BS ISO 24479:2024

BSI Standards Publication

Biotechnology — Cellular morphological analysis — General requirements and considerations for cell morphometry to quantify cell morphological features

Documents
Document Sharing Hub

Full document details: BS ISO 24479:2024

National foreword

This British Standard is the UK implementation of ISO 24479:2024.

The UK participation in its preparation was entrusted to Technical Committee BTI/1, Biotechnologies.

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

Contractual and legal considerations

This publication has been prepared in good faith, however no representation, warranty, assurance or undertaking (express or implied) is or will be made, and no responsibility or liability is or will be accepted by BSI in relation to the adequacy, accuracy, completeness or reasonableness of this publication. All and any such responsibility and liability is expressly disclaimed to the full extent permitted by the law.

This publication is provided as is, and is to be used at the recipient's own risk.

The recipient is advised to consider seeking professional guidance with respect to its use of this publication.

This publication is not intended to constitute a contract. Users are responsible for its correct application.

© The British Standards Institution 2024
Published by BSI Standards Limited 2024

ISBN 978 0 539 25258 3

ICS 07.080

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 October 2024.

Amendments/corrigenda issued since publication

Date	Text affected

Documents
Document Sharing Hub

Full document details: BS ISO 24479:2024

International Standard

ISO 24479

First edition
2024-10

Biotechnology — Cellular morphological analysis — General requirements and considerations for cell morphometry to quantify cell morphological features

Biotechnologie — Analyse morphologique cellulaire — Exigences générales et considérations pour la morphométrie cellulaire afin de quantifier les caractéristiques morphologiques des cellules

Reference number
ISO 24479:2024(en)

© ISO 2024

Documents
Document Sharing Hub

Full document details: BS ISO 24479:2024

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

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

Contents

Page

Foreword ..... **v**
Introduction ..... **vi**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 Abbreviations **4**
5 General Concept **5**
5.1 Cell morphometry 5
5.2 Steps for cell morphometry 5
6 Target of interest (TOI) **5**
7 Image capture **6**
7.1 Microscopic observation method 6
7.1.1 General 6
7.1.2 Cell properties to be observed 7
7.1.3 Sample preparation 7
7.2 Microscope system and its settings 9
7.2.1 General 9
7.2.2 Light source 11
7.2.3 Objective lens and condenser lens 11
7.2.4 Components in optical path 11
7.2.5 Image capture device 12
7.2.6 Image data 12
7.2.7 Environmental conditions 12
7.2.8 Observing position 12
7.2.9 Image capturing conditions 13
7.3 Execution of image capture 13
7.3.1 Single image capture 13
7.3.2 Multiple image capture 13
8 Segmentation of the TOI **13**
9 Quantification **14**
9.1 General 14
9.2 Procedure to quantify morphological features of segmented object 14
10 Qualification of Measurement **14**
11 Reporting **15**
11.1 General 15
11.2 Reporting of sample properties and sample preparation 15
11.3 Reporting of the microscopic observation method 15
11.4 Reporting of the image data, image pre-processing, and image analysis for morphometric analysis 16
11.5 Reporting of the morphological features for morphometric analysis 17
Annex A (informative) **Check sheet regarding selection of contrast-enhancing techniques for optical microscopy** **18**
Annex B (informative) **Check sheet regarding selection of microscope system** **20**
Annex C (informative) **List of cell morphological descriptors, definitions and formulae for cell shape, size** **21**
Annex D (informative) **List of cell morphological descriptors, definitions and formulae for cell texture** **29**

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

Annex E (informative) **Points to consider when acquiring phase-contrast images of cells suitable for image analysis** **34**

Bibliography **43**

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub

Full document details: BS ISO 24479:2024

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 276, *Biotechnology*, Subcommittee SC 1, *Analytical methods.*

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 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

Introduction

Optical microscopy is a widely practiced technique for characterization of processed cells. Morphology of cells, such as shape, size, texture of whole or parts of cells, can provide information about various aspects of cells including identity, phenotype, viability, doubling time, as well as the states of stress and drug responses. Morphological evaluation of cells is widely employed in basic research, drug discovery, in-process control and release testing for cell manufacturing and cell banks for cell-based therapeutic products.

Therefore, it is desired to establish a common understanding of definitions and formulae regarding cell morphological descriptors in which specialists in research and business fields can refer to and compare information, within an institution, and with other interested parties.

The current situation is that characteristics of cell morphology obtained from microscopic images are frequently described qualitatively in expressions such as "unevenness around", "elongated", "rounded". Even when cell morphological descriptors characterizing the morphology of the cell are measured and quantified from the cell image, these cell morphological descriptors are not consistently used.

This document allows to check whether the numerical values assigned as cell morphological descriptors are calculated by an appropriate method, and to improve the reliability of the measured value and the evaluation result. It is expected that the "common language of definitions and mathematical formulae" based on this document will enable the accumulation of more reliable data, and such language will provide a basis for assessment whether individually acquired data can be quantitatively compared to each other.

This document is intended primarily for users, both in academia and industry, who evaluate cell characteristics. However, it can also be referred to by suppliers of tools such as microscopes, image processing devices, and software, suppliers of database that handle information on cell morphology and users who write scientific papers regarding cell morphology.

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

Biotechnology — Cellular morphological analysis — General requirements and considerations for cell morphometry to quantify cell morphological features

1 Scope

This document provides general requirements for cell morphometry to quantify cell morphological features including cell shape, size and texture.

This document addresses aspects of cell image capture using optical microscopy and image processing for morphometry.

This document does not address the statistics associated with a morphological analysis of a cellular sample.

This document also gives terms and definitions corresponding to cell morphological descriptors, and lists examples and their formulae, that represent quantitative cellular morphological features for evaluation of cell morphology in cell analysis.

This document primarily applies to morphological analysis of cultured mammalian cells.

This document is not intended for imaging used in clinical diagnostics.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

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

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

3.1
aberration
<optical system> failure of an optical system to produce a perfect image

EXAMPLE Spherical aberration, chromatic aberration

[SOURCE: ISO 10934:2020, 3.1.4, modified — “objective lens aberration” removed a preferred term and examples added.]

3.2
bit depth
maximum number of discrete levels available for the digitized representation of the signal intensity, represented as a power of 2

[SOURCE: ISO 22493:2014, 5.2.2.2.1, modified — “colour depth” and “pixel depth” are deleted from the preferred term, Notes 1 and 2 to entry are deleted.]

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

3.3
bounding box
rectangular region enclosing annotated object

Note 1 to entry: The major and minor axes of the rectangle are parallel to the edges of the images.

[SOURCE: ISO/IEC 30137-4:2021, modified — second sentence of Note 1 to entry deleted.]

3.4
cell morphology
form and structure of either the cell, subcellular components, or both

Note 1 to entry: In view of *cell morphometry* (3.6), cell morphology can be represented by a single or multiple *cell morphological descriptor(s)* (3.5) associated with *morphological feature(s)* (3.15).

3.5
cell morphological descriptor
quantitative representation of *cell morphology* (3.4)

3.6
cell morphometry
process of measuring dimensional, shape, and structural characteristics of cells including analysis of derived properties

Note 1 to entry: Steps for cell morphometry starts with determination of a purpose of the cell morphology analysis and ends with analysis of quantified results for the intended purpose. By taking these steps, cell morphometry can derive properties, e.g. phenotype such as immunosuppressive activity. See Table 1 for details.

3.7
cell shape
external geometric form of a cell

[SOURCE: ISO/TR 13014:2012 2.26, modified — term “shape” has been changed to “cell shape” and “particle” has been changed to “cell”.]

3.8
cell texture
spatial arrangement of colours or intensities in an image associated with cellular characteristics

Note 1 to entry: The pattern can have a specific spatial scale or colour.

Note 2 to entry: The cell texture can result from a specific arrangement of sub-cellular components.

3.9
convex hull
smallest convex set containing a given geometric object

Note 1 to entry: “convex set” is a geometric set where any line segment connecting two points in the set lies entirely within the set.

[SOURCE: ISO 19107:2019, 3.15, modified – Note 1 to entry replaced.]

3.10
depth of focus
axial depth of the space on both sides of the image within which the image appears acceptably sharp, while the positions of the object plane and of the objective are maintained

[SOURCE: ISO 10934:2020, 3.1.37, modified — Note 1 to entry is deleted.]

3.11
formula
recipe for calculating a value

[SOURCE: ISO/IEC 29500-1:2016, 12.1.7, modified — the explanatory statement has been deleted.]

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

3.12
field of view
FOV
field which is observed by the microscope

Note 1 to entry: The full image frame of a digital imaging device corresponds to its field of view.

[SOURCE: ISO 13322-1:2014, 3.1.6, modified — the term “viewing” has been changed to “microscope”.]

3.13
image capture
image acquisition
process of creating a two-dimensional original image of an object

[SOURCE: ISO 21227-1:2003, 3.4]

3.14
measurement
process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity

[SOURCE: ISO/IEC GUIDE 99:2007, 2.1, modified — Notes to entry have been deleted.]

3.15
morphological feature(s)
shape, size, and texture of cellular components

3.16
numerical aperture
NA
number originally defined by Abbe for objectives and condensers, which is given by the expression $n \sin u$, where n is the refractive index of the medium between the lens and the object and u is half the angular aperture of the lens

Note 1 to entry: Unless specified by “image-side”, the term refers to the object side.

[SOURCE: ISO 10934:2020, 3.1.10.4]

3.17
optical resolution
numerical measure of the image quality of an optical system

[SOURCE: ISO 8600-5:2020, 3.12]

3.18
pixel resolution
number of imaging pixels per unit distance of the detector

[SOURCE: ISO 15253:2021, 3.7, modified —hyphen between “pixel” and “resolution” was deleted.]

3.19
region of interest
ROI
parts of an image to which discrete observations are applied

Note 1 to entry: Region is selected by observer’s intended purpose.

[SOURCE: ISO 10934:2020, 3.2.28, modified — Note 1 to entry has been added.]

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

3.20
segmentation
partitioning images into distinct regions

Note 1 to entry: A distinct region is determined in attention of target of interest.

Note 2 to entry: The partitioning process includes filter application to the image.

Note 3 to entry: Segmentation can be of individual pixels. In such case, segmentation means partitioning pixels within images into distinct groups.

3.21
spatial resolution
smallest separation between two details in the object for which they can be detected as being separate under a given set of conditions

[SOURCE: ISO 15253:2021, 3.7, modified – "recognized" was replaced to "detected".]

3.22
tile capture
capturing method to extend a field of view by recording a series of tile images with limited field size

Note 1 to entry: Tile images can be recorded by systematically changing the relative position between the sample and the objective lens by mechanical drive.

Note 2 to entry: If tiles overlap, the tile images can be stitched to a larger overview image based on motor position or tile image correlation.

3.23
time lapse capture
image-recording method, in which multiple images are captured at specific time interval

Note 1 to entry: time lapse capture can be used for tracing changes in cell states or activities (such as cell division, fusion or phagocytosis of processed cells).

3.24
target of interest
TOI
part, region or both of cell(s) for morphological examinations defined by observer's intended purpose

3.25
Z-stack capture
image-recording method, in which multiple images are captured in the direction of the optical axis at a selected distance interval

Note 1 to entry: Z-stacks can be used to create a 3D image or for capturing objects in different optical focal planes.

4 Abbreviations

DIC differential interference contrast

FOV field of view

NA numerical aperture

TOI target of interest

ROI region of interest

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

5 General Concept

5.1 Cell morphometry

Cell morphology represents various features and states of cells, such as cell division and apoptosis, depending on the life cycle of the cell and environmental factors such as media contents and culture vessel. Cell morphometry can provide information on phenotype, viability, proliferation, stages of differentiation, function, and other cell characteristics. In addition, time-lapse observation of cell morphology can characterize dynamic properties of cells such as migration ability. It can also reflect a stimulatory response in a living biological system, including dynamic measurements of cellular morphology as an indication of toxicity in drug screening[6].

NOTE There are cases where acquiring 3D-images and time series images includes specific sample preparation and device handling, which are not covered in this document[7-9].

5.2 Steps for cell morphometry

Table 1 describes the steps for cell morphometry.

Table 1 — Steps for cell morphometry

Step#	Summary	Relevant clause
Step-1	Determine purpose of the cell morphology analysis	- (out of scope)
Step-2	Define appropriate TOI(s) for the intended purpose	6
Step-3	Select observation methods and sample preparation according to the TOI(s)	7.1
Step-4	Select or establish microscope system	7.2, 7.3
Step-5	Adjust settings of microscope system in order to acquire images	7.2, 7.3
Step-6	Perform segmentation (including pre/post image processing) of the TOI(s) from acquired images	8
Step-7	Select appropriate cell morphological descriptors which characterize segmented TOI(s) and determine their numerical values	9, 10
Step-8	Preparation of a report for results of morphometric analysis	11
Step-9	Analysis with quantified results for the intended purpose	(out of scope)

6 Target of interest (TOI)

The TOI should be defined according to intended purpose of the morphology analysis.

The TOI should be defined before conducting the image capture step. This is important to select observation methods, microscope components and image capture devices, and their settings. Imaging conditions can be optimized by visual observation.

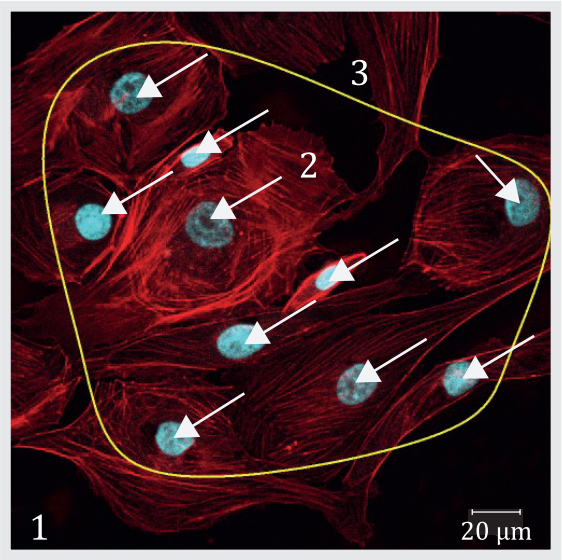
Images should not be optimized for human perception but for downstream image processing and analysis.

NOTE When the image capture conditions are determined by human perception, and when the image is checked after capture, the brightness, capture position, focus, image resolution, and other conditions can vary, which makes it difficult to analyse the image.

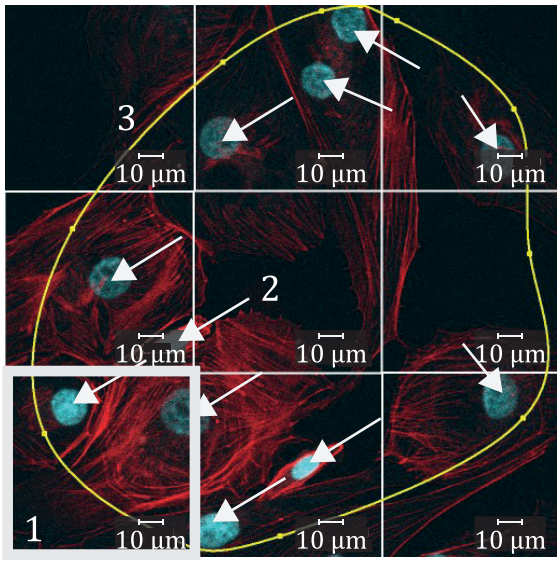
Figure 1 describes the relationship between FOV, TOI, and ROI.

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024



a) Relationship between FOV, ROI, and TOI in a single image made of a single FOV



b) Relationship between FOV, ROI, and TOI in a tiled image made of 9 FOVs

Key

1 FOV

2 TOI

3 ROI

NOTE For the purpose of Figure 1, TOI is cell nucleus, and ROI is set to include TOIs.

Figure 1 — Relationship between FOV, TOI, and ROI

7 Image capture

7.1 Microscopic observation method

7.1.1 General

Optical microscopy is one of the most widely applied method for cell observation. Manual microscopy has been used in the past but is currently replaced by digital imaging and use of information technology[10].

In order to observe cells, users should consider the contrast of their sample and select methods (and microscope system) that support sufficient contrast and resolution for imaging.

A contrast-enhancing technique for optical microscopy should be selected so that the TOI can be visualized in sufficient contrast for the intended purpose prior to initiating measurements. Contrast-enhancing techniques that utilize difference in refractive indices, such as phase contrast or differential interference can be used. Staining (labelling, dyeing) can also be used. In addition, a method combining transmitted light observation and digital image processing can be used. Users should be aware that those techniques can influence morphometric measurements.

A microscope system including its components should be selected so that the TOI can be visualized in sufficient spatial and temporal resolution for the intended purpose.

NOTE 1 Understanding the dimension of the TOI helps the proper selection and settings for microscope system.

NOTE 2 Proper system and its adjustments of settings can be selected using predetermined reference materials for visual observation, such as positive/negative control cells or cell images. The points to consider for the selection are listed in 7.1.2. A summary is given in Table A.1.

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

NOTE 3 Points to consider when acquiring phase-contrast images of cells suitable for image analysis are described in Annex E. Checklists for adherent cells that do not form colonies, adhesive cells that form colonies, and spheroids are given in Tables E.1, E.2, and E.3, respectively.

The microscopic observation method shall be documented.

7.1.2 Cell properties to be observed

7.1.2.1 Adherence and suspension

Microscopic observation method should be selected while taking the cell culturing condition (e.g adherence, floating, or suspension) into account.

Cells adhered to the vessel wall tend to form a thin layer(s). Forming of a thin layer(s) decreases contrast of these cells. Therefore, appropriate contrast-enhancing techniques should be applied (see Table A.1). Assuming a flat homogenous substrate/image field, change in the location of cells during observation has little impact on the quality of the acquired image of these cells.

Since cells suspended in the culture medium can change their locations during the observation, the observation techniques should be selected in consideration of the temporal resolution.

7.1.2.2 Stack and stratification

Layering, stratification, and three-dimensional (3D) structuring of cells can affect cell observation.

NOTE 1 Most transmission-type methods are not adequate for observation of deep 3D structures because the illumination light is scattered, and less light is transmitted through the sample. Imaging with infrared light can reduce the influence of scattered light.

NOTE 2 When single-layer or multi-layer cells have significant variations in cell-layer thickness and height, a "halo" image effect appears around the cells. This effect lowers image quality in phase-contrast observation.

NOTE 3 For fluorescence-type measurements, light emitted from cell outside the imaging plane can reduce image quality. This problem can be reduced using confocal microscopy or related techniques. It is important to be aware that changing of microscope type can change performance characteristics/traceability.

7.1.2.3 Intracytoplasmic structures

Intracytoplasmic structures (such as pigments, granules, and vacuoles) can affect image capture. Intracytoplasmic structures increase cell contrast, therefore cells with sufficient amount of intracytoplasmic structures can be observed not only by phase-contrast, differential-interference methods but also by other transmitted light methods such as a brightfield.

7.1.3 Sample preparation

7.1.3.1 Sample preparation - general

Cell cultures are affected by the surrounding environment e.g., ambient temperature, humidity, CO_2 concentration. Changes in those conditions can lead to morphological changes of the cells up to cell death, in some cases. Therefore, care should be taken that the observation environment is equivalent to the culture environment, and that the cells do not undergo morphological changes during observation.

In addition, since morphological features of the cell changes depending on cell density and passages during culture, care should be taken to use experimental conditions which fit the intended purpose.

In the case when a portion of cells is taken out from the cell population and used for observation, it should be considered depending on the intended purpose, whether that portion includes the cells to be observed or whether that portion is representative of the whole cell population. The intended purpose should be described.

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

In the case when a portion of cells is transferred from the cell population to other vessels, procedures, materials such as type of vessels and pipetting devices, and reagents applied for the transfer should be properly selected.

Methods that can be applied for the observation depend on whether the observed or image-acquired cells are to be discarded, continued in culture for further observation, or used for other purposes.

Procedure and condition of sample preparation shall be documented.

7.1.3.2 Sample preparation for specific observation - Labelling, dyeing, and chemical treatments

7.1.3.2.1 General

Histochemical staining, fluorescent labelling, immunostaining, or other chemical treatments can contribute to TOI determination. Therefore, appropriate reagents and absorption/excitation wavelength and microscope components should be selected. Histochemical staining, fluorescent labelling, immunostaining, or other chemical treatments can alter cell membrane and nuclear properties, as well as other cell characteristics. As a result, the morphological characteristics of the treated cells can be different from those of untreated living cells.

If cells are to be used after microscopic observation, a processing method that minimally interferes with the intended use shall be applied.

7.1.3.2.2 Cell fixation

When fixing cells using a chemical fixative, a method that preserves cellular structures of interest should be applied.

NOTE Chemical treatments such as cell fixation can alter cell membrane and nucleus properties, as well as other cell characteristics. As a result, morphological features of treated cells can differ from those of living, untreated cells. Further guidance can be found in ISO 20166-4.

7.1.3.2.3 Fluorescent labelling or immunostaining

When applying a staining process, the intensity of staining should be sufficient to allow the signal to be detected over background.

NOTE 1 The term “detection” in the above sentence includes detection by visual observation and that by using an image capture device. There are cases where subvisible dyes, i.e. dyes that are not seen with visual observation, can be detected by using image capture device.

NOTE 2 If the light intensity is increased as a countermeasure to the insufficient staining, autofluorescence or phototoxicity can occur.

Non-specific staining can occur depending on the nature of the labelling or dyeing procedure, e.g., concentration of the reagents used.

Some types of staining also affect cell characteristics, making it impossible to continue culturing after observation and imaging.

Elapsed time and culture environment can affect cell activity and sensitization during observation or cause a photochemical change of the stained material.

Labels used for immunostaining or other fluorescent labelling shall be documented. This should include their excitation and emission characteristics.

If there is an intention to continue the cell culture after cell staining, the effect of cell staining on the culturability of the cells should be considered.

7.1.3.3 Observation vessels

The material and design of vessel applied for microscopy can affect the observation of cells.

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024

In the case when same vessel is used both for cell culture and observation, its material and surface treatment should be suitable for both purposes.

On the other hand, there are cases that culturing is performed applying vessels which are incompatible for microscopy. When vessel is changed for observation in such cases, it is necessary to make sure that the state and morphological features of the cells are not affected by transportation between the vessels.

NOTE 1 The observation vessel is sometimes referred to as the sample holder, where the sample holder is the device (or sample carrier, container or vessel) on which the specimen was mounted, cultured, or adhered for microscopical examination. It is typically flat and can be made of glass or plastic (e.g., microscope slides, petri dishes, and multi-well plates)[11,12].

NOTE 2 Vessels made of polymeric material are often not appropriate for differential interference microscopy because of birefringence.

A vessel whose thickness entering the optical path is less than the working distance should be used.

NOTE 3 Working distance, i.e., the distance from a front lens element of the objective to a target of interest when the target of interest is in focus, depends on the optical design of the objective, such as magnification and NA.

7.1.3.4 Contamination

In the case when the cell sample is to be applied for subsequent culture after observation, measures should be taken to avoid contamination with microorganisms during the entire process from sample extraction, preparation, observation, and return to the culture environment.

In cases where cells cannot be automatically observed in the culture environment, it is recommended that a sample be removed from the culture, if possible, to avoid contamination of cells that will continue to undergo culturing.

7.2 Microscope system and its settings

7.2.1 General

Microscope system consists of light source, objective lens, image capture device, stage and a set of other filters and mirrors. The configuration of the instrument depends on the specific purpose of observation.

NOTE 1 This document presupposes that the microscope system is maintained properly.[13] For this, several resources exist. For example, traceable microscopic rulers and calibrated graticules can be used to calibrate size measurements[4,14]. Normalizations for non-uniformity of the field can be applied[3,5].

The spatial resolution of the image is determined by the combined optical resolution of the lens and the pixel resolution of the image capture device. Captured image of the ROI needs sufficient spatial resolution. The camera should have the pixel resolution necessary to achieve it.

NOTE 2 The optical resolution of the lenses depends on its NA. The camera resolution is determined by the size of the pixel and magnification.

Points to consider for each component depend on specific microscopic observation method.

The points to consider when selecting a microscope system and its settings are given in 7.2.2 to 7.2.9. A summary is given in Table B.1.

© ISO 2024 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS ISO 24479:2024