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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):

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

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

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

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:

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

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

$$x = \frac{X}{X+Y+Z} \tag{5}$$

$$y = \frac{Y}{X+Y+Z} \tag{6}$$

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.