
**Microscopes — Microscopes
with digital imaging displays —
Information provided to the user
regarding imaging performance**

*Microscopes — Microscopes avec écrans d'affichage numérique —
Informations fournies à l'utilisateur concernant la performance
d'affichage*

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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.

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The committee responsible for this document is ISO/TC 172, *Optics and photonics*, Subcommittee SC 5, *Microscopes and endoscopes*.

Microscopes — Microscopes with digital imaging displays — Information provided to the user regarding imaging performance

1 Scope

This International Standard specifies the minimum information to be provided to the user by the manufacturers of microscopes with digital displays regarding imaging performance.

It further specifies terms and definitions for describing the optical performance of the digital imaging path of microscopy systems including the observation of the image on digital displays.

NOTE Terms and definitions for the direct visual observation with eyepieces are specified in ISO 8039 and ISO 10934-1.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10934-1, *Optics and optical instruments — Vocabulary for microscopy — Part 1: Light microscopy*

ISO 10934-2, *Optics and optical instruments — Vocabulary for microscopy — Part 2: Advanced techniques in light microscopy*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 10934-1, ISO 10934-2 and the following apply.

NOTE Terms shown in italics within a definition or a note are defined elsewhere within this International Standard or in ISO 10934.

3.1

digital microscopy system

instrument consisting of an *objective*, an *image sensor* (3.2) and a *digital display* (3.3) to make visible minute details that are not seen with unaided eye

Note 1 to entry: The system might include means for image enhancement, image analysis, archiving, etc.

3.2

image sensor

device to transform an optical image into a *digital image*

3.3

digital display

output device for the visual presentation of a *digital image* and other information

3.4

resolving power limit

maximum spatial frequency distinguishable in object space, expressed in lp/mm

Note 1 to entry: The resolving power actually achieved will always be lower than the resolving power limit due to reduced contrast, moiré patterns, etc. In addition, it depends on sensor type, object structure, illumination, etc.

3.5

pixel pitch

distance between the centres of adjacent pixels (picture elements) in an *image sensor* (3.2) or a *digital display* (3.3)

3.6

line pair

one line and one space between the lines of a spatial grid with equal line thickness and space width between lines

3.7

viewing distance

distance between the *digital display* (3.3) and the observer's eye

4 Symbols and abbreviated terms

c_1	coefficient related to the operation mode of the image sensor
$d_{AX\ SYS}$	depth of field for observation of the image presented on the digital display
d_{VIEW}	actual viewing distance
$d_{VIEW\ USEF}$	useful range of viewing distance
$h_{LIM\ DIS}$	height of the object field limited by the image area in the display
$h_{LIM\ SEN}$	height of the object field limited by the sensor
h_{SYS}	height of the object field of the microscopy system
λ	wavelength
M_{DIS}	display lateral magnification
$M_{DIS\ VIS}$	visual display magnification
$M_{DIS\ VIS\ USEF}$	useful range of the perceived visual display magnification
$M_{TOT\ PROJ}$	lateral magnification at the image projected onto the sensor
$M_{TOT\ VIS}$	total visual magnification of the virtual microscope image formed by the eyepiece
NA	numerical aperture
$N_x\ DIS$	number of image pixels in the display in x -direction
$N_x\ SEN$	number of active sensor pixels in x -direction
$N_y\ DIS$	number of image pixels in the display in y -direction
$N_y\ SEN$	number of active sensor pixels in y -direction
n	refractive index of the object or of the immersion media
pp_{DIS}	pixel pitch of the digital display
pp_{SEN}	pixel pitch of the image sensor
RL_{DIS}	display resolving power limit in object space
RL_{OPT}	optical resolving power limit in object space
RL_{SEN}	sensor resolving power limit in object space
RL_{SYS}	system resolving power limit in object space
$w_{LIM\ DIS}$	width of the object field limited by the image area in the display
$w_{LIM\ SEN}$	width of the object field limited by the sensor
w_{SYS}	width of the object field of the microscopy system

5 Magnification

5.1 Optical magnification, $M_{TOT PROJ}$

$M_{TOT PROJ}$ is the lateral magnification at the image projected onto the image sensor. It should be expressed in proportional form, e.g. 10:1.

5.2 Display magnification, M_{DIS}

M_{DIS} is the lateral magnification at the digital image presented on the digital display or monitor. It should be expressed in proportional form, e.g. 100:1.

NOTE The display magnification, M_{DIS} , is the ratio of a given distance in the image presented on the monitor to the corresponding distance in the object.

5.3 Visual display magnification, $M_{DIS VIS}$

$M_{DIS VIS}$ is the display lateral magnification by observing the digital image presented on the digital display. It should be expressed in numerical form with the multiplication sign, e.g. 50×.

The visual display magnification is given by:

$$M_{DIS VIS} = \frac{M_{DIS} \cdot 250}{d_{VIEW}} \quad (1)$$

where

- $M_{DIS VIS}$ is the visual display magnification;
- M_{DIS} is the display lateral magnification;
- 250 is the reference viewing distance in mm;
- d_{VIEW} is the actual viewing distance in mm.

NOTE The value of $M_{DIS VIS}$ is comparable with the value of $M_{TOT VIS}$ of light microscopes with eyepieces.

6 Resolving power limit

6.1 Optical resolving power limit, RL_{OPT}

RL_{OPT} is the lateral resolving power limit in object space that is limited by the optical system. It should be expressed in line pairs per millimetre, e.g. 200 lp/mm. It is given by:

$$RL_{OPT} = \frac{2 \cdot NA \cdot 10^6}{\lambda} \quad (2)$$

where

- RL_{OPT} is the optical resolving power limit in object space in line pairs per millimetre;
- NA is the effective numerical aperture at object side of the optical system projecting the image onto the sensor;
- λ is the wavelength in nm.

NOTE 1 The formula for RL_{OPT} does not apply for super-resolution.

NOTE 2 In consideration of consistency with the definition of resolving limit of electric equipment, the optical resolving limit defined in this International Standard is based on the cut-off frequency of an optical system.

6.2 Sensor resolving power limit, RL_{SEN}

RL_{SEN} is the lateral resolving power limit in object space that is limited by the image sensor. It should be expressed in line pairs per millimetre, e.g. 100 lp/mm. It is given by:

$$RL_{SEN} = \frac{500 \cdot M_{TOT\ PROJ}}{c_1 \cdot pp_{SEN}} \quad (3)$$

where

RL_{SEN} is the sensor resolving power limit in object space in line pairs per millimetre;

$M_{TOT\ PROJ}$ is the lateral magnification at the image projected onto the sensor;

c_1 is a coefficient related to the operation mode of the image sensor;

pp_{SEN} is the pixel pitch of the image sensor in μm .

EXAMPLES for c_1

1 corresponds to a standard or full frame mode;

2 corresponds to a 2×2 binning mode;

3 corresponds to a 3×3 binning mode;

0,5 corresponds to a pixel shift mode with a lateral movement of the image sensor of $0,5 pp_{SEN}$.

6.3 Display resolving power limit, RL_{DIS}

RL_{DIS} is the lateral resolving power limit in object space that is limited by the digital display. It should be expressed in line pairs per millimetre, e.g. 50 lp/mm. It is given by:

$$RL_{DIS} = \frac{M_{DIS}}{2 \cdot pp_{DIS}} \quad (4)$$

where

RL_{DIS} is the display resolving power limit in object space in line pairs per millimetre;

M_{DIS} is the display lateral magnification;

pp_{DIS} is the pixel pitch of the digital display in mm.

6.4 System resolving power limit, RL_{SYS}

RL_{SYS} is the lateral resolving power limit in object space of the microscopy system. It should be expressed in line pairs per millimetre, e.g. 50 lp/mm. Its value corresponds to the smallest value of either the optical resolving power limit or the sensor resolving power limit or the display resolving power limit, for example:

$$RL_{SYS} = \min(RL_{OPT}; RL_{SEN}; RL_{DIS}) \quad (5)$$

where

RL_{SYS} is the system resolving power limit in object space in line pairs per millimetre;

RL_{OPT} is the optical resolving power limit in line pairs per millimetre;

RL_{SEN} is the sensor resolving power limit in object space in line pairs per millimetre;

RL_{DIS} is the display resolving power limit in object space in line pairs per millimetre.

NOTE If RL_{SYS} is smaller than RL_{OPT} , the image quality might be limited by moiré effects, especially when inspecting periodically structured specimens.

7 Useful range of magnification

7.1 Useful range of viewing distance

Within the useful range of viewing distance, resolved details are seen by the observer's eye under an angle between 2,3' and 4,6' (minutes of arc). Its value is given by:

$$d_{VIEW\ USEF} = \frac{60}{(2,3..4,6)} \cdot \frac{180}{\pi} \cdot \frac{M_{DIS}}{RL_{SYS}} \quad (7)$$

where

$d_{VIEW\ USEF}$ is the useful range of viewing distance in mm;

2,3 .. 4,6 is the range of angles in minutes of arc that allows to resolve 1 line pair by the observer's eye;

M_{DIS} is the display lateral magnification;

RL_{SYS} is the system resolving power limit in object space in line pairs per millimetre.

7.2 Useful range of visual magnification

The perceived visual magnification is within the useful range of magnification if the actual viewing distance is in its useful range. The value of the useful range of magnification is given by:

$$M_{DIS\ VIS\ USEF} = \frac{(2,3..4,6)}{60} \cdot \frac{\pi}{180} \cdot 250 \cdot RL_{SYS} = \frac{RL_{SYS}}{(3..6)} \quad (8)$$

where

$M_{DIS\ VIS\ USEF}$ is the useful range of the perceived visual display magnification;

2,3 .. 4,6 is the range of angles in minutes of arc that allows to resolve 1 line pair by the observer's eye;

250 is the reference viewing distance in mm;

RL_{SYS} is the system resolving power limit in object space in line pairs per millimetre.

NOTE 1 $M_{DIS\ VIS\ USEF}$ is comparable with the useful range of magnification for visual observation of light microscopes with eyepieces which is usually taken to lie between 500 and 1 000 times the numerical aperture of the objective.

NOTE 2 When the actual value of the perceived visual display magnification is less than the lower limit of $M_{DIS\ VIS\ USEF}$, then the resolving power limit of the digital microscopy system cannot be fully utilized.

7.3 Empty magnification

Empty magnification occurs when the actual value of the perceived visual display magnification is higher than the upper limit of $M_{DIS VIS USEF}$ or when the actual viewing distance is shorter than the lower limit of useful range of viewing distance.

NOTE Exceeding the range of useful magnification gives no further information about the object.

8 Object field

8.1 Object field limited by the sensor

The sensitive area of the sensor is of rectangular shape and is usually expressed by the number of active pixels in x -direction times y -direction, e.g. $1\,920 \times 1\,280$. The dimensions of this area are the number of pixels times the pixel pitch. The object field limited by the sensor is the conjugate area of the sensitive sensor area in object space. It should be expressed in mm in the form “width \times height”, e.g. $1,6\text{ mm} \times 0,9\text{ mm}$ and it is given by:

$$w_{LIM\ SEN} \times h_{LIM\ SEN} = \frac{pp_{SEN} \cdot (N_{x\ SEN} \times N_{y\ SEN})}{1000 \cdot M_{TOT\ PROJ}} \quad (9)$$

where

$w_{LIM\ SEN}$ is the width of the object field limited by the sensor in mm;

$h_{LIM\ SEN}$ is the height of the object field limited by the sensor in mm;

pp_{SEN} is the pixel pitch of the image sensor in μm ;

$N_{x\ SEN}$ is the number of active sensor pixels in x -direction;

$N_{y\ SEN}$ is the number of active sensor pixels in y -direction;

$M_{TOT\ PROJ}$ is the lateral magnification at the image projected onto the sensor.

Sometimes, the number of pixels of the sensor is called “sensor resolution”. This term should not be used in order to avoid any confusion with the resolving power.

8.2 Object field limited by the display

The image area in the display is of rectangular shape and is usually expressed by the number of image pixels in x -direction times y -direction, e.g. $1\,024 \times 768$. It might be smaller than the full display area. The dimensions of the image area are the number of pixels times the pixel pitch. The object field limited by the display is the image area divided by the display lateral magnification. It should be expressed in mm in the form “width \times height”, e.g. $1,2\text{ mm} \times 0,9\text{ mm}$ and it is given by:

$$w_{LIM\ DIS} \times h_{LIM\ DIS} = \frac{pp_{DIS} \cdot (N_{x\ DIS} \times N_{y\ DIS})}{M_{DIS}} \quad (10)$$

where

$w_{LIM\ DIS}$ is the width of the object field limited by the image area in the display in mm;

$h_{LIM\ DIS}$ is the height of the object field limited by the image area in the display in mm;

pp_{DIS} is the pixel pitch of the digital display in mm;

- $N_{x\text{ DIS}}$ is the number of image pixels in the display in x -direction;
- $N_{y\text{ DIS}}$ is the number of image pixels in the display in y -direction;
- M_{DIS} is the display lateral magnification.

Sometimes, the number of pixels of the digital display is called “display resolution”. This term should not be used in order to avoid any confusion with the resolving power.

8.3 Object field of the microscopy system

The object field of the microscopy system is of rectangular shape and should be expressed in mm in the form “width × height”, e.g. 1,2 mm × 0,9 mm. The values of width and height correspond to the smaller values of either the object field limited by the sensor or the object field limited by the display, for example:

$$w_{\text{SYS}} \times h_{\text{SYS}} = \min(w_{\text{LIM SEN}}; w_{\text{LIM DIS}}) \times \min(h_{\text{LIM SEN}}; h_{\text{LIM DIS}}) \quad (11)$$

where

- w_{SYS} is the width of the object field of the microscopy system in mm;
- h_{SYS} is the height of the object field of the microscopy system in mm;
- $w_{\text{LIM SEN}}$ is the width of the object field limited by the sensor in mm;
- $w_{\text{LIM DIS}}$ is the width of the object field limited by the image area in the display in mm;
- $h_{\text{LIM SEN}}$ is the height of the object field limited by the sensor in mm;
- $h_{\text{LIM DIS}}$ is the height of the object field limited by the image area in the display in mm.

NOTE It is assumed that the optical system neither introduces vignetting nor limits the object field of the digital microscopy system.

9 Depth of field

The axial depth of the space on both sides of the object plane within which the object can be moved without detectable loss of sharpness in the digital image presented on the display should be expressed in μm . Its value is given by:

$$d_{\text{AX SYS}} = \frac{n \cdot \lambda}{1000 \cdot NA^2} \quad (12)$$

where

- $d_{\text{AX SYS}}$ is the depth of field for observation of the image presented on the digital display in μm ;
- n is the refractive index of the object or of the immersion media;
- λ is the wavelength in nm;
- NA is the effective numerical aperture of the optics projecting the image onto the sensor;

If RL_{SYS} is not equal to RL_{OPT} , the manufacturer shall indicate that the actual depth of field might be different. The stated value shall still be calculated with [Formula \(12\)](#).

NOTE If RL_{SYS} is not equal to RL_{OPT} , the depth of field usually increases as compared with the value given by the [Formula \(12\)](#).^[3]

10 Information to be provided to the user by manufacturers

10.1 General information

The manufacturer of the microscopy system should provide a system description including the necessary data of the components in order to allow the user to reproduce the imaging performance data mentioned in [Clauses 5 to 9](#).

In the case of optional components or multiple operation modes, all specified configurations shall be covered.

The format of the data shall be in accordance with the format described in [Clauses 5 to 9](#).

10.2 Imaging performance data

The manufacturer of the microscopy system shall provide the following imaging performance data for all specified configurations. Where magnifications are continuously changeable, all data shall be given at least for the maximum and minimum values.

- a) Display lateral magnification, M_{DIS} .
- b) Useful range of the perceived visual display magnification, $M_{DIS\ VIS\ USEF}$.
- c) System resolving power limit in object space, RL_{SYS} .
- d) Width of the object field of the microscopy system, w_{SYS} .
- e) Height of the object field of the microscopy system, h_{SYS} .
- f) Depth of field for observation of the image presented on the digital display, $d_{AX\ SYS}$.

NOTE These data may be compiled as shown in [Tables A.1 to A.5](#).

Annex A (informative)

Examples for information to be provided to the user

A.1 Microscopy system with optical zoom

A.1.1 Example for general information

Optical system consisting of the following:

- a) three exchangeable objectives: $5\times / 0,125$; $10\times / 0,25$; $20\times / 0,5$;
- b) optical zoom with a magnification range from $0,25\times$ to $1\times$. At $0,25\times$, the effective NA is reduced to 50 % of the objective NA ;
- c) iris diaphragm to balance resolution and depth of field with an opening range from 25 % to 100 % (100 % corresponds to the NA of the objective).

Imaging sensor with the following features:

- a) five megapixel RGB sensor;
- b) pixel pitch: $pp_{SEN} = 3,2 \mu\text{m}$;
- c) number of pixels in x -direction: $N_{x,SEN} = 2\ 600$;
- d) number of pixels in y -direction: $N_{y,SEN} = 1\ 950$;
- e) standard mode for full frame image capturing and 2×2 binning mode for fast live image;
- f) digital zoom with a magnification range from $1\times$ to $4\times$.

Digital display with the following features:

- a) HD display with an aspect ratio of 16:9 and $1\ 920 \times 1\ 080$ pixels;
- b) pixel pitch: $pp_{DIS} = 0,27 \text{ mm}$;
- c) standard imaging mode with an active image window in aspect ratio 4:3 consisting of the following:
 - 1) number of pixels in x -direction: $N_{x,DIS} = 1\ 200$;
 - 2) number of pixels in y -direction: $N_{y,DIS} = 900$;
- d) full screen imaging mode with an aspect ratio of 16:9 consisting of the following:
 - 1) number of pixels in x -direction: $N_{x,DIS} = 1\ 920$;
 - 2) number of pixels in y -direction: $N_{y,DIS} = 1\ 080$.

A.1.2 Examples for imaging performance data

A.1.2.1 Standard imaging mode

Standard imaging mode in aspect ratio 4:3, full frame image capturing and open iris diaphragm.

Table A.1 — Standard imaging mode

Objective	Optical zoom	Digital zoom	Display magnification M_{DIS}	Useful range of visual magnification $M_{DIS\ VIS\ USEF}$ from .. to	System resolving power limit RL_{SYS} lp/mm	Object field $w_{SYS} \times h_{SYS}$ mm × mm	Depth of field $d_{AX\ SYS}$ µm
5×	0,25×	1×	49:1	15× .. 30×	90 ^b	6,66 × 4,99	141 ^a
10×	0,25×	1×	97:1	30× .. 60×	180 ^b	3,33 × 2,50	35 ^a
20×	0,25×	1×	195:1	60× .. 120×	361 ^b	1,66 × 1,25	8,8 ^a
5×	1×	1×	195:1	60× .. 120×	361 ^b	1,66 × 1,25	35 ^a
10×	1×	1×	389:1	120× .. 240×	721 ^b	0,83 × 0,62	8,8 ^a
20×	1×	1×	779:1	240× .. 481×	1 442 ^b	0,42 × 0,31	2,2 ^a
5×	1×	4×	779:1	76× .. 152×	455	0,42 × 0,31	35
10×	1×	4×	1 558:1	152× .. 303×	909	0,21 × 0,16	8,8
20×	1×	4×	3 115:1	303× .. 606×	1 818	0,10 × 0,08	2,2

This table is created using coefficients $c_1 = 1$.

^a The actually perceived depth of field might be larger since $RL_{SYS} \neq RL_{OPT}$.

^b The display resolving power is the limiting factor since $RL_{SYS} = RL_{DIS}$.

A.1.2.2 Full screen imaging mode

Full screen imaging mode in aspect ratio 16:9, full frame image capturing and open iris diaphragm.

Table A.2 — Full screen imaging mode

Objective	Optical zoom	Digital zoom	Display magnification M_{DIS}	Useful range of visual magnification $M_{DIS\ VIS\ USEF}$ from .. to	System resolving power limit RL_{SYS} lp/mm	Object field $w_{SYS} \times h_{SYS}$ mm × mm	Depth of field $d_{AX\ SYS}$ µm
5×	0,25×	1×	78:1	24× .. 48×	144 ^b	6,66 × 3,74	141 ^a
10×	1×	1×	623:1	152× .. 303×	909	0,83 × 0,47	8,8
20×	1×	4×	4 985:1	303× .. 606×	1 818	0,10 × 0,06	2,2

This table is created using coefficients $c_1 = 1$.

^a The actually perceived depth of field might be larger since $RL_{SYS} \neq RL_{OPT}$.

^b The display resolving power is the limiting factor since $RL_{SYS} = RL_{DIS}$.