015, 2.5]

3.1.4

nanoparticle

nano-object with all external dimensions in the nanoscale where the lengths of the longest and the shortest axes of the nano-object do not differ significantly

Note 1 to entry: If the dimensions differ significantly (typically by more than three times), terms such as nanofibre or nanoplate may be preferred to the term nanoparticle.

[SOURCE: ISO/TS 80004-2:2015, 4.4, modified — the abbreviation "NP" has been deleted.]

3.2 Instrument-related terms

3.2.1

scanning electron microscopy

SEM

method that examines and analyses the physical information (such as secondary electron, backscattered electron, absorbed electron and X-ray radiation) obtained by generating electron beams and scanning the surface of the sample in order to determine the structure, composition and topography of the sample

[SOURCE: ISO/TS 80004-6:2021, 4.5.5]

3.2.2

scanning transmission electron microscopy

STEM

method that produces magnified images or diffraction patterns of the sample by a finely focused electron beam, scanned over the surface and which passes through the sample and interacts with it

Note 1 to entry: Typically uses an electron beam with a diameter of less than 1 nm.

Note 2 to entry: Provides high-resolution imaging of the inner microstructure and the surface of a thin sample (or small particles), as well as the possibility of chemical and structural characterization of micrometre and sub-micrometre domains through evaluation of the X-ray spectra and the electron diffraction pattern.

[SOURCE: ISO/TS 80004-6:2013, 4.5.7]

3.2.3

transmission electron microscope

TEM

method that produces magnified images or diffraction patterns of the sample by an electron beam which passes through the sample and interacts with it

[SOURCE: ISO/TS 80004-6:2021, 4.5.6]

3.2.4

focused ion beam instrument

FIBI

instrument and method that allows to fabricate objects at nanoscale using a focused ion beam (FIB), typically Gallium, and observe the fabricated area using an SEM column located in the same instrument chamber

Note 1 to entry: For FIB lithography, refer to ISO/TS 80004-8:2020, 7.1.9.

Note 2 to entry: For FIB focused ion-beam deposition refer to ISO/TS 80004-8:2020, 7.2.12.

3.2.5

dual beam instrument

DBI

instrument combining the instruments used in the *SEM* (3.2.1) and *FIB* (3.2.4) methods

3.3 Measurement-related terms

3.3.1

Feret diameter

distance between two parallel tangents on opposite sides of the image of a particle

[SOURCE: ISO 26824:2013, 8.6]

[ISO TS 22292:2021](https://standards.iteh.ai/catalog/standards/sist/4e6ab78a-3d5b-4380-8241-db576a34420c/iso-ts-22292-2021)

<https://standards.iteh.ai/catalog/standards/sist/4e6ab78a-3d5b-4380-8241-db576a34420c/iso-ts-22292-2021>

3.3.2

maximum Feret diameter

maximum value of Feret diameter of an object, whatever its orientation

[SOURCE: ISO/TR 945-2:2011, 2.1]

3.3.3

minimum Feret diameter

minimum value of Feret diameter of an object whatever its orientation

[SOURCE: ISO 21363:2020, 3.4.5]

3.3.4

pixel

smallest non-divisible image-forming unit on a digitized TEM image

[SOURCE: ISO/TS 24597:2011, 3.1, modified — the abbreviation "TEM" has been changed to "SEM".]

3.3.5

measurand

quantity intended to be measured

[SOURCE: ISO/IEC Guide 99: 2007, 2.3]

4 Sample considerations

4.1 General

[Clause 4](#) discusses physical properties of the sample rod. For methods that can be applied to prepare the sample rod, see [Annexes A, E and F](#).

4.2 Choice of sample rod diameter

Sample rod diameter considerations apply to both TEM and scanning TEM (see [Annex B](#)) as follows:

- a) The sample shall be rod-shaped with cross section shape no more than 50 % different from circular cross section (1:1,5 ratio of axis length for elliptical sample rod cross section);

NOTE 1 Rectangular cross-section that does not exceed the 1:1,5 aspect ratio is acceptable.

- b) The sample rod shall be made of low atomic number material such as carbon;
- c) The sample rod diameter shall be less than one inelastic mean free path for the incident electron energy in the TEM chosen. For example, at 300 keV incident electron energy a carbon rod with less than 250 nm diameter shall be utilized;
- d) Sample rod diameter that exceeds twice the inelastic mean free path shall be avoided to reduce the effect of plural electron scattering in the sample rod and the associated loss of spatial resolution^[5] [\[6\]](#)^[7];
- e) The effect of geometrical broadening of the electron beam shall be kept at a small fraction of desired resolution of the final 3D reconstructed volume. The geometrical broadening can be estimated from instrument convergence semi-angle and collection semi-angle ^[4];
- f) To ensure adequate image resolution, the sample rod diameter shall not exceed two times the depth of focus ^[15].

NOTE 2 Typical imaging conditions in conventional TEM mode at 300 keV electron energy allow for about 250 nm sample rod diameter.

NOTE 3 The choice of imaging parameters for TEM and STEM tomography, depth focus, and rod diameter is described in detail in Reference [\[15\]](#).

5 Instrument factors

5.1 Microscope set up

5.1.1 General

This clause provides guidance on conventional parallel beam transmission electron microscope (TEM) instrumentation set up for data acquisition. For scanning TEM (STEM) instrumentation set up, see [Annex B](#).

The critical set up parameters for TEM data acquisition are:

- a) acceleration voltage (see [5.1.2](#));
- b) convergence semi-angle (see [5.1.3](#));
- c) collection angle (see [5.1.4](#));
- d) microscope magnification (see [5.1.5](#));
- e) number of pixels of the detector (see [5.1.6](#));

f) image acquisition time (see 5.1.7).

5.1.2 Acceleration voltage

The acceleration voltage shall be selected as described in A.2.4 d) on sample thickness. Typically, 300 kV or 200 kV should be used.

Using the maximum voltage available on a particular TEM is preferred. Using the maximum available voltage means maximum allowable sample rod diameter and maximum depth of focus. For example, using 300 kV rather than 200 kV allows for 250 nm diameter carbon rod rather than 200 nm diameter rod at 200 kV.

5.1.3 Convergence semi-angle

The convergence semi-angle shall be selected so that the illumination at the sample plane is uniform over the imaged area of the sample. Furthermore, the illumination uniformity has to be such that the apparent sample focus does not visibly change over the observed area.

NOTE The illumination uniformity can be verified by performing an intensity profile across the image (e.g. diagonally from corner to corner of the image). The uniformity of the defocus value is typically not a concern and it can be verified by performing a fast Fourier transform (FFT) of sub areas of the image. For example, a 2048×2048 image can be divided into 256×256 pixels regions of interest. The image composed of absolute value of FFT can be compared among the various regions of interest to ensure that the FFT does not vary too much from region to region. An FFT that has same numbers of circular rings over an arbitrary sub-field of view is an indication that illumination is sufficiently uniform.

5.1.4 Collection angle

In conventional TEM mode the collection angle is selected by the objective aperture size. The collection angle is determined as the square root of the sum of squares of the convergence angle and acceptance angle of the objective aperture. In practise the convergence angle in TEM mode is much smaller than the acceptance angle of the objective aperture. Therefore, the collection angle is determined by the objective aperture acceptance angle alone.

The main criteria for collection angle is the avoidance of diffraction contrast while maximizing the contrast in the image. The contrast increases with decreasing collection angle,^{[9][10]} but at the same time, the diffraction contrast increases with decreasing collection angle. The presence of diffraction contrast could make the data unsuitable for 3D reconstruction.^{[9][10]} The number of counts collected by the detector decreases with decreasing collection angle leading to increase in acquisition time.

5.1.5 Microscope magnification

Microscope magnification and detector pixel size are critical parameters to ensure correct sampling of the object for 3D reconstruction. The higher the desired number sampling, the lower the effect of the detector point spread function. At the same time high sampling, i.e. high microscope magnification and small pixel size, decreases the field of view that can be acquired without region stitching by subsequent offset acquisitions. High magnification also decreases the number of counts per pixel at a given beam current density and acquisition time. Typically, the magnification is chosen so that the desired projected image resolution is sampled by 5 pixels or more^[11].

EXAMPLE If a desired projected image resolution is 1 nm, the pixel size is chosen to be about 0,2 nm. A 2 048 pixel \times 2 048 pixel camera can then cover a 410 nm \times 410 nm field of view that is adequate for most practical purposes. For example, a camera with physical 5 μm \times 5 μm pixel size and 0,2 nm pixel size at the sample plane requires microscope magnification 25,000 \times . Typically, a somewhat higher magnification, for example, 30,000 \times , can be chosen to slightly oversample the object.

5.1.6 Number of pixels of the detector

The highest number of pixels on the camera should be used to achieve optimum image resolution and field of view. For example, it is advisable to use binning 1 for a 2 048 pixel \times 2 048 pixel camera so that

there are 2 048 pixel × 2 048 pixel in the images. Number of pixels, their size, the field of view and the image resolution are related. See [5.1.5](#) for an example of magnification estimate.

If the point spread function of the camera is poor, it can be necessary to combine camera pixels (bin the pixels). In such case, the necessary magnification should be estimated for the combined pixel size. Combining (binning) the camera pixels results in a corresponding decrease of the field of view as compared to binning 1 images. For example, a 1 000 nm² × 1 000 nm² field of view with 0,5 nm per pixel obtainable with 1 000 pixel × 1 000 pixel² camera would be reduced to 500 nm × 500 nm if the camera is binned by 2 while maintaining sampling 0,5 nm per pixel.

5.1.7 Image acquisition time

Image acquisition time shall be chosen such that enough signal to noise ratio is obtained in the projected images to allow for projected image alignment and for reconstruction. The acquisition time needs to be chosen such that the sample drift is less than the pixel size at the sample plane. If the number of counts per pixel is too low at the drift limited acquisition time, either the microscope beam current can be increased or multiple images at each tilt can be acquired. Typically, about 100 electrons per pixel of the detector are sufficient for alignment and reconstruction^[12].

5.2 Microscope calibration

To ensure correct microscope calibration, the microscope shall be calibrated under the same imaging conditions as used for the tomography data acquisition. The calibration shall be performed either immediately before or immediately after the data acquisition. Identical conditions in this case refers to using the same lens currents, the objective lens and the intermediate and projector lens system currents, as used for the tomography data acquisition. The calibration of the TEM instrument shall be performed as described in ISO 21363.

NOTE Calibrating before or after collection of projected images is equivalent as long as the lens settings are not changed between calibration and collection of projected images.

For precise size measurement by using TEM, the same condition of TEM lens and specimen height between a measurement specimen and a calibration specimen is important. An internal reference length for TEM instruments must be calibrated using calibration standards. All size measurements should be done with the same lens and specimen height conditions of calibration. Focusing condition of the lens can affect the size measurement. A Scherzer defocus condition is generally used. The zero defocus is defined using Fresnel fringe at first, then focusing goes to Scherzer defocus. Specimen height should be at the eucentric position. It is important to obtain images at eucentric height and the same defocus condition at magnifications as used explicitly for the instrument's calibration.

For most microscopes the selection of identical conditions is achieved by selecting the same nominal magnification and same focus value of the objective lens current as used for the tilt series acquisition. The sample focusing shall be achieved by utilizing the mechanical Z height of the stage. Focusing by changing the imaging lens in a TEM or probe forming lens in STEM should be limited to a range no more than ±1 μm to prevent magnification change or image rotation. The lens currents must be same for microscope calibration as for data collection. Calibration should be performed at the eucentric height and at the limits of the range of lens current focusing. For calibration a suitable calibration sample with known dimensions shall be used. An example of such sample is available from several suppliers^[13].

The pixel size obtained using the above calibration procedure shall be used to calibrate the reconstructed 3D volume.

See [Annex D](#) for recommended microscope and data collection parameters to record. See [Annex H](#) for an example of uncertainty budget.

6 Image capture (data acquisition)

6.1 General

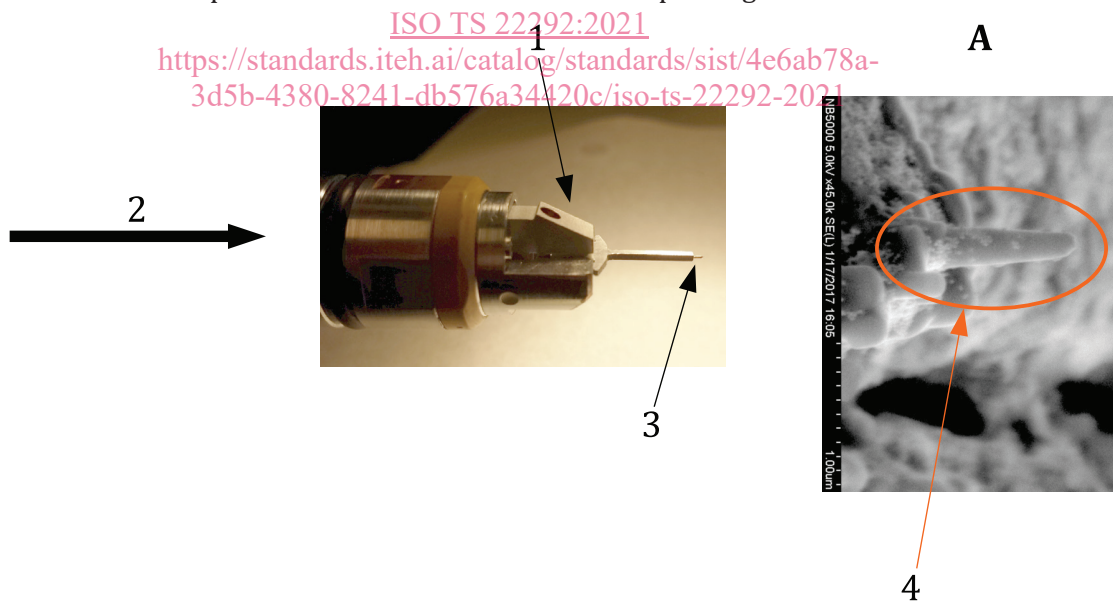
This clause covers image capture to obtain projected images in 2D.

NOTE ISO 21363 provides extensive information on collection of projected images, that is also applicable to collection of projected images for electron tomography reconstruction.

6.2 Procedure

The procedure is as follows.

- The data shall be collected in a transmission electron microscope (TEM) in conventional TEM mode. Alternatively, a scanning TEM (STEM) mode can be used, see [Annex B](#). The TEM shall be operated at incident energy between 100 kV and 400 kV. The microscope can have thermal electron source (LaB₆), field assisted thermal electron source (Schottky electron source) or a field emission electron source. The point resolution of the microscope shall be 0,3 nm or less. The information limit of the microscope shall be 0,2 nm or less.
- The microscope shall allow sample to be tilted over -90° to $+90^{\circ}$ around at least one axis perpendicular to the incident beam. The microscope vacuum pressure near the sample chamber shall be 1×10^{-7} torr or less.
- Data acquisition shall be bright field TEM. Other methods such as scanning TEM (STEM) bright field (BF-STEM), annular dark field (ADF-STEM), and high angle annular dark field HAADF-STEM are acceptable if they provide contrast that is predominantly monotonic with sample thickness regardless of tilt angle. See [Annexes B](#) and [C](#) for examples.
- The sample shall be mounted as depicted in [Figure 2](#) with the sample long axis perpendicular to the electron beam and parallel with the tilt axis of the sample stage.



Key

- | | | | |
|---|--------------------------------|---|---|
| 1 | sample stage clamp | 4 | carbon rod with nanoparticles |
| 2 | tilt axis = sample holder axis | A | SEM image of sample tip with sample rod |
| 3 | sample tip with carbon rod | | |

Figure 2 — Sample rod at the tip of a TEM holder