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Microscopes — Definition and measurement of illumination properties —

Part 2:
Illumination properties related to the colour in bright field microscopy

Microscopes — Définition et mesurage des propriétés d'éclairage —

Partie 2: Propriétés d'illumination liées à la couleur en microscopie à champ lumineux

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Foreword

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This document was prepared by Technical Committee ISO/TC 172, *Optics and photonics*, Subcommittee SC 5, *Microscopes and endoscopes*.

A list of all parts in the ISO 19056 series can be found on the ISO website.

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.

Microscopes — Definition and measurement of illumination properties —

Part 2: Illumination properties related to the colour in bright field microscopy

1 Scope

This document specifies measurands and measurement procedures of colour properties for bright field microscopy with transmitted light illumination. These measurements are defined in image planes or intermediate image planes.

This document also specifies how the information is provided to the user.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 11664-1:2019, *Colorimetry — Part 1: CIE standard colorimetric observers*

ISO 11664-3, *Colorimetry — Part 3: CIE tristimulus values*

3 Terms and definitions

No terms and definitions are listed in this document.

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

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

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

4 Measurands

4.1 General

Since various light sources such as halogen lamps, tungsten lamps, LEDs, and OLEDs are used for bright field microscopy, it is essential to understand the difference in colour properties due to the light sources. This applies for different applications and various types of instruments.

4.2 Spectral measurement

The spectral radiant flux (W) shall be measured by placing an instrument equipped with an integrating sphere and an optical spectrometer function in an image plane or in an intermediate image plane. The measurements shall be performed in the measurement spectral range from 380 nm to 780 nm with 5 nm intervals or less.

4.3 Chromaticity

The chromaticity coordinates (x, y) shall be calculated from the measured spectral properties by calculating the tristimulus values of the XYZ colour space (CIE 1931 colour space) by using the CIE 1931 2° colour-matching functions.

The tristimulus values X, Y and Z shall be defined according to ISO 11664-1 and ISO 11664-3 by the following Formulae (1) to (3):

$$X = k\int_{380}^{780} P(\lambda)\cdot\bar{x}(\lambda)\mathrm{d}\lambda \quad (1)$$

$$Y = k\int_{380}^{780} P(\lambda)\cdot\bar{y}(\lambda)\mathrm{d}\lambda \quad (2)$$

$$Z = k\int_{380}^{780} P(\lambda)\cdot\bar{z}(\lambda)\mathrm{d}\lambda \quad (3)$$

where

$\bar{x}(\lambda)$, $\bar{y}(\lambda)$ and $\bar{z}(\lambda)$	are the CIE 1931 2° colour-matching functions according to ISO 11664-1:2019, Table 1;
$P(\lambda)$	is the spectral radiant flux obtained by spectral measurements of the measurand;
k	is a constant defined according to Formula (4).

The constant k is defined as follows:

$$k = \frac{100}{\int_{380}^{780} P(\lambda)\cdot\bar{y}(\lambda)\mathrm{d}\lambda} \quad (4)$$

The chromaticity coordinates x and y are calculated from the tristimulus values X, Y, Z as given in Formulae (5) and (6):

$$x = \frac{X}{X+Y+Z} \quad (5)$$

$$y = \frac{Y}{X+Y+Z} \quad (6)$$

The diagram produced by plotting x as abscissa and y as ordinate is defined as the CIE (x,y) chromaticity diagram. An example is given in Figure 1.

According to ISO 11664-1 and ISO 11664-3, it is sufficient to use the values of colour-matching functions in the spectral range from 380 nm to 780 nm with 5 nm intervals in this document. Therefore, if the measurement of the spectral radiant flux is performed with an interval of less than 5 nm, the measured data shall be converted to 5 nm intervals by either using the least squares method or the data shall be extracted by 5 nm intervals only.

NOTE There are two types of colour-matching functions defined according ISO 11664-1, the CIE 1931 2° colour-matching functions and the CIE 1964 10° colour-matching functions. This document applies only the CIE 1931 2° colour matching functions which have been used in general.

4.4 Correlated colour temperature, T_{cp}

4.4.1 General

Although there are various methods available, for calculating the correlated colour temperature according to this document either the method described in 4.4.2 or 4.4.3 shall be used.

The colour temperature is an index to express the colour by a single parameter when the chromaticity of the light source is on the black body locus. However, actual light sources do not completely match with the black body radiation. Therefore, the correlated colour temperature, i.e. the colour temperature of the nearest colour on the black body locus shall be used.

4.4.2 Correlated colour temperature using the CIE 1960 UCS chromaticity diagram and its procedure

The tristimulus values of the XYZ colour space (CIE 1931 colour space), calculated from the spectral properties obtained by the spectral measurements and the CIE 1931 2° colour-matching functions according to 4.3 shall be converted to (u, v) chromaticity values on the CIE 1960 UCS chromaticity diagram.

The (u, v) chromaticity values are calculated by the following Formulae using the tristimulus values X, Y and Z[4].

$$u=\frac{4X}{X+15Y+3Z} \quad (7)$$

$$v=\frac{6Y}{X+15Y+3Z} \quad (8)$$

Afterwards, the correlated colour temperature shall be expressed as an absolute temperature which corresponds to the nearest point on the black body locus from the (u, v) chromaticity values in the diagram[4].

The correlated colour temperature shall only be indicated if the distance from the black body locus is ±0,05 or less in the (u, v) chromaticity diagram[4].

4.4.3 Correlated colour temperature using (x, y) chromaticity coordinates

The correlated colour temperature shall be obtained by Formula (9) proposed by McCamy[6].

$$T_{cp}=-437n^3+3601n^2-6861n+5514,31 \quad (9)$$

Where, n is defined as follows using the (x, y) chromaticity coordinates (according to 4.3).

$$n=\frac{x-0,3320}{y-0,1858} \quad (10)$$

The error caused by this approximation is regarded as negligible for practical use.

5 Measurement procedures

5.1 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 on the measurement results.

5.2 Measurement environment

The microscope system shall be placed in a darkroom, and careful attention shall be paid so that, for example, street light, sun light, and room light do not affect the measurands. The measurement shall be performed at an operation temperature as specified by the manufacturer since it is possible the light intensity and spectral properties can vary due to the heat, not only for halogen lamps but also for LEDs.

5.3 Integrating sphere

The integrating sphere shall be attached to the aperture port that corresponds to the image plane to be measured. The integrating sphere to be used shall be the one whose reflectance is high enough in the visible spectral range used for measurements and also the traceability of the spectral reflectance has been obtained in the spectral range from 380 nm to 780 nm with 5 nm intervals or less.

5.4 Microscope settings

5.4.1 Diaphragm settings

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

The field diaphragm of the illumination systems shall be limited to a circular diameter of approximately 10 mm in the image plane. The aperture diaphragm of the illumination systems shall be opened to the size of the conjugated pupil size matched with the numerical aperture (NA) of the objective lens to be used.

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 a case, the actual illumination aperture shall be indicated (also see Clause 6 "Information provided to the user").

5.4.2 Adjustment of samples and focusing for diaphragms

The focus, the field diaphragm, and the aperture diaphragm shall be adjusted using the slide glass with the cover glass. The spectral properties shall be measured at the location without samples.

5.4.3 Other combinations

If optical elements, such as filters, or units, such as magnification changers, are used in measurements, their names or product series names shall be given. In the case of binocular tube with two sleeves (right and left) for eyepieces, the measurements shall be performed respectively and both values for the chromaticity or the correlated colour temperature shall be provided.

5.4.4 Adjustment of light source

For measuring the chromaticity and the correlated colour temperature, the light source shall be adjusted according to the recommended operating condition given by the microscope manufacturer. When using a halogen lamp as a light source, the spectral properties vary significantly with the input voltage. Therefore, in order to obtain the specified white light, the appropriate (as recommended by the manufacture) input voltage and the light balancing filters shall be used.

NOTE For most of white LEDs, the colour slightly shifts toward violet as the light intensity increases due to the influence of the saturation absorption of the phosphor.

5.5 Stability of measurement

The measurement shall be performed under the condition where the variation of the light intensity and spectral properties of the light source are sufficiently ignored within the measurement time period using the optical spectrometer.

6 Information provided to the user

6.1 General

If the information on chromaticity or correlated colour temperature (T_{cp}) is provided to the user, this information shall be indicated by numeric values, or shall be indicated in the (x,y) diagram, and shall contain additional information with respect to the image plane position as well as the dimensions of image field, on the microscope configuration, and on the illumination sources and related parameters. Details are given in 6.2 and 6.3.

6.2 Information of chromaticity and correlated colour temperature

a) If the chromaticity and correlated colour temperature, T_{cp}, are expressed in numeric values;

1) The chromaticity shall be given with three decimal places.

EXAMPLE (x ,y) = (0,355 , 0,365)

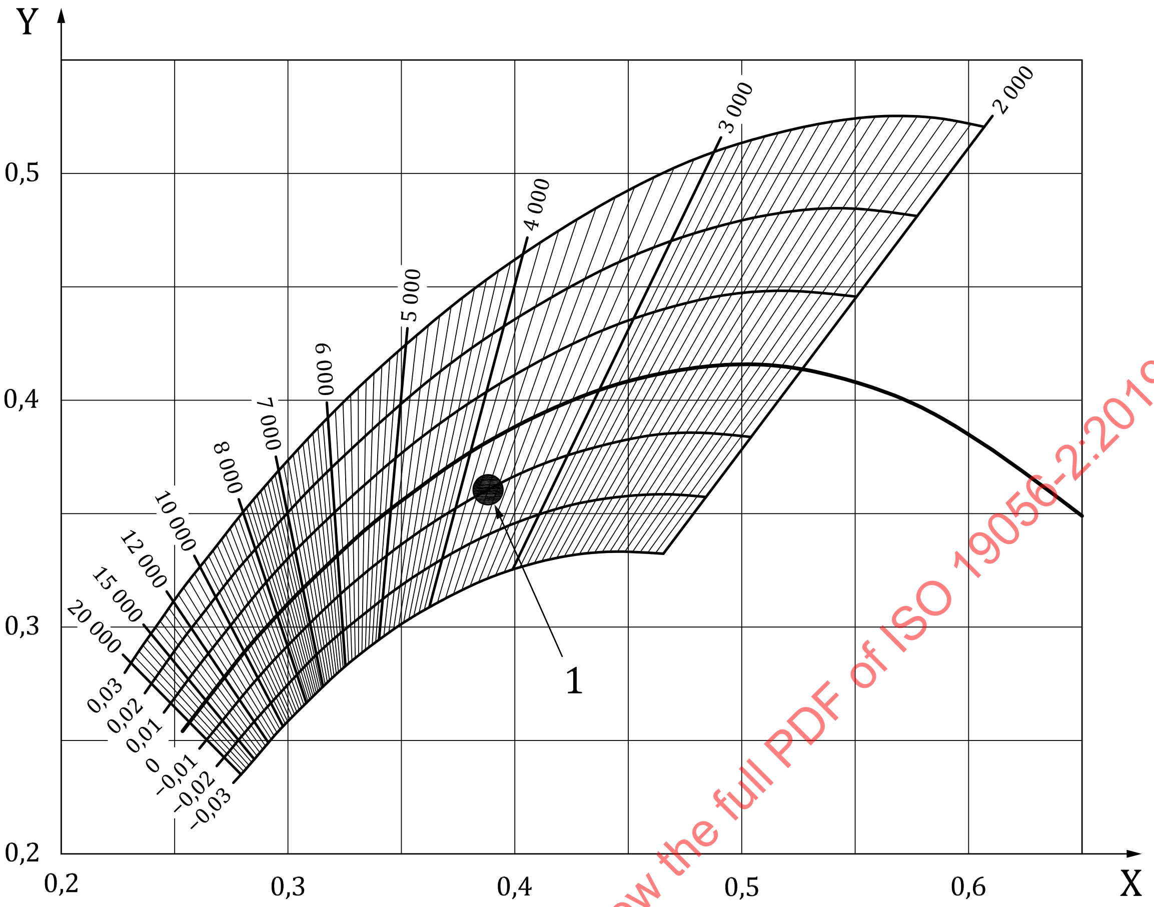
2) The correlated colour temperature, T_{cp}, shall be expressed at 50 K steps.

EXAMPLE T_{cp} = 5500 K

b) If the chromaticity and correlated colour temperature, T_{cp}, is expressed in diagrams;

The range shall be expressed using the CIE (x, y) chromaticity diagram. It is necessary to express the black body locus together with the isotemperature line/isanomal line in the (x, y) chromaticity diagram.

NOTE See Figure 1.



Key

X *x* coordinates of the chromaticity

Y *y* coordinates of the chromaticity

1 colour of illumination light

Figure 1 — Example of the CIE (x,y) chromaticity diagram

6.3 Additional information

The following additional information shall be provided to the user:

a) Information on the image plane position and the dimensions of the image field;

This information shall refer to the place where chromaticity and correlated colour temperature, T_{cp}, are measured.

b) Information on the microscope configuration;

The following information shall be included as they are significant, especially when additional optical elements (such as filters, objective lenses, condenser, etc.) are applied, since these optical elements can be exchanged by the user and become part of the light path.

1) List of configuration and the number of additional parts;

2) List of objective lens, condenser, binocular tube and light balancing filters as necessary.

3) The size of the illumination aperture, if this cannot be increased up to the NA value of the objective.

c) Information on the illumination sources;

This information shall include the type of light source and its spectral properties. For halogen lamps, the brightness settings shall be provided.