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Microscopes — Definition and measurement of illumination properties —

Part 3: Incident light fluorescence microscopy with incoherent light sources

Microscopes — Définition et mesurage des propriétés d'éclairage — Partie 3: Microscopie par fluorescence à lumière incidente avec sources lumineuses incohérentes

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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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 documents 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 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.

Introduction

This document defines important quantities and methods to assess the quality of incident light fluorescence illumination for microscopes as well as methods to measure them. Details covered will enable users to compare different microscopes quantitatively with respect to their fluorescence illumination prior to a purchase decision and chose an appropriate instrument according to their application. Data obtained by following these methods also enables users to compare existing instruments with each other or to track performance variations over time, assuring proper performance. Additionally, what information is to be provided to the user is covered.

Microscopes — Definition and measurement of illumination properties —

Part 3: Incident light fluorescence microscopy with incoherent light sources

1 Scope

This document specifies procedures for the measurement of illumination brightness, temporal stability and uniformity for incident light fluorescence microscopy. The measurement for uniformity is defined in image planes or intermediate image planes only, when these planes are suitable for detection by electronic imaging devices.

This document defines how illumination brightness, temporal stability 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 ambiguities in the description of measurement procedures. Nevertheless, this document will give useful estimates for the uniformity with visual observation as in this case an eyepiece is used to observe an intermediate image plane (which is under the scope of this document).

2 Normative references

There are no normative references in this document.

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 https://www.iso.org/obp

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

4 Measurands

4.1 General

For obtaining the desired image quality, when using a light microscope, the brightness, temporal stability and uniformity of the image play an important role. This holds true for different applications and various types of instruments. The measurement of illumination brightness and uniformity is mandatory. The temporal stability is an optional measurement because it is only suitable for certain types of light sources (e.g. arc lamps).

4.2 Illumination brightness

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

As this document is based on measurement procedures, the image brightness shall be expressed in the corresponding SI unit. The radiometric unit irradiance shall be used because it is well suited for the essentially monochromatic or narrow-band illumination spectral range used in fluorescence microscopy.

The value is expressed as given by Formula (1):

$$E = \frac{P}{A} \tag{1}$$

where

E is the irradiance averaged over the area of the diaphragm used in W/mm^2;

P is the radiant flux recorded by the power meter in W;

A is the area of diaphragm used in mm^2.

4.3 Temporal stability

4.3.1 General

The temporal stability of the radiant flux is an important factor when conducting experiments in a time series. If experimental results are to be derived from variations in the image brightness, any variation in illumination brightness due to temporal instability shall not contribute to these results.

The temporal stability shall be expressed for at least one of two different time scales.

4.3.2 Short-term stability of radiant flux

The radiant flux shall be recorded for a time interval of 5 min. During this time interval the detector of the power meter is illuminated continuously and a power reading is recorded every second. The short-term stability of radiant flux, S_{short}, is expressed as a percentage by Formula (2):

$$S_{short} = 100 \times \left(1 - \frac{P_{max} - P_{min}}{P_{max} + P_{min}} \right) \tag{2}$$

where

S_{short} is the short term stability in percent;

P_{max} is the maximum radiant flux recorded during the time interval of 5 min;

P_{min} is the minimum radiant flux recorded during the time interval of 5 min.

Care should be taken that the light source is permanently on during the complete time interval of 5 min, because some light sources are only switched on during actual image acquisition.

4.3.3 Long term stability of radiant flux

The radiant flux shall be recorded for a time interval of 120 min. During this time interval the detector of the power meter is illuminated every 30 s for one second. During the one second illumination of the detector a power reading is recorded. The long term stability of radiant flux, S_{long}, is expressed as a percentage by the Formula (3):

$$S_{long} = 100 \times \left(1 - \frac{P_{max} - P_{min}}{P_{max} - P_{min}} \right) \tag{3}$$

where

S_{long} is the long term stability in percent;

P_{max} is the maximum radiant flux recorded during the time interval of 120 min;

P_{min} is the minimum radiant flux recorded during the time interval of 120 min.

4.4 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 condition.

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

The uniformity of the brightness, U, in the image field is expressed as a percentage by Formula (4):

$$U = 100 \times \frac{B_{IF,min}}{B_{IF,max}} \quad (4)$$

Where

U is the uniformity of brightness in the image field, in percent;

$B_{IF,min}$ is the minimum brightness in image field;

$B_{IF,max}$ is the maximum brightness in image field.

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 a suitable averaging method in the computation of the uniformity.

5 Measurement procedure

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 of illumination brightness, temporal stability and uniformity.

5.2 Microscope settings

The microscope shall be set up in the desired configuration regarding excitation wavelength (band), dichroic filters and/or emission filters.

If the microscope offers an adjustable field stop, it shall be set to the smallest setting for which the field of view of the imaging sensor in 5.5 is fully illuminated.

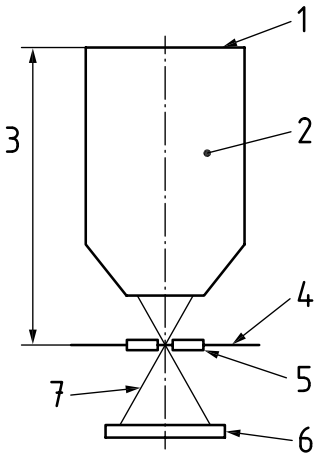
If the microscope offers an adjustable aperture stop, it shall be set to the smallest setting for which the pupil of the objective lens used is overfilled.

For the measurement of illumination brightness and temporal stability the illumination shall be warmed-up according to the manufacturer's specifications. If the manufacturer does not specify a warm-up time, the illumination shall be warmed-up for at least one hour prior to measurement. The light output of the illumination shall be set to its maximum value and, if a filter is used for extinction during measurement, this filter should be specified.

5.3 Illumination brightness

The measurement is performed with a ×10 objective and a calibrated power meter placed so that the detection area of the power meter is slightly underfilled with light. The detection range of the power meter shall be in the linear range. The detector of the power meter shall not be saturated. The integration time of the power meter shall be between 0,2 s and 1 s.

The focal plane of the objective shall include a circular, centred diaphragm of (0,4 ± 0,1) mm in diameter. Figure 1 shows the position of the power meter during the measurement.



Key

1 objective-locating surface of the nosepiece
2 ×10 objective
3 parfocalizing distance of the objective
4 focal plane
5 circular diaphragm
6 detection area of power meter
7 marginal ray

Figure 1 — Position of power meter during measurement

NOTE 1 Because the actual measured value of the area of the diaphragm enters the calculation for illumination brightness in 4.2 the diameter need not have a tight tolerance.

NOTE 2 If no diaphragm is used, the measured value of radiant flux can still be used to compare the same instrument at different points in time.

5.4 Temporal stability

The measurement is performed with a ×10 objective and a power meter placed so that the detection area of the power meter is underfilled with light. The detection range of the power meter shall be in the linear range. If a ×10 objective is not available, the measurement shall be performed without an objective and the power meter shall be placed at the position of the pupil of the illumination, thus the objective needs to be removed. The power meter shall be mounted in a fixed position with regard to the objective or the objective nosepiece respectively. External influences on the measurement, e.g. changes in room temperature or changes in the ambient lighting conditions, have to be eliminated. Figure 2 shows an alternative position of the power meter when used without objective.

The detector of the power meter shall not be saturated. The integration time of the power meter shall be between 0,2 s and 1 s.