



**International
Standard**

ISO 24479

**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*

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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 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).

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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.

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.

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.]

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.]

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.]

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

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)

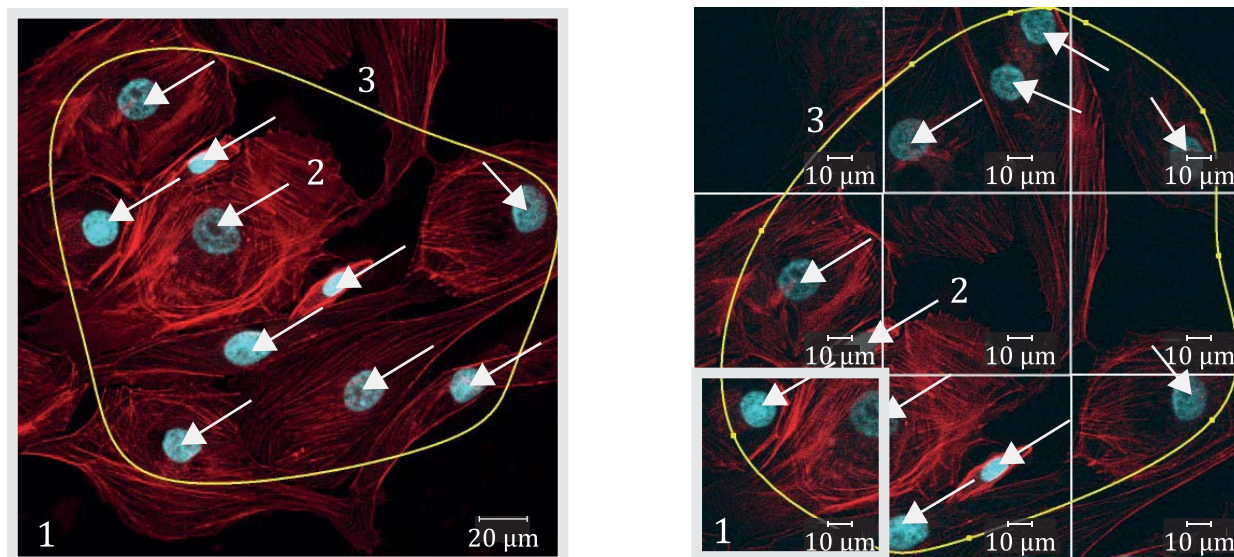
The TOI should be defined according to intended purpose of the morphology analysis.

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.



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](#).

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, CO₂ 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.

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.

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.

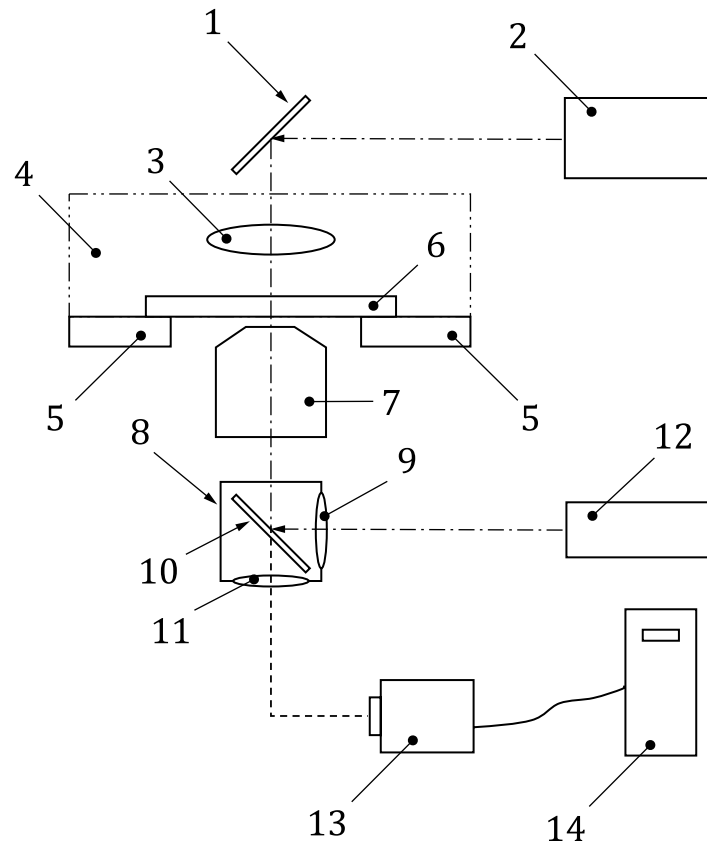
NOTE 1 This document presupposes that the microscope system is maintained properly. [\[13\]](#) For this, several resources exist. For example, traceable microscopic rulers and calibrated graticules can be used to calibrate size measurements [\[4,14\]](#). Normalizations for non-uniformity of the field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



Key

- 1 mirror
- 2 light source
- 3 condenser lens
- 4 environment
- 5 stage
- 6 sample
- 7 objective lens
- 8 set of fluorescent filters
- 9 excitation filter
- 10 dichroic mirror
- 11 fluorescence filter
- 12 light source
- 13 image capture device
- 14 image storage device

Figure 2 — Example of microscope system

If the initially obtained image does not sufficiently differentiate or characterize features, one should consider whether the microscope or system settings should be readjusted for increased image quality, the sample preparation process is sufficient, or the approach is otherwise not fit for purpose.