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Microscopes — Vocabulary for light microscopy

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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*.

This first edition cancels and replaces ISO 10934-1:2002 and ISO 10934-2:2007, which have been combined and technically revised.

The main changes compared to the previous edition are as follows:

— update of the title;

— added new terms for light microscopy: focal length of normal tube lens, objective field number, pixel, pixel size, Airy unit, excitation wavelength, excitation wavelength band, detection wavelength band, OSTD added as new terms;

— added new terms for advanced techniques in light microscopy: coherent anti-stokes Raman scattering microscopy, stimulated Raman scattering microscopy, structured illumination microscope, super-resolution microscopy, localization microscopy, stimulated emission depletion microscopy, super-resolution SIM, light sheet microscopy, digital holographic microscopy, optical coherence (tomography) microscopy;

— Terms amended: diffraction limit of resolving power, resolution;

— Editorially revised.

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 — Vocabulary for light microscopy

1 Scope

This document specifies terms and definitions to be used in the field of light microscopy and advanced techniques in light microscopy.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

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

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

3.1 Terms and definitions relating to light microscopy

3.1.1
Abbe test plate
device for testing the *chromatic* (3.1.4.2) and *spherical aberration* (3.1.4.7) of *microscope* (3.1.99) *objectives* (3.1.106)

Note 1 to entry: When testing for spherical aberration, the cover glass thickness for which the objective is best corrected is also found. The test plate consists of a slide on which is deposited an opaque metal layer in the form of parallel strips arranged in groups of different width. The edges of these strips are irregularly serrated to allow the aberrations to be judged more easily. In its original and most common form, the slide is covered with a wedge-shaped cover glass, the increasing thickness of which is marked on the slide. Additional versions without the cover glass and/or with reflective stripes are also in use.

3.1.2
Abbe theory of image formation
explanation of the mechanism by which the *microscope* (3.1.99) *image* (3.1.75) is formed

Note 1 to entry: It assumes coherent illumination and is based on a three-step process involving diffraction.

a) First step: the object diffracts light coming from the source.

b) Second step: the objective collects some of the diffracted beams and focuses them, according to the laws of geometrical optics, in the back focal plane of the objective to form the primary diffraction pattern of the object.

c) Third step: the diffracted beams continue on their way and are reunited; the result of their interference is called the primary image of the microscope.

This explains the necessity for the maximum number of rays diffracted by the object to be collected by the objective, so that they may contribute to the image. Fine detail will not be resolved if the rays it diffracts are not allowed to contribute to the image.

3.1.3
aberration
⟨material and geometric form⟩ deviation from perfect imaging by an optical system, caused by the properties of the material of the *lenses* (3.1.87) or by the geometric forms of the refracting or reflecting surfaces

3.1.4
aberration
⟨optical system⟩ failure of an optical system to produce a perfect *image* (3.1.75)

3.1.4.1
astigmatism
aberration (3.1.4) which causes rays in one plane containing an off-axis *object* (3.1.104) point and the *optical axis* (3.1.107) to focus at a different distance from those in the plane at right angles to it

3.1.4.2
chromatic aberration
aberration (3.1.4) of a *lens* (3.1.87) or *prism* (3.1.119), due to *dispersion* (3.1.47) by the material from which it is made

Note 1 to entry: This defect may be corrected by using a combination of lenses made from glasses or other materials of different dispersion.

3.1.4.2.1
axial chromatic aberration
aberration (3.1.4) by which *light* (3.1.88) of different wavelengths is focused at different points along the *optical axis* (3.1.107)

3.1.4.2.2
lateral chromatic aberration
chromatic difference of magnification
aberration (3.1.4) by which the *images* (3.1.75) formed by *light* (3.1.88) of different wavelengths, although they may be brought to the same *focus* (3.1.65) in the *optical axis* (3.1.107), are of different sizes

3.1.4.3
coma
aberration (3.1.4) in which the *image* (3.1.75) of an off-axis point *object* (3.1.104) is deformed so that the image is shaped like a comet

3.1.4.4
curvature of image field
aberration (3.1.4) resulting in a curved *image field* (3.1.54.4) from a plane *object field* (3.1.54.5)

Note 1 to entry: Curvature of the image field is particularly obvious with objectives of high magnification and large numerical aperture, which have a restricted depth of field. It may largely be eliminated by additional correction.

3.1.4.5
distortion
aberration (3.1.4) in which *lateral magnification* (3.1.90.8) varies with distance from the *optical axis* (3.1.107) in the *image field* (3.1.54.4)

3.1.4.5.1
barrel distortion
negative distortion
difference in *lateral magnification* (3.1.90.8) between the central and peripheral areas of an *image* (3.1.75) such that the lateral magnification is less at the periphery

EXAMPLE A square object in the centre of the field thus appears barrel shaped (i.e. with convex sides).

3.1.4.5.2
pincushion distortion
positive distortion
difference in *lateral magnification* (3.1.90.8) between the central and the peripheral areas of an *image* (3.1.75) such that the lateral magnification is greater towards the periphery

EXAMPLE A square object in the centre of the field thus appears pincushion shaped (i.e. with concave sides).

3.1.4.6
monochromatic aberrations
collective term for all *aberration* (3.1.4) outside the Gaussian space which appear for *monochromatic* (3.1.123.2) *light* (3.1.88)

Note 1 to entry: The monochromatic aberrations are: spherical aberration, coma, astigmatism, curvature of image field and distortion.

3.1.4.7
spherical aberration
aberration (3.1.4) resulting from the spherical form of the wavefront arising from an *object* (3.1.104) point on the *optical axis* (3.1.107), on its emergence from the optical system

Note 1 to entry: As a consequence, the rays emanating from an object point on the optical axis at different angles to the axis, or rays entering the lens parallel to the optical axis but at differing distances from it, intersect the optical axis in the image space before (undercorrection) or behind (overcorrection) the ideal image point formed by the paraxial rays.

3.1.5
achromat
⟨lens element⟩ *lens* (3.1.87) in which the *axial chromatic aberration* (3.1.4.2.1) is corrected for two wavelengths

EXAMPLE One wavelength less than about 500nm, the other greater than about 600 nm.

3.1.6
achromat
⟨microscope objective⟩ *microscope* (3.1.99) *objective* (3.1.106) in which *chromatic aberration* (3.1.4.2) is corrected for two wavelengths and *spherical aberration* (3.1.4.7) and other aperture-dependent defects are minimized for one other wavelength which is usually about 550nm

EXAMPLE One wavelength less than about 500 nm, the other greater than about 600nm.

Note 1 to entry: This term does not imply any degree of correction for curvature of image field; coma and astigmatism are minimized for wavelengths within the achromatic range.

3.1.7
Airy pattern
image (3.1.75) of a primary or secondary *point source* (3.1.135.1) of *light* (3.1.88) which, due to *diffraction* (3.1.41) at a circular *aperture* (3.1.10) of an aberration-free *lens* (3.1.87), takes the form of a bright disc surrounded by a sequence of concentric dark and bright rings

3.1.7.1
Airy disc
diffraction disc
central area bounded by the first dark ring of the *Airy pattern* (3.1.7)

Note 1 to entry: The Airy disc contains 84 % of the energy of the Airy pattern.

3.1.7.2
Airy unit
AU
diameter of the theoretical first minimum of the *Airy pattern* (3.1.7) in the low *numerical aperture* (3.1.10.4) approximation

Note 1 to entry: $AU = 1{,}22\frac{\lambda_{ref}}{NA}$

3.1.8
anisotropic
having a non-uniform spatial distribution of properties

Note 1 to entry: In polarized light microscopy, this usually refers to the preferential orientation of optical properties with respect to the vibration plane of the polarized light.

3.1.9
apertometer
device for measuring the *numerical aperture* (3.1.10.4) of *microscope* (3.1.99) *objectives* (3.1.106)

3.1.10
aperture
area of a *lens* (3.1.87) which is available for the passage of *light* (3.1.88)

Note 1 to entry: In microscopy, it is usually expressed as the numerical aperture.

3.1.10.1
angular aperture
⟨microscopy⟩ maximum plane angle subtended by a *lens* (3.1.87) at the centre of an *object field* (3.1.54.5) or *image field* (3.1.54.4) by two opposite marginal rays when the lens is used in its correct working position

Note 1 to entry: The term may be qualified by the side of the lens to which it refers (e.g. object side, illumination side, image side).

3.1.10.2
condenser aperture
illuminating aperture
aperture (3.1.10) of the illuminating system which is defined by the diameter of the *illuminating aperture diaphragm* (3.1.38.6)

3.1.10.3
imaging aperture
aperture (3.1.10) of the imaging system

Note 1 to entry: The imaging aperture is generally defined by the numerical aperture of the objective.

3.1.10.4
numerical aperture
NA
number originally defined by Abbe for *objectives* (3.1.106) and *condensers* (3.1.28), which is given by the expression $n \sin u$, where n is the *refractive index* (3.1.125) of the medium between the *lens* (3.1.87) and the *object* (3.1.104) and u is half the *angular aperture* (3.1.10.1) of the lens

Note 1 to entry: Unless specified by “image-side”, the term refers to the object side.

3.1.11
aplanatic
corrected for *spherical aberration* (3.1.4.7) and *coma* (3.1.4.3)

3.1.12
apochromat
⟨lens element⟩ *lens* (3.1.87) in which *axial chromatic aberration* (3.1.4.2.1) is corrected for three wavelengths

EXAMPLE Wavelengths of about 450nm, 550nm and 650nm.

3.1.13
apochromat
⟨microscope objective⟩ *microscope* (3.1.99) *objective* (3.1.106) in which the *chromatic aberration* (3.1.4.2) is corrected for three or more wavelengths and the *spherical aberration* (3.1.4.7) and other aperture-dependent defects are minimized for about 550nm as with *achromats* (3.1.6)

EXAMPLE Wavelengths of about 450nm, 550nm and 650nm.

Note 1 to entry: This term does not imply any degree of correction for curvature of image field.

3.1.14
aspherical
not forming part of the surface of a sphere

Note 1 to entry: This term is also used to describe the shape of a refracting or a reflecting surface designed to minimize spherical aberration and some other aberrations.

3.1.15
beam splitter
means whereby a beam of *light* (3.1.88) may be divided into two or more separate beams

3.1.16
birefringence
Δn
quantitative expression of the maximum difference in *refractive index* (3.1.125) due to *double refraction* (3.1.48)

3.1.17
bright field
system of *illumination* (3.1.73) and imaging in which the *direct light* (3.1.45) passes through the *objective* (3.1.106) *aperture* (3.1.10) and illuminates the background against which the *image* (3.1.75) is seen

3.1.18
bulb
envelope of a lamp (3.1.85), which is usually out of glass or fused silica

Note 1 to entry: This term is commonly used to describe the lamp itself.

3.1.19
catadioptric
having optical arrangements or optical elements which operate by both reflection and refraction

3.1.20
catoptric
having optical arrangements or optical elements which operate by reflection

3.1.21
centring telescope
auxiliary telescope
two-stage magnifier, designed for use in place of the *eyepiece* (3.1.52) to enable an *image* (3.1.75) of the *back focal plane* (3.1.62.1) of the *objective* (3.1.106) to be inspected

Note 1 to entry: The centring telescope is used principally for adjustment of the microscope illuminating system, especially with phase contrast and modulation contrast. May also be used for conoscopic observation.

3.1.22
circle of least confusion
smallest diameter *image* (3.1.75) spot formed from a point *object* (3.1.104) when *spherical aberration* (3.1.4.7) and *astigmatism* (3.1.4.1) are present

3.1.23
clear focusing screen
sheet of clear glass or plastic material used for *focusing* (3.1.67) in photography and *photomicrography* (3.1.115) in which a figure on the *screen* (3.1.132) (e.g. cross lines) serves to define the *plane* (3.1.117) in which the *aerial image* (3.1.75.1) observed with a *focusing magnifier* (3.1.92.1) shall be located

3.1.24
coarse adjustment
focusing mechanism (3.1.68) designed to make large and rapid alterations in the distance along the *optical axis* (3.1.107) between the *object* (3.1.104) and the *objective* (3.1.106)

3.1.25
coating of optical surfaces
deposit of one or more thin dielectric and/or metallic layers on a surface of an optical element for the purpose of decreasing or increasing reflection and/or transmission

EXAMPLE Optical elements such as a lens, mirror, prism, or filter.

3.1.26
collector
lens (3.1.87) which serves to project a suitably sized *image* (3.1.75) of the *source* (3.1.135) into a given *plane* (3.1.117) (e.g. in *Köhler illumination* (3.1.73.3) into the *aperture plane* (3.1.117.1) of the *condenser* (3.1.28))

Note 1 to entry: Sometimes known as the “lamp collector”.

3.1.27
compensator
retardation plate (3.1.130) of fixed or variable *optical path length difference* (3.1.108.1) used to measure the optical path length differences within an *object* (3.1.104)

Note 1 to entry: Many types of compensator exist, often designated by the name of their originator e.g. Babinet, Berek, Senarmont.

3.1.27.1
first-order red compensator
first-order red plate
sensitive tint plate
retardation plate (3.1.130) producing an *optical path length difference* (3.1.108.1) of one wavelength, giving rise to the *interference colour* (3.1.82) having the typical tint of the *first-order red* (3.1.57)

3.1.27.2
half-wave compensator
half-wave plate
retardation plate (3.1.130) producing an *optical path length difference* (3.1.108.1) of half a wavelength, the reference wavelength being taken to be 550nm

3.1.27.3
quarter-wave compensator
quarter-wave plate
retardation plate (3.1.130) producing an *optical path length difference* (3.1.108.1) of a quarter of a wavelength

Note 1 to entry: The reference wavelength is selected according to the application and is individually indicated. When oriented at 45° to the plane of polarization, it changes plane-polarized light into circularly-polarized light and vice versa.

3.1.27.4
quartz-wedge compensator
retardation plate (3.1.130) consisting of a wedge of quartz (or two such wedges in the subtraction position) producing *optical path length differences* (3.1.108.1) continuously variable between 0 λ and 3 λ or 4 λ along its length

Note 1 to entry: This property results in the production of a series of interference colours in the form of fringes perpendicular to the length of the wedge. With monochromatic light, the coloured fringes are seen as alternating dark and bright bands.

3.1.28
condenser
part of the illuminating system of the *microscope* (3.1.99) which consists of one or more *lenses* (3.1.87) (or mirrors) and their mounts, usually containing a *diaphragm* (3.1.38), and designed to collect, control and concentrate *radiation* (3.1.123) into the illuminating *numerical aperture* (3.1.10.4)

Note 1 to entry: In bright field microscopy by epi-illumination, the objective serves as its own condenser.

3.1.28.1
Abbe condenser
condenser (3.1.28) of simple design introduced by Abbe, in which there is only limited *correction* (3.1.33) for *spherical aberration* (3.1.4.7) and none for *chromatic aberration* (3.1.4.2)

3.1.28.2
achromatic-aplanatic condenser
condenser (3.1.28) in which *chromatic aberrations* (3.1.4.2) and *spherical aberrations* (3.1.4.7) have been reduced

Note 1 to entry: Achromatic-aplanatic correction is particularly advantageous for high numerical aperture, oil immersion condensers.

3.1.28.3
cardioid condenser
dark-field condenser (3.1.28.4) for *transmitted-light illumination* (3.1.73.6), in which the *correction* (3.1.33) for *spherical aberration* (3.1.4.7) and *coma* (3.1.4.3) is calculated for a reflecting surface with the shape of a cardioid of revolution

Note 1 to entry: In practice, the correction is achieved by using a zone of a spherical surface which differs imperceptibly in its corrective effect from a true cardioid surface.

3.1.28.4
dark-field condenser
dark-ground condenser
condenser (3.1.28) designed for *dark-field* (3.1.35) microscopy

Note 1 to entry: For transmitted-light microscopy, this condenser is a separate component; for reflected-light microscopy, it is generally within the mount of the objective, surrounding the imaging system of the objective.

3.1.28.5
pancratic condenser
condenser (3.1.28) containing a variable “zoom” (pancratic) *lens* (3.1.87) which allows the size of the *illuminated field* (3.1.54.3) at the *object* (3.1.104) to be varied while the *illuminated field diaphragm* (3.1.38.5) remains of constant size

Note 1 to entry: The size of the illuminating aperture varies inversely with that of the illuminated field at the object, and the product of both sizes remains a constant.

3.1.28.6
phase-contrast condenser
condenser (3.1.28) designed for *phase contrast* (3.1.32.4) microscopy which forms on the *phase plate* (3.1.112) in the *back focal plane* (3.1.62.1) of the *objective* (3.1.106) a suitably sized *image* (3.1.75) of a *diaphragm* (3.1.38) (generally annular) positioned in the *front focal plane* (3.1.62.2) of the *condenser*

3.1.28.7
substage condenser
condenser (3.1.28) designed to fit beneath the *stage* (3.1.136) of a *microscope* (3.1.99)

3.1.28.8
swing-out top lens condenser
condenser (3.1.28) designed so that its top *lens* (3.1.87) can conveniently be removed from the optical path by operating a lever, thus increasing the *condenser's* (3.1.28) *focal length* (3.1.61) in order to increase the area of the *illuminated field* (3.1.54.3) and decrease the illuminating *numerical aperture* (3.1.10.4) for use with *objectives* (3.1.106) of low *magnification* (3.1.90)

3.1.28.9
universal condenser
condenser (3.1.28) designed for multiple contrast techniques such as *bright field* (3.1.17), *dark-field* (3.1.35), *phase contrast* (3.1.32.4), *differential interference contrast* (3.1.32.2.1), *polarized light* (3.1.88.1) and *modulation* contrast (3.1.32.3)

3.1.29
conjugate planes
planes (3.1.117) perpendicular to the *optical axis* (3.1.107) which are imaged onto another in accordance with the rules of geometrical optics

3.1.30
conoscopic figure
interference pattern of curves linking points of equal *retardation* (3.1.129), formed in the *back focal plane* (3.1.62.1) of the *objective* (3.1.106) when an optically *anisotropic* (3.1.8) *object* (3.1.104) is placed between *crossed polars* (3.1.118.2) or, exceptionally, *parallel polars* (3.1.118.3)

3.1.31
conoscopy
observation of the *conoscopic figure* (3.1.30) by means of a pinhole *diaphragm* (3.1.38) or a *centring telescope* (3.1.21) in place of the *eyepiece* (3.1.52), or by means of a *Bertrand lens* (3.1.87.2)

3.1.32
contrast
distinction between regions in an *image* (3.1.75) due to differences in brightness and/or colour

3.1.32.1
interference contrast
⟨term⟩ *contrast* (3.1.32) in the *image* (3.1.75) caused mainly by interference

3.1.32.2
interference contrast
⟨phenomenon⟩ enhancing the *contrast* (3.1.32) between features having different *optical path lengths* (3.1.108)

3.1.32.2.1
differential interference contrast
contrast (3.1.32) due to *double-beam interference* (3.1.81.1) in which two waves which fall on the *object plane* (3.1.117.5) or *image plane* (3.1.117.3) are separated laterally by a distance similar to the *minimum resolvable distance* (3.1.128.2)

Note 1 to entry: This kind of contrast is characterized by an impression of unilateral oblique illumination. Variations in optical path length due to gradients in surface relief (reflected light) or in physical thickness or refractive index (transmitted light) appear as relief contrast in the image.

3.1.32.2.2
Nomarski differential interference contrast
form of *differential interference contrast* (3.1.32.2.1) using *Nomarski prisms* (3.1.119.2)

3.1.32.3
modulation contrast
contrast (3.1.32) technique due to Hoffman which uses a modulator in the *back focal plane* (3.1.62.1) of the *objective* (3.1.106) or in a succeeding *conjugate plane* (3.1.29), and a slit *aperture* (3.1.10) in the *front focal plane* (3.1.62.2) of the *condenser* (3.1.28)

Note 1 to entry: The modulator is a filter composed of three regions: a dark region, a grey region onto which the slit in the condenser is imaged and a bright region. The modulator influences the direct light and diffracted light in order to increase contrast.

3.1.32.4
phase contrast
form of *interference contrast* (3.1.32.2) (in its widest sense) due to Zernike, in which the image *contrast* (3.1.32) of a *phase object* (3.1.111) is enhanced by altering *phase* (3.1.110) and amplitude of the *direct light* (3.1.45) with respect to those of the *diffracted light* (3.1.40) and which is achieved by the action of a *phase plate* (3.1.112), usually in the form of an annulus, placed in the *back focal plane* (3.1.62.1) of the *objective* (3.1.106) (or in a succeeding *plane conjugate* (3.1.29) with this) conjugate with an appropriate *illuminating aperture diaphragm* (3.1.38.6) in the *front focal plane* (3.1.62.2) of the *condenser* (3.1.28)

Note 1 to entry: The phase plate has two properties: it shifts the phase of the direct light by 90° and absorbs some of its intensity. Contrast is achieved by conversion of phase differences within the light leaving the object into intensity differences in the image. Two kinds of phase contrast are available, depending on the characteristics of the phase plate; in positive phase contrast, objects which retard the phase of the diffracted light by a small amount appear darker than the background, while in negative phase contrast they appear brighter.

3.1.32.5
relief contrast
form of *contrast* (3.1.32) which presents gradients of geometrical or *optical path length differences* (3.1.108.1) in the *object* (3.1.104) in the form of a distribution of brightness in the *image* (3.1.75) which gives an impression of *relief* (3.1.126)

Note 1 to entry: This impression occurs because the distribution of brightness in a relief contrast image is similar to the distribution of light and shadow in the image of a three-dimensional object illuminated from one side.

3.1.33
correction
process whereby the *aberrations* (3.1.4) of an optical system are minimized

3.1.33.1
correction class
type of *correction* (3.1.33) of an optical system (achromatic, plan, etc.)

3.1.33.2
correction collar
mechanism provided on some *objectives* (3.1.106) in order to adapt their *correction* (3.1.33) for *spherical aberration* (3.1.4.7) to compensate for deviations from correct *optical path length* (3.1.108) in the *cover glass* (3.1.34), wall of culture chamber and/or other media between the *object* (3.1.104) and the objective

3.1.33.3
correction for object to primary image distance
calculation of a *microscope* (3.1.99) *objective* (3.1.106) to optimize its corrections for a given standardized *object to primary image* (3.1.80.2.2) distance

3.1.33.4
overcorrection
error in the *correction* (3.1.33) of *spherical aberration* (3.1.4.7), leading to lack of *contrast* (3.1.32) in the *image* (3.1.75)

Note 1 to entry: In microscopy it may be caused by the use of a cover glass thicker than, or a mechanical tube length longer than, the values assumed in the computation of the objective. The term may be used also in connection with other aberrations, e.g. chromatic aberration.