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Microscopes — Definition and measurement of illumination properties —

Part 1:
Image brightness and uniformity in bright field microscopy

Microscopes — Définition et mesurage des propriétés d'éclairage —

Partie 1: Luminosité et uniformité de l'image en microscopie à champ clair

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Foreword

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

ISO 19056 consists of the following parts, under the general title *Microscopes — Definition and measurement of illumination properties*:

— *Part 1: Image brightness and uniformity in bright field microscopy*

Microscopes — Definition and measurement of illumination properties —

Part 1:
Image brightness and uniformity in bright field microscopy

1 Scope

This part of ISO 19056 specifies procedures for the measurement of image brightness and uniformity for bright field light microscopy. These measurements for image brightness and uniformity are defined in image planes or intermediate image planes only, when these planes are suitable for detection by electronic imaging devices.

This part of ISO 19056 defines how image brightness and uniformity are measured and how this information is provided to the user.

NOTE The scope is intentionally limited to electronic imaging devices and (intermediate) image planes. The visual observation by means of eyepieces would require a different measurement procedure and hence result in unambiguities in the description of measurement procedures. Nevertheless, this part of ISO 19056 will give useful estimates for the image brightness with visual observation as in this case, an eyepiece is used to observe an intermediate image plane (which is under the scope of this part of ISO 19056).

2 Measurands

2.1 General

For obtaining the desired image quality when using a light microscope, the brightness and uniformity of the image play an important role. This holds true for different applications and various types of instruments.

Intentionally, this part of ISO 19056 is limited to bright field microscopy and detection by means of electronic imaging devices but within this limitation, this part of ISO 19056 does apply to transmitted or reflected light microscopy and conventional or laser illumination.

2.2 Brightness

The image shall be bright enough to allow the detection of the details of the object under investigation.

NOTE This is especially true for microscopic contrasting techniques like phase contrast, differential interference contrast, dark field microscopy, or fluorescence microscopy. Although these contrasting techniques are beyond the scope of this part of ISO 19056, the given concepts can be easily extended.

As this part of ISO 19056 is based on measurement procedures, the image brightness shall be expressed in the corresponding SI units. The photometric unit Illuminance (measured in lm/m^2) shall be used whenever the illumination source covers the visible spectral range (380 nm to 780 nm) continuously, which is usually the case for filament and halogen lamps as well as for phosphor-type white LEDs. The radiometric unit irradiance (measured in W/m^2) shall be used for all other illumination sources (like discharge arc lamps, lasers, and single colour LEDs) and are more generally applicable, e.g. to the ultraviolet and infrared spectral range.

2.3 Uniformity

The microscope's optical system shall achieve a certain degree of image uniformity in order to allow the detection of the details of the object under investigation. A severe drop of the image brightness towards the edge of the image field can result in brightness values that are not sufficient in the above mentioned sense.

Furthermore, spatial variations of the image brightness over the image field cannot always be distinguished from spatial variations of the properties of the object under investigation.

The uniformity of the brightness in the image field is expressed as

$$\mathit{Uniformity}\ (\%) = 100 \times \frac{\text{minimum brightness in image field}}{\text{maximum brightness in image field}} \qquad (1)$$

NOTE Depending on the size of the image field, the objective magnification and the size of the sensor pixels, it might be necessary to apply an adequate averaging method in the computation of the uniformity.

3 Measurement procedure

3.1 General

In addition to defining the measurement geometry and procedure, it is necessary to describe essential settings of the microscope in order to eliminate their influence to the measurement of image brightness and uniformity.

3.2 Diaphragm settings

In order to obtain results that are as unambiguous as possible, the settings of relevant diaphragms shall be defined as follows.

Any field diaphragm in the beam path shall be opened to the size that fully includes the desired image field or image sensor. If this is not possible, the smallest diaphragm defines the size of the field.

In addition to the aperture diaphragm, that is inherent to the objective lens, the measurement requires an additional aperture diaphragm that is placed into the microscope's illumination beam path, as the image brightness heavily depends on the aperture size.

This additional aperture diaphragm shall be opened to the size of the conjugated aperture size (NA) of the objective lens used as this size can be set quite exactly.

Setting the illumination aperture size to a smaller value (e.g. approximately 2/3 of the size of the objective's aperture as required for proper Köhler illumination) would result in high measurement uncertainties. Allowing for illumination aperture sizes larger than the objective's aperture would also lead to high measurement but furthermore increase the measured image brightness by only adding stray light.

Especially when using objective lenses with high numerical aperture, it is not always possible to increase the illumination aperture to the corresponding value. In such cases, the actual illumination aperture shall be indicated (see Clause 4).

3.3 Brightness (illuminance and irradiance)

The light flux is measured at the centre of the given image field using an integrating sphere. The centre of the image field shall be limited by means of a measurement aperture with a circular diameter of (4,0 ± 0,05) mm placed in the plane of the image. The integrating sphere shall have an entrance aperture of at least two times the diameter of the measurement aperture.

If the mechanical design of the microscope does not allow the entrance aperture of the integrating sphere to be placed in the plane of the image field, auxiliary optics may be used in order to project the image plane into the entrance aperture of the integrating sphere.

3.4 Uniformity

The measurement for uniformity shall be performed by using an electronic image sensor that can capture the whole image field. This electronic image sensor shall consist of at least 50 rows and columns of individual elements (pixels). Furthermore, the image sensor shall be designed for an entrance pupil at infinity.

If the mechanical design of the microscope does not allow the image sensor to be placed in the plane of the image field, no auxiliary optics shall be used. In this case, a uniformity value cannot be measured according to this part of ISO 19056.

This image sensor does not need to be calibrated in radiometric or photometric units as its output can be related to the brightness measurement described in 3.3. However, gamma correction shall not be applied to the signal from the image sensor in order to retain a linear relationship between irradiance and sensor signal (hence $\gamma = 1$).

3.5 Spectral information

Whenever the illumination source covers the visible spectral range (380 nm to 780 nm) continuously (as for halogen lamps or for phosphor-type white LEDs), additional spectral information can be omitted.

For all other illumination sources (like discharge arc lamps, lasers, and single colour LEDs), the image brightness and uniformity shall be referred to a specific spectral range. Examples are given in Clause 4.

4 Information provided to the user

If information on image brightness and uniformity is provided to the user, this information shall be given according to the definitions given in 3.3 and 3.4 and shall contain the following additional data:

a) information on the dimensions and shape of the image field, e.g.

   1) the image field is rectangular with a width of 10 mm and a height of 8 mm,

   2) the image field is circular and has a diameter of 20 mm, and

   3) the image uniformity is 80 % (at FN 17);

b) information on the microscope configuration, especially additional optical elements (like filters, objective lenses, condenser, etc.) that can be exchanged by the user but were part of the light train when measuring the illumination brightness:

   1) list configuration and additional part numbers;

   2) list especially light source, objective lens and condenser if not part of the configuration given in Clause 4 b);

c) information on the illuminating light source used and its relevant parameters (spectral properties, voltage or current, etc.);

d) information on the size of the illumination aperture and in particular, if this cannot be increased up to the NA value of the objective;

e) information on the spectral range that the measured image brightness and uniformity are measured in, e. g.

   1) 150 lm/m^2 in the visible spectral range,

2) 0,8 W/m^2 in the range of 850 nm to 880 nm, and

3) 1,1 W/m^2 at (532 ± 10) nm.

Bibliography

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

[2] ISO 12853, *Microscopes — Information provided to the user*

[3] ISO 80000 (all parts), *Quantities and units*

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