



International
Standard

ISO 20263

**Microbeam analysis — Analytical
electron microscopy — Method
for the determination of interface
position in the cross-sectional
image of the layered materials**

*Analyse par microfaisceaux — Microscopie électronique
analytique — Méthode de détermination de la position
d'interface dans l'image de coupe transversale des matériaux en
couches*

**Second edition
2024-11**

Reference number
ISO 20263:2024(en)

Full document details: [ISO 20263-2024](#)

© ISO 2024  **Documents**
Document Sharing Hub



COPYRIGHT PROTECTED DOCUMENT

© ISO 2024

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	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms, definitions and abbreviated terms	1
3.1 Terms and definitions	1
3.2 Abbreviated terms	4
4 Specimen preparation for cross-sectional imaging	4
4.1 General	4
4.2 Requirements for the cross-sectional specimen	5
5 Determination of an interface position	6
5.1 General	6
5.2 Preliminary considerations	6
5.2.1 Ideal model of an interface	6
5.2.2 More realistic model of an interface	6
5.2.3 Dealing with intensity fluctuations in the image	7
6 Detailed procedure for determining the position of the interface	8
6.1 General	8
6.2 Preparing cross-sectional TEM/STEM image	10
6.2.1 Preparing digitized Image	10
6.2.2 Displaying the digitized image	11
6.3 Setting the ROI	11
6.3.1 General	11
6.3.2 Classification of image	11
6.3.3 Procedure of setting the ROI	12
6.4 Acquisition of the averaged intensity profile	16
6.5 Moving-averaged processing	19
6.6 Differential processing	20
6.7 Determination of interface position	21
7 Uncertainty	21
7.1 Uncertainty accumulating from each step of the procedure	21
7.2 Uncertainty of measurement result on image analysis	22
Annex A (informative) Examples of processing the real TEM/STEM images for three image types	24
Annex B (informative) Two main applications of the method	38
Annex C (informative) Calibration of scale unit: Pixel size calibration	45
Bibliography	47

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 document 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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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 by Technical Committee ISO/TC 202, *Microbeam analysis*, Subcommittee SC 3, *Analytical electron microscopy*.

This second edition cancels and replaces the first edition (ISO 20263:2017), which has been technically revised.

The main changes are as follows:

- introduction has been revised;
- terms and sources in Clause 3 have been revised;
- [subclauses 5.2.2](#), [5.2.3](#), [6.1](#), [6.2.1](#), [6.7](#), [7.1](#), [7.2](#), [A.4.1](#), [B.2.3](#) and [B.2.4](#) have been revised;
- figures have been revised.

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

Multi-layered materials are widely used in the production of semiconductor devices, various kinds of sensors, coating films for optical element, new functional materials, etc. One of the factors used to determine the characteristics of multi-layered materials is the layer thickness, for evaluation of products and verification of the production process. In practice, measuring the total thickness and/or the thickness of each layer and checking the uniformity of thickness and/or flatness of the interface are often done using recorded images of the materials. Evaluations can be made from the cross-sectional TEM/STEM images by accurately determining the averaged interface position between two different layered materials.

In relation to the determination of the interface position in the HR atomic imaging, analysis by the multi-slice simulation (MSS) method can be applied for the target measurement, if the atomic structural models can be constructed. However, in real materials, there are a lot of cases when they cannot, such as:

- the interface between amorphous layers, or layers of amorphous substance and crystal;
- the interface recorded in low-resolution image in which the atomic columns cannot be identified:
 - for very thick single-layered material;
 - for thick multi-layered material.

This document gives the method to determine the averaged interface position using a differential processing of the accumulated intensity profile obtained from the ROI set in the cross-sectional TEM/STEM image of the multi-layered material. The thickness of the layer that can be applied ranges from a few nanometers to a few micrometers. Thus, this document is not intended for the determination of the simulated position of the layer interface analysed by the MSS method.

Microbeam analysis — Analytical electron microscopy — Method for the determination of interface position in the cross-sectional image of the layered materials

1 Scope

This document specifies a procedure for the determination of the averaged interface position between two different layered materials recorded in the cross-sectional image of the multi-layered material. This document does not apply for determining the simulated interface of the multi-layered materials expected through the multi-slice simulation (MSS) method.

This document is applicable to the cross-sectional images of multi-layered materials recorded using a transmission electron microscope (TEM) or a scanning transmission electron microscope (STEM) and cross-sectional elemental mapping images using an energy dispersive X-ray spectrometer (EDS) or an electron energy loss spectrometer (EELS). This document is also applicable to digitized images recorded on an image sensor built into a digital camera, a digital memory set in the PC or an imaging plate, where the digitalized image is obtained by converting an analogue image recorded on photographic film using an image scanner.

2 Normative references

There are no normative references in this document.

3 Terms, definitions and abbreviated terms

3.1 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/>

3.1.1

cross-sectional image

TEM/STEM image of the multi-layered materials along a plane perpendicular to the stacking direction

3.1.2

differential processing

calculation of the difference between the values of adjacent pixel data in the intensity profile

3.1.3

digital camera

device that detects the image using a chip-arrayed *image sensor* (3.1.12), such as a charge-coupled device (CCD) or complementary metal-oxide semiconductor (CMOS), which converts a visual image to an electric signal

[SOURCE: ISO 29301:2023, 3.7]

3.1.4

dot pitch

distance between adjacent pixels in pixel-based devices

3.1.5

elemental mapping image

image produced by the selected signal which is attributed to a particular element, from the EDS/EELS spectrum

3.1.6

FIB thinning

site-specific thinning technique by using focused ion beam to thin a particular region of the specimen

3.1.7

filtering mask

mask to define the cut-off frequency in the reciprocal space

3.1.8

fast Fourier transformation

FFT

efficient algorithm to compute the discrete Fourier transform

[SOURCE: ISO 15932:2013, 5.4.1.1]

3.1.9

inverse fast Fourier transformation

IFFT

efficient algorithm to compute the inverse of the discrete Fourier transform

[SOURCE: ISO 15932:2013, 5.4.1.2]

3.1.10

image file format

format for saving an image as a computer file according to a predetermined rule

3.1.11

image scanner

device that converts an analogue image into a digitized image with the desired resolution

EXAMPLE There are mainly two different types of scanners: flatbed type and drum type.

[SOURCE: ISO 29301:2023, 3.17, modified — example has been added.]

3.1.12

image sensor

device, such as a charge-coupled device (CCD) array or complementary metal-oxide semiconductor (CMOS) sensor, which converts visual image information to an electric signal, built-in *digital camera* ([3.1.3](#)) or other imaging devices

3.1.13

intensity profile

signal intensity distribution along a line specified in the image

3.1.14

interface

boundary surface at the junction of two different layers of materials recorded in the *cross-sectional image* ([3.1.1](#)) of the multi-layered materials

3.1.15

ion-milling

thinning technique of sputtering the specimen with an inert gas

[SOURCE: ISO 15932:2013, 4.1]

3.1.16

imaging plate IP

electron image detector consisting of a film with a thin active layer embedded with specifically designed phosphors

[SOURCE: ISO 29301:2023, 3.16]

3.1.17

low pass filter

filter to pass signals of frequencies lower than the cut-off frequency

3.1.18

moving average

calculation for averaging the selected dataset which is picked out from equal number of dataset on either side of a central data

3.1.19

multi-slice simulation

MSS

computer simulation method of high-resolution TEM images, which treats electrons as incoming waves and treats the interactions with matter as occurring on multiple successive single slices of the specimen

[SOURCE: ISO 15932:2013, 6.4.1, modified — “algorithm for the simulation” has been replaced by “computer simulation method”.]

3.1.20

multi-layered material

laminated material which is fabricated by alternating layers of at least two kinds of materials on the substrate

3.1.21

photographic film

sheet or a roll of thin plastic coated by photographic emulsion for recording an image

[SOURCE: ISO 29301:2023, 3.23]

3.1.22

pixel

smallest unit element that makes up the digital image

3.1.23

region of interest

ROI

sub-dataset picked out from the entire dataset for a specific purpose

3.1.24

ultra-microtome

thin sectioning instrument to prepare the specimen thin enough for TEM observation by using glass or diamond knives

3.1.25

zone axis

crystallographic direction, designated $[u \ v \ w]$, defined by the intersection of a number of crystal planes $(h_1 k_1 l_1, \dots, h_p k_p l_p)$ such that all of the planes satisfy the so-called Weiss zone law; $hu + kv + lw = 0$

[SOURCE: ISO 29301:2023, 3.36]

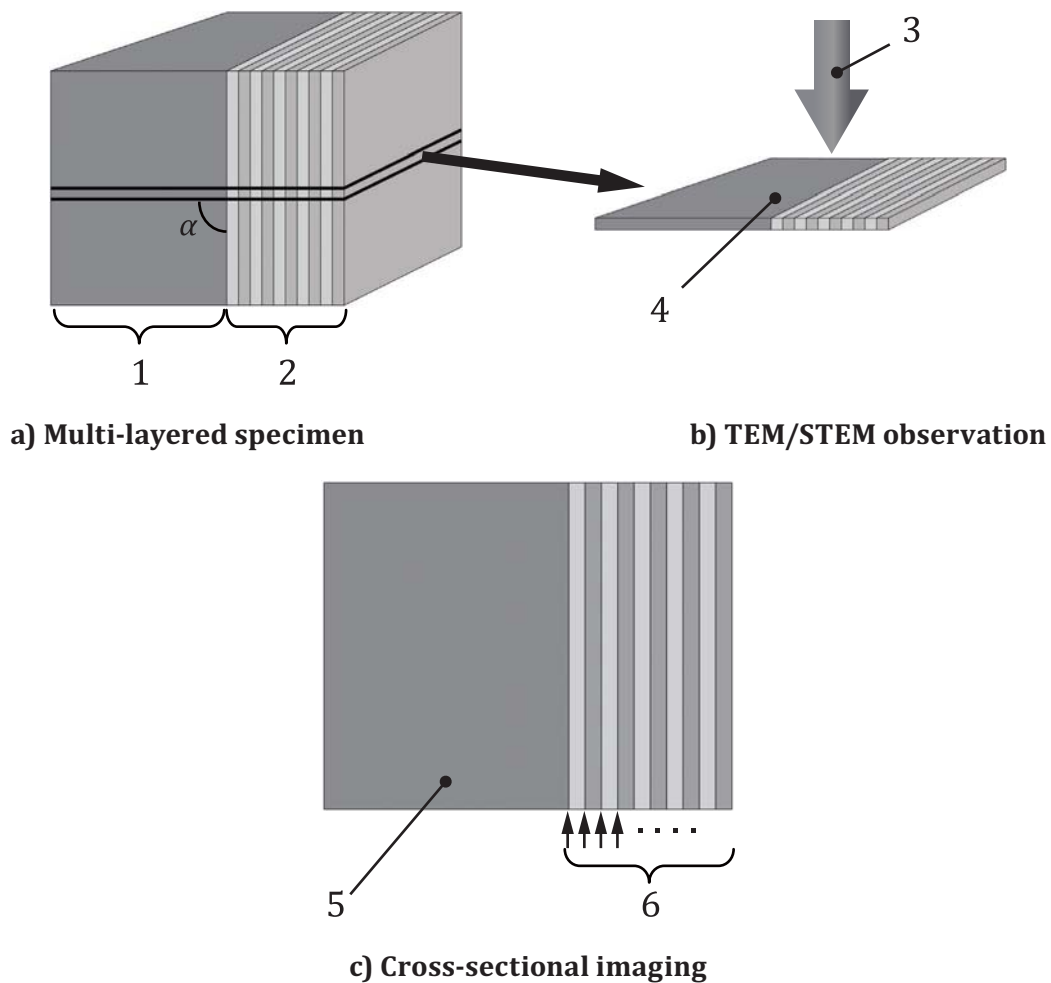
3.2 Abbreviated terms

AEM	Analytical electron microscope/microscopy
CCD	Charge coupled device
CMOS	Complementary metal oxide semiconductor
CRT	Cathode ray tube
EDS/EDX	Energy dispersive X-ray spectrometer/spectroscopy Although "EDX" and "EDS" are interchangeable, this document uses "EDS".
EELS	Electron energy loss spectrometer/spectroscopy
FFT	Fast Fourier transformation
FIB	Focused ion beam
HREM	High-resolution transmission electron microscope/microscopy
IFFT	Inverse fast Fourier transformation
MSS	Multi-slice simulation
ROI	Region of interest
STEM	Scanning transmission electron microscope/microscopy
TEM	Transmission electron microscope/microscopy

4 Specimen preparation for cross-sectional imaging

4.1 General

To determine the interface position of the multi-layered materials stacked on a substrate, the specimen observed by TEM/STEM shall be cut into a cross-sectional thin slice perpendicular to the stacking direction of the multi-layered thin film, using the techniques of ultra-microtome, ion-milling, FIB thinning, chemical etching and so on. In order to keep the thickness information of the layered materials with an accuracy of 1 %, the cut out angle α [shown in [Figure 1, a](#)] shall be $90^\circ \pm 6^\circ$.

**Key**

- 1 substrate
- 2 multi-layered materials
- 3 direction of electron beam
- 4 thin slice of the specimen
- 5 cross-sectional TEM/STEM image
- 6 arrows indicate interface positions

Figure 1 — Specimen preparation for cross-sectional imaging

4.2 Requirements for the cross-sectional specimen

Ensure that the specimen:

- provides a good and sharp contrast and clear and straight interface for the multi-layered materials in the TEM/STEM/elemental mapping image,
- can be cleaned to remove contamination without causing mechanical/electrical damage or distortion,
- has a smooth surface on both sides and identical thickness, at least within the area used for the determination process of interface position,
- is aligned to a low-index zone axis along the electron optical axis, if the specimen region is a single crystal.

5 Determination of an interface position

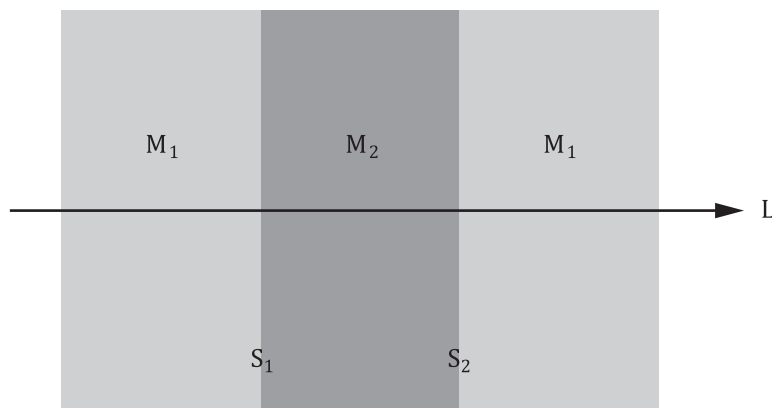
5.1 General

It is important to determine the position of an interface in a layered material from its cross-sectional TEM/STEM/elemental mapping image, objectively and uniquely. In [Clause 6](#), the main scheme for the determination of the interface position, as prescribed by this document, is explained.

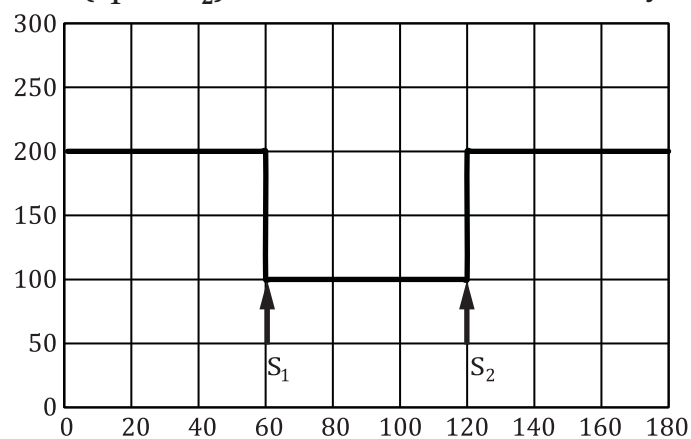
5.2 Preliminary considerations

5.2.1 Ideal model of an interface

Ideally, the interface between two kinds of materials, M_1 and M_2 , show straight edge [see [Figure 2 a](#)]. In this case, it is easy to find the interface positions (S_1 and S_2) uniquely from the intensity profile [see [Figure 2 b](#)] along a line (L) perpendicular to the interface.



a) Ideal interface (S_1 and S_2) model between two kinds of layers, M_1 and M_2



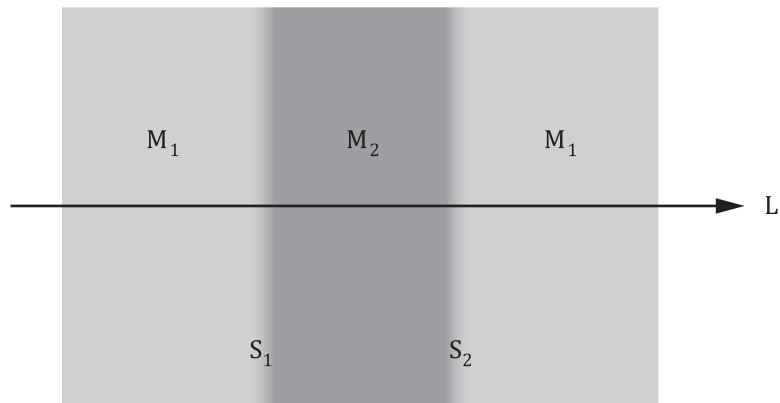
b) Intensity profile along an arrow line, L , in a), perpendicular to the interfaces (S_1 and S_2)

Figure 2 — Ideal interface model

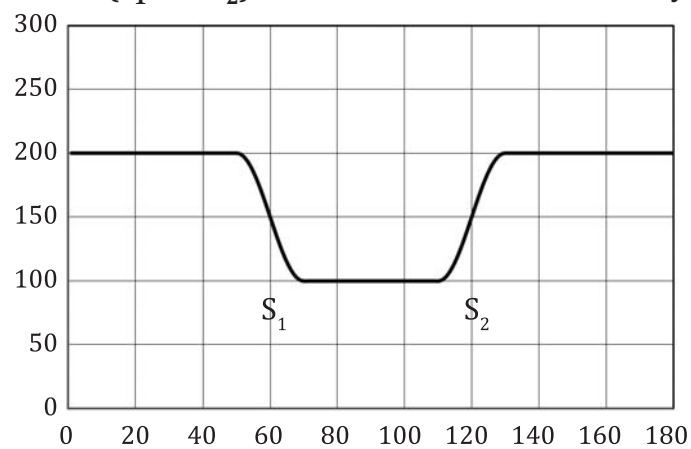
5.2.2 More realistic model of an interface

In general, the interface will not be in a straight line. It typically appears as a region with a gradated intensity distribution between layers M_1 and M_2 [see [Figure 3 a](#)]. In this case, it is not easy to find the accurate interface position from its intensity profile which is normally s-shaped [see [Figure 3 b](#)]. This document defines the interface position at the steepest tilt angle in the slope. Differential processing of the intensity profile is the most suitable method to determine the interface position as defined above. [Figure 3 c](#) shows the differential curve of the intensity profile on in [Figure 3 b](#). Pixel positions on the x-axis corresponding to

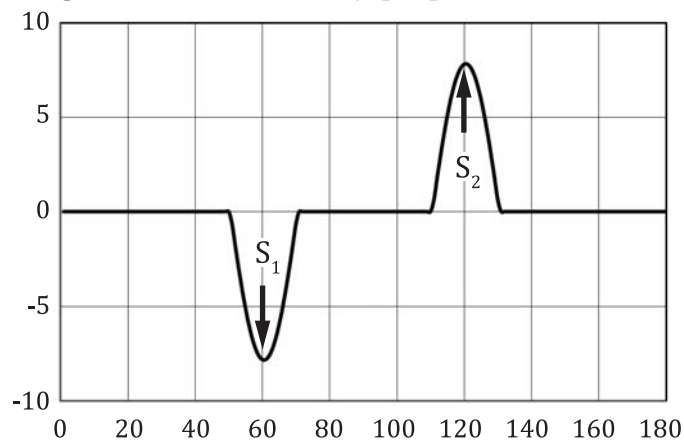
the minimal value and maximal value in the curve show the interface positions (S_1 and S_2) on both sides of the layer M_2 .



a) Realistic interface (S_1 and S_2) model between two kinds of layers, M_1 and M_2



b) Intensity profile along an arrow line, L , in a), perpendicular to the interfaces (S_1 and S_2)



c) Differential curve of intensity profile

NOTE Positions of minimal and maximal value correspond to the interfaces (S_1 and S_2) defined in this document.

Figure 3 — Realistic interface model

5.2.3 Dealing with intensity fluctuations in the image

Unlike models described in 5.2.1 and 5.2.2, the actual cross-sectional TEM/STEM/elemental mapping image has a domain-like intensity fluctuation, background noise and sometimes (in the high-resolution images)

periodic modulation of the intensity due to atomic column structures. Because of this non-uniformity in the intensity in the image, follow the steps a) to g) sequentially for obtaining the desired smooth intensity profile with a plateau and well-defined slope.

NOTE 1 Details of the procedure are described in [Clause 6](#).

- a) Prepare the cross-sectional TEM/STEM/elemental mapping digital image.
- b) Set the direction of the interface parallel to the y-axis of the monitor screen.
- c) Set the ROI area in the image.
- d) Obtain the averaged intensity profile by adding up and averaging the line profiles in the direction perpendicular to the interface (parallel to the x-axis of the monitor screen) over the entire interface direction (parallel to the y-axis of the monitor screen) in the ROI.
- e) Apply “moving-average” processing to the averaged intensity profile produced by the previous step, d). This will remove small noise from the boundary region contributing to the slope of the interface.
- f) Apply differential processing to the moving-averaged intensity profile obtained in e).
- g) Determine the interface position as the pixel coordinate on the x-axis which either corresponds to the maximal or minimal value in the differential curve.

6 Detailed procedure for determining the position of the interface

6.1 General

As described in the previous clause, the interface position can be determined through the differential processing of the intensity profile in the ROI.

In the differential processing, a noise component existing in the intensity profile becomes an obstacle to find the correct interface position. Therefore, it is necessary to remove the noise components in advance through the average processing and the moving-averaged processing.

Also, in an atomic resolution image with an atomic column arrangement along the interface, the oscillated component depends on the atomic column which cannot be eliminated even in the averaged intensity profile. This is an obstacle to the extraction of the correct interface position by differential processing. Therefore, for such an image, pre-processing for obscuring the atomic column structure is essential through the processing of FFT/low pass filtering/IFFT.

[Figure 4](#) shows a flow chart of the interface position determination procedure described in [5.2.3](#). In [Clause 6](#) details of each procedure will be described.

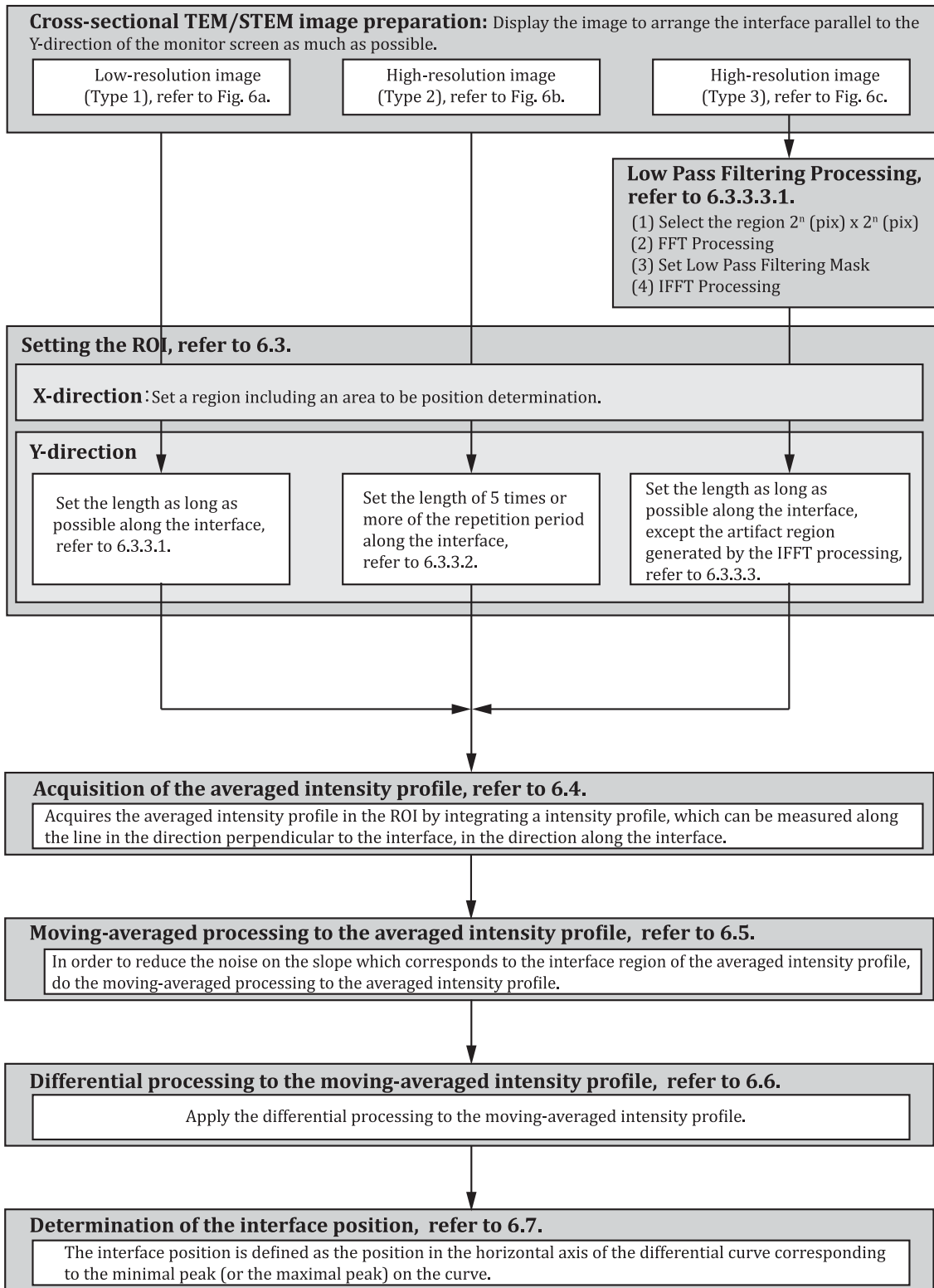


Figure 4 — Flow chart of interface position determination procedure

6.2 Preparing cross-sectional TEM/STEM image

6.2.1 Preparing digitized Image

It is necessary to prepare a digitized cross-sectional image to determine the interface position. The bit depth of digitization of the image shall be 8 bits or more. There are four ways of digitizing the image corresponding to each image detection system shown in [Table 1](#).

Table 1 — Comparison table for image detection

Image detection system	Apparatus for digitization	Pixel size
Photographic film	Flatbed image scanner	Determined by dot pitch applied to image scanner
Imaging plate	Dedicated scanner	Determined by LASER beam diameter for readout
Image sensor (Digital camera)	Built in the digital camera	Same size as that of the image sensor
Digital memory	Built in the PC connected to the scanning device	Determined by scanning condition of electron beam

- a) Photographic film: Analogue image recorded on the photographic negative film shall be converted to a digitized image using an image scanner with dot pitch less than 20 μm .

NOTE 1 Use a flatbed image scanner because it is easy to set the transparent scale in it for pixel size calibration (Refer to ISO 29301).

- b) Imaging plate (IP): The recorded image shall be read out with a dedicated image digitizer (IP reader) connected to a PC.
- c) Image sensor: The digitized image, captured by a digital camera, shall be saved on the memory in the PC system as an image file with a reversible format.

NOTE 2 Ensure that the procedure for normalization of gain is performed to have a uniform background of the image.

- d) Digital memory: STEM and elemental mapping image, captured by the digital memory built into a PC, shall be saved as an image file with a reversible format.

Before and during the execution of the digitization procedure, ensure the following.

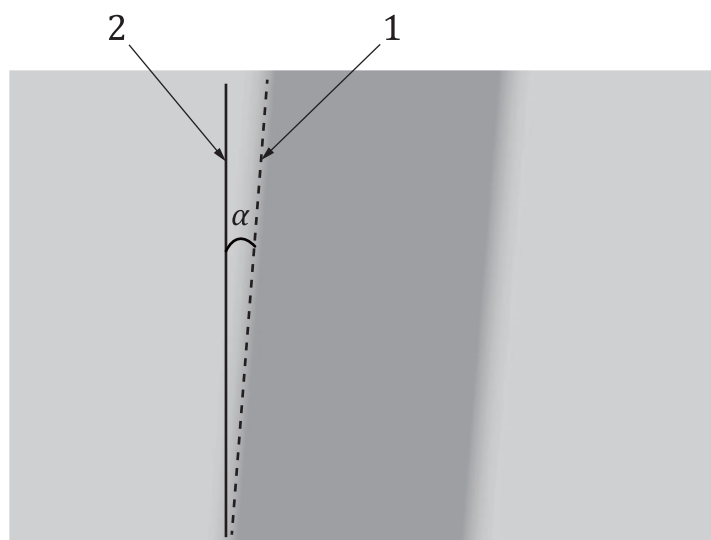
- The correct sensitivity setting is used for the photographic film used, to generate the negative image with proper density and contrast.
- Keep the exposure time to minimize the drift and reduce blurring in the recorded image.
- Do not use “binning” in the readout process of the magnified digital image from the digital camera.
- When using an image montage function, ensure that the seams of the image do not overlap with any of the interface of the specimen.
- For saving the digitized images, an uncompressed image file format (ESP, PICT, TIFF or Windows bitmap), or reversible (lossless) compressed image file format (GIF or PING) shall be used.
- Ethical digital imaging requires that the original uncompressed image file be stored on archival media, e.g. CD-R, without any image manipulation or processing operation. All parameters of the production and acquisition of this file and any subsequent processing steps shall be documented and reported to ensure reproducibility.

Generally, acceptable imaging operations include gamma correction, histogram stretching and brightness and contrast adjustments need not be reported. All other operations (such as Unsharp-Masking, Gaussian Blur, etc.) shall be directly identified by the author as part of the experimental methodology. However, for

diffraction data or any other image data that is used for subsequent quantification, all imaging operations shall be reported.

6.2.2 Displaying the digitized image

The digitized image used for further processing shall be orientated so that the interface is, as far as possible, parallel to the y-axis of the monitor screen. If the interface is tilted with an angle, α , to the y-axis of the monitor screen, it is necessary to measure and to record the tilting angle, α (see [Figure 5](#)).



Key

- 1 interface
- 2 y-axis of monitor screen

Figure 5 — Example of a tilted target image

6.3 Setting the ROI

6.3.1 General

This depends on the type of the image classified in [6.3.2](#).

6.3.2 Classification of image

Firstly, it is necessary to obtain an intensity profile in a direction across the interface. Ensure that the intensity profile is as smooth as possible. To do this, set the ROI in the target image in advance. Then, integrate the line profile measured across the interface, pixel by pixel, to the appropriate range along the interface. In practice, the setting procedure depends on the image type classified by image resolution and the atomic column arrangement recorded in high resolution image, as follows.

- Type 1: Low resolution images in which atomic column arrangements cannot be recognized. [Figure 6 a\)](#) shows an example.
- Type 2: High resolution images in which atomic column arranged at an angle θ to the interface [see [Figure 6 b\)](#)].
- Type 3: High resolution images in which atomic column is parallel to the interface [see [Figure 6 c\)](#)].