
Microscopes — Vocabulary for light microscopy

Microscopes — Vocabulaire relatif à la microscopie optique

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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 microscopy, super-resolution microscopy, localization microscopy, stimulated emission depletion microscopy, super-resolution structured illumination microscopy, light sheet microscopy, digital holographic microscopy, optical coherence 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:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

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 *aberrations* (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 Usually the correction is made for a wavelength below 500 nm and for a wavelength above 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 550 nm

EXAMPLE Usually the correction is made for a wavelength below 500 nm and for a wavelength above 600 nm.

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: $AU = 1,22 \frac{\lambda_{\text{ref}}}{NA}$

Where λ_{ref} is the reference wavelength and NA the numerical aperture.

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 450 nm, 550 nm and 650 nm.

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 550 nm as with *achromats* (3.1.6)

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

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

Note 2 to entry: For more information see ISO 19012-2.

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. It 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) is 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.

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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

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)

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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.

3.1.33.5

undercorrection

error in *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, undercorrection may be caused by the use of a cover glass thinner than, or a mechanical tube length shorter than, the values assumed in the computation of the objectives. The term may be used also in connection with other aberrations, e.g. chromatic aberration.

3.1.34

cover glass

rectangular or circular piece of thin glass used to cover a microscopical preparation

Note 1 to entry: Because its thickness, refractive index and dispersion affect calculation and correction, the cover glass is regarded as part of the objective for the purpose of design. The tolerances of its thickness, refractive index and dispersion should be considered in relation to the demands of the objective, as standardized in ISO 8255-1.

3.1.35

dark-field

system of *illumination* (3.1.73) and imaging in which the *direct light* (3.1.45) is prevented from passing through the *aperture* (3.1.10) of the *objective* (3.1.106)

Note 1 to entry: The image is formed from light scattered by features in the object, the detail thus appearing bright against a dark background. It may be qualified as transmitted-light or reflected-light dark-field.

3.1.35.1

dark-field stop

central opaque disc usually used in the *front focal plane* (3.1.62.2) of a *condenser* (3.1.28) to occlude all the *direct light* (3.1.45) which would fall within the *aperture* (3.1.10) of the *objective* (3.1.106)

3.1.36

depth of field

<object space> axial depth of the space on both sides of the *object plane* (3.1.117.5) within which the *object* (3.1.104) can be moved without detectable loss of sharpness in the *image* (3.1.75), while the positions of the *image plane* (3.1.117.3) and of the *objective* (3.1.106) are maintained

Note 1 to entry: See Note to 3.1.37.

3.1.37

depth of focus

<image space> axial depth of the space on both sides of the *image* (3.1.75) within which the image appears acceptably sharp, while the positions of the *object plane* (3.1.117.5) and of the *objective* (3.1.106) are maintained

Note 1 to entry: In some publications, the term “depth of focus” is used to refer to object space. It is recommended that, when the distinction is important, the full terms “depth of field (in object space)” and “depth of focus (in image space)” be used.

3.1.38**diaphragm**

mechanical limitation of an opening normal to the *optical axis* (3.1.107) which restricts the cross-sectional area of the *light* (3.1.88) path at a defined place in an optical system and which may be fixed or variable in size, shape (although usually circular) and position

3.1.38.1**aperture diaphragm**

diaphragm (3.1.38) in any *aperture plane* (3.1.117.1)

3.1.38.2**Bertrand diaphragm**

field diaphragm (3.1.38.4) placed after a *Bertrand lens* (3.1.87.2) to restrict the *field* (3.1.54) from which a *conoscopic figure* (3.1.30) is formed

3.1.38.3**condenser diaphragm**

diaphragm (3.1.38) which controls the effective size and shape of the *condenser aperture* (3.1.10.2) and which normally functions as the *illuminating aperture diaphragm* (3.1.38.6) in transmitted light

3.1.38.4**field diaphragm**

diaphragm (3.1.38) in any *field plane* (3.1.117.2)

Note 1 to entry: Field diaphragms are usually fitted just after the lamp collector and in the eyepiece.

3.1.38.5**illuminated field diaphragm**

field diaphragm (3.1.38.4) whose *image* (3.1.75) defines the *illuminated field* (3.1.54.3) at the *object* (3.1.104)

3.1.38.6**illuminating aperture diaphragm**

aperture diaphragm (3.1.38.1) which defines the *illuminating aperture* (3.1.10.2) or the *pupil* (3.1.122) of an illuminating system

Note 1 to entry: For transmitted light, this is usually incorporated in the front focal plane of the condenser; in reflected-light microscopes it is found in the epi-illuminator in a plane conjugate with the back focal plane of the objective. It is commonly known simply as the “aperture diaphragm” or the “aperture stop”.

3.1.38.7**iris diaphragm**

diaphragm (3.1.38) bounded by multiple leaves, usually metal, arranged so as to provide an opening of variable size, which is adjustable by means of a control

3.1.38.8**visual field diaphragm**

diaphragm (3.1.38) which defines the *field* (3.1.54) of view and which is usually contained within the *eyepiece* (3.1.52)

3.1.39**dichromatic mirror**

dichroic mirror

special type of *interference filter* (3.1.55.8) used as an essential part of a *fluorescence microscope* (3.1.99.5) using *epi-illumination* (3.1.73.2) and which is designed to reflect selectively the shorter wavelength exciting *radiation* (3.1.123) and transmit the longer wavelength *fluorescence* (3.1.58)

Note 1 to entry: A similar device is often used as a lamp reflector, in order to transmit the longer wavelength heat (infrared) radiation while reflecting the visible light.