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Microscopes — Vocabulary for light microscopy

Microscopes — Vocabulaire relatif à la microscopie optique

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

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.

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)

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.

3.1.40
diffracted light
light (3.1.88) which has undergone *diffraction* (3.1.41) at the *object* (3.1.104) and which gives rise to the first-order, second-order, etc. components of the *diffraction pattern* (3.1.41.1)

3.1.41
diffraction
phenomenon of deviation of the direction of propagation of *light* (3.1.88) or other wave motion when a wavefront passes any discontinuity in an *object* (3.1.104)

3.1.41.1
diffraction pattern
distribution of *light* (3.1.88) due to *diffraction* (3.1.41), which depends on the geometrical and optical properties of the *object* (3.1.104), the *aberrations* (3.1.3) of the *lens* (3.1.87) and the shape of its exit *pupil* (3.1.122), and the wavelength of the light

3.1.41.1.1
primary diffraction pattern
primary diffraction image
diffraction pattern (3.1.41.1) of an *object* (3.1.104) which takes the form of multiple *images* (3.1.75) of the *source* (3.1.135)

Note 1 to entry: In Köhler illumination it is formed in the back focal plane of the objective.

3.1.42
diffraction grating
set of regularly repeating structures which, when illuminated, produce, by reflection or transmission, maxima and minima of *intensity* (3.1.79) as a consequence of *diffraction* (3.1.41) and interference

Note 1 to entry: These maxima and minima vary in position according to wavelength. Radiation of any given wavelength may thus be selected from complex radiation allowing the grating to be used for producing monochromatic light.

3.1.43
dioptre
unit of refractive power expressed as the reciprocal of the *focal length* (3.1.61) of a *lens* (3.1.87) in metres

3.1.44
dioptric
describing optical arrangements or optical elements which operate by refraction, i.e. using *lenses* (3.1.87)

3.1.45
direct light
light (3.1.88) which enters the *objective* (3.1.106) after undergoing no change in direction of propagation on passing through the *object field* (3.1.54.5) (transmitted light), or on specular reflection at a flat surface in the object field oriented normally to the direction of propagation of the light (reflected light)

3.1.46
dispersion
<wave group> change in phase velocity of a wave group as a function of its wavelength (or frequency) when passing from one medium to another which causes a separation of the monochromatic components of a complex *radiation* (3.1.123)

3.1.47
dispersion
<refractive index> variation in *refractive index* (3.1.125) of a medium which causes a separation of the monochromatic components of a complex *radiation* (3.1.123)

Note 1 to entry: The quantity characterizing this property may have a special name, e.g. the Abbe number, or the dispersive power, of the medium.

3.1.47.1
dispersion curve
graph of *refractive index* (3.1.125) of a medium as a function of wavelength or a related parameter, at a given temperature

3.1.48
double refraction
effect of anisotropy, by which electromagnetic waves are divided into *plane-polarized* (3.1.88.1.2) components having mutually perpendicular vibration directions and being propagated with different velocities

Note 1 to entry: Double refraction may be due to structure, orientation of particles, or strain. The quantitative expression of double refraction is birefringence.

3.1.49
excitation
input of energy to matter leading to the emission of *radiation* (3.1.123)

3.1.50
exposure
total quantity of *light* (3.1.88) allowed to fall upon a photosensitive emulsion which is measured in lux per second

3.1.50.1
exposure meter
device for determining the required *exposure* (3.1.50) for photographic materials

3.1.51
extinction
condition in which an optically anisotropic *object* (3.1.104) appears dark when observed between *crossed polars* (3.1.118.2)

3.1.52
eyepiece
lens (3.1.87) system in a separate mount, which magnifies the *microscope's* (3.1.99) final *real image* (3.1.75.3), formed in a *viewing tube* (3.1.144.6), and projects it to infinity or to a distance comfortable for viewing by the human eye

3.1.52.1
compensating eyepiece
eyepiece (3.1.52) designed to correct residual aberrations of the *objective* (3.1.106),

EXAMPLE Compensation of *chromatic difference of magnification* (3.1.4.2.2) or *astigmatism* (3.1.4.1).

3.1.52.2
external-diaphragm eyepiece
eyepiece (3.1.52) in which the *field diaphragm* (3.1.38.4) is located in front of the *lenses* (3.1.87)

Note 1 to entry: This type of eyepiece is suitable for the insertion of graticules.

3.1.52.3
filar eyepiece
micrometer-screw eyepiece
micrometer eyepiece (3.1.52.9) in which reference marks in the *primary image plane* (3.1.117.4) may be adjusted by means of a micrometer screw and the resultant indicated displacement is used to derive dimensions

3.1.52.4
focusable eyepiece
eyepiece (3.1.52) with a mechanism to focus on a *graticule* (3.1.70) or *field diaphragm* (3.1.38.4) mounted within it

3.1.52.5
high-eyepoint eyepiece
eyepiece (3.1.52) computed so that the *exit pupil of the microscope* (3.1.122.2) is sufficiently far from the *eye lens* (3.1.87.3.) to facilitate use of the *microscope* (3.1.99) by wearers of spectacles and/or for special applications

3.1.52.6
Huygens eyepiece
term originally used for an *eyepiece* (3.1.52) consisting of two planoconvex *lenses* (3.1.87) (the *field lens* (3.1.87.4) and the *eye lens* (3.1.87.3)) mounted with their convex sides facing the *objective* (3.1.106) and with the *field diaphragm* (3.1.38.4) between them

3.1.52.7
internal-diaphragm eyepiece
eyepiece (3.1.52) in which the *field diaphragm* (3.1.38.4) is located between the *field lens* (3.1.87.4) and the *eye lens* (3.1.87.3)

3.1.52.8
Kellner eyepiece
improved type of *Ramsden eyepiece* (3.1.52.11) in which the distances between the *field lens* (3.1.87.4) and the *diaphragm* (3.1.38), and from the *eye lens* (3.1.87.3) to the *exit pupil of the microscope* (3.1.122.2), are both increased

3.1.52.9
micrometer eyepiece
focusable eyepiece (3.1.52.4) used for measuring

Note 1 to entry: In its most common form, a measuring graticule is fitted in the primary image plane. It is calibrated against a stage micrometer.

3.1.52.10
pointer eyepiece
eyepiece (3.1.52) containing a pointer in its *primary image plane* (3.1.117.4)

3.1.52.11
Ramsden eyepiece
eyepiece (3.1.52) consisting of two planoconvex *lenses* (3.1.87) of the same *focal length* (3.1.61) [the *field lens* (3.1.87.4) and the *eye lens* (3.1.87.3)], mounted with their convex sides together and separated by a distance equal to the *focal length* of the lenses

3.1.52.12
widefield eyepiece
eyepiece (3.1.52) specially computed to provide a *field* (3.1.54) of view greater than that of a normal *eyepiece* of the same *magnification* (3.1.90)

3.1.53
eyepoint height
eye relief
distance measured along the *optical axis* (3.1.107) from the last surface of the *eyepiece* (3.1.52) to the *exit pupil of the microscope* (3.1.122.2) where the eye is located

Note 1 to entry: Its value may be affected by optical systems which are inserted between objective and eyepiece.

3.1.54
field
area in the *object plane* (3.1.117.5) or any other *plane conjugate* (3.1.29) with it

Note 1 to entry: The term may be qualified by its location (e.g. object field, image field) or its function (e.g. illuminated field, photometric field).

3.1.54.1
eyepiece field of view
part of the *primary image* (3.1.75.2) which is defined by the *field diaphragm* (3.1.38.4) of the *eyepiece* (3.1.52)

3.1.54.2
field-of-view number
field number
FN
number which specifies the *eyepiece field of view* (3.1.54.1) and which is the actual diameter in millimetres of the *field diaphragm* (3.1.38.4) in an *external-diaphragm eyepiece* (3.1.52.2) or the apparent diameter of the *virtual image* (3.1.75.4) of the *field diaphragm* (3.1.38.4) in an *internal-diaphragm eyepiece* (3.1.52.7)

Note 1 to entry: The field-of-view number is now one of the standard markings of the eyepiece and may be used to calculate the diameter of the microscope field of view (object field).

3.1.54.3
illuminated field
part of the *object field* (3.1.54.5) which receives *illumination* (3.1.73)

3.1.54.4
image field
any *field* (3.1.54) in which an *image* (3.1.75) of the *object* (3.1.104) is formed

3.1.54.5
object field
microscope field of view
FOV
part of the *object* (3.1.104) which is reproduced in the final *image* (3.1.75) which is defined by

a) the *field diaphragm* (3.1.38.4) of the *eyepiece* (3.1.52), or

b) the dimensions of the receiving device,

together with the total *magnification* (3.1.90) of the optical elements lying between the object and a) or b)

3.1.54.6
objective field number
OFN
maximum *field-of-view number* (3.1.54.2) of the *eyepiece* (3.1.52) for which the *objective* (3.1.106) is designed to be used

3.1.55
filter
optical device designed to control selectively the wavelengths, colour temperature, vibration direction, and/or *intensity* (3.1.79) of the *radiation* (3.1.123) which it transmits or reflects

3.1.55.1
barrier filter
filter (3.1.55) used in fluorescence microscopy which is designed to prevent the passage towards the *image* (3.1.75) of those wavelengths of *light* (3.1.88) used for *excitation* (3.1.49) but to allow the light produced by *fluorescence* (3.1.58) of the *object* (3.1.104) to pass

3.1.55.2
broad-band-pass filter
broad-band filter
filter (3.1.55) which allows the passage of *radiation* (3.1.123) with a broad wavelength band (greater than about 50 nm) around a given central wavelength

Note 1 to entry: The concept of a “broad” band is arbitrary.

3.1.55.3
colour filter
filter (3.1.55) which allows the passage of *light* (3.1.88) of selected colour (chromaticity) or wavelength characteristics

3.1.55.4
colour-conversion filter
conversion filter
filter (3.1.55) used to change the colour temperature of *light* (3.1.88) received from a *source* (3.1.135)

3.1.55.5
contrast filter
filter (3.1.55) used to adjust the *contrast* (3.1.32) in an *image* (3.1.75) between features of an *object* (3.1.104) or between the object and the background

3.1.55.6
exciter filter
filter (3.1.55) used in fluorescence microscopy designed (ideally) to pass only those wavelengths which excite *fluorescence* (3.1.58)

3.1.55.7
heat filter
heat protection filter
filter (3.1.55) designed to prevent the passage of *radiation* (3.1.123) in the infrared or near infrared ranges

3.1.55.8
interference filter
filter (3.1.55) designed to transmit or reflect selectively a limited part of the spectrum by *multiple-beam interference* (3.1.81.3)

3.1.55.9
long-wave-pass filter
long-pass filter
filter (3.1.55) designed to allow the passage of *radiation* (3.1.123) of wavelengths longer than a given limit

3.1.55.10
narrow-band-pass filter
narrow-band filter
filter (3.1.55) (often an *interference filter* (3.1.55.8)) which allows the passage of *radiation* (3.1.123) only within a very narrow wavelength band around a given central wavelength

Note 1 to entry: The concept of a “narrow” band is arbitrary.

3.1.55.11
neutral-density filter
ND filter
neutral filter
filter (3.1.55) designed to reduce as equally as possible the *intensity* (3.1.79) of *radiation* (3.1.123) across the whole visible spectrum

3.1.55.12
polarizing filter
filter (3.1.55) acting as a *polar* (3.1.118) by total or partial absorption of *light* (3.1.88) vibrating in certain directions

3.1.55.13
short-wave-pass filter
short-pass filter
filter (3.1.55) designed to allow the passage of *radiation* (3.1.123) of wavelengths shorter than a given limit

3.1.56
fine adjustment
focusing mechanism (3.1.68) designed to make small and precise alterations in the relative positions along the *optical axis* (3.1.107) between the *object* (3.1.104) and the *objective* (3.1.106)

Note 1 to entry: The precision of positioning which it provides should be better than the depth of field of the objective.

3.1.57
first-order red
sensitive tint
characteristic reddish-violet *interference colour* (3.1.82) selected from *light* (3.1.88) of a continuous spectrum by the *extinction* (3.1.51) of other wavelengths

3.1.58
fluorescence
phenomenon of selective absorption of *radiation* (3.1.123) of relatively short wavelength (i.e. of relatively high energy) by matter, resulting in the emission of radiation of longer wavelengths (i.e. of lower energy), persisting for only a very short time after the cessation of the *excitation* (3.1.49)

Note 1 to entry: In the special case of multiple-photon excitation, longer wavelength (lower energy) radiation may excite fluorescence with the effect of radiation of shorter wavelength.

3.1.58.1
primary fluorescence
autofluorescence
fluorescence (3.1.58) exhibited by virtue of the inherent properties of an *object* (3.1.104)

3.1.58.2
secondary fluorescence
fluorescence (3.1.58) exhibited by an *object* (3.1.104) after treatment with a *fluorochrome* (3.1.60)

3.1.58.3
excitation wavelength
specific wavelength of *light* (3.1.88) required to excite a *fluorochrome* (3.1.60), such as a fluorescent antibody or fluorescent protein, to emit light at emission wavelengths

3.1.58.3.1
excitation wavelength band
specific wavelength range of *light* (3.1.88) used to excite a *fluorochrome* (3.1.60)

3.1.58.4
detection wavelength band
specific wavelength range of *light* (3.1.88) collected by the photo detector

3.1.59
fluorite
crystalline calcium fluoride (CaF_2)

Note 1 to entry: This material, or others with similar optical properties, is used as an additional lens material for the correction of chromatic aberration and improvement in light transmission in some microscope objectives of the higher correction classes.

3.1.60
fluorochrome
substance used to impart *fluorescence* (3.1.58) to structures within a specimen for subsequent examination by *fluorescence microscopy* (3.1.99.5)

3.1.61
focal length
f or *f'*
distance measured along the optical axis from the principal *plane* (3.1.117) of a *lens* (3.1.87) to the appropriate *focal plane* (3.1.62)

3.1.62
focal plane
surface in which bundles of parallel rays are brought to a point by an ideal *lens* (3.1.87)

EXAMPLE The focal plane is the surface at right angles to the optical axis of a lens (or mirror) in which the image of an object lying at infinity is formed.

3.1.62.1
back focal plane
<converging lens> *focal plane* (3.1.62) of a *lens* (3.1.87) which lies behind it when viewed in the direction of passage of *light* (3.1.88)

3.1.62.2
front focal plane
<converging lens> *focal plane* (3.1.62) of a *lens* (3.1.87) which lies in front of it when viewed in the direction of the passage of *light* (3.1.88)

3.1.63
focal point
F or *F'*
point of intersection of the *focal plane* (3.1.62) with the *optical axis* (3.1.107), and where rays entering an ideal *lens* (3.1.87) parallel to the optical axis cross the optical axis (converging lens) or appear to originate from (diverging lens)

3.1.64
focus
<lens> *focal point* (3.1.63) of a *lens* (3.1.87)

3.1.65
focus
<imaging> state of sharpest imaging

3.1.66
focus
<ray tracing> point in the *object plane* (3.1.117.5) at which those rays intersect which, after refraction and/or reflection in an optical system also intersect in the *image plane* (3.1.117.3) to give rise to a sharp *image* (3.1.75) of the conjugate point

3.1.67
focusing
<control> act of bringing the optical system into *focus* (3.1.65), i.e. bringing it to the position at which it forms an *image* (3.1.75) of the utmost sharpness in the proper *image plane* (3.1.117.3)

Note 1 to entry: In accordance with the character of the focusing mechanism used for this, the word may be qualified by the adjective “coarse” or “fine”.

3.1.68
focusing mechanism
mechanism used to change the distance between the *object* (3.1.104) and the optical system forming an *image* (3.1.75), with the purpose of obtaining maximum sharpness in that image

Note 1 to entry: In the case of the microscope, the *focusing mechanism* (3.1.68) is often mediated by a rack and pinion and converts the rotary motion of a knob into linear motion along the *optical axis* (3.1.107) of either the *objective* (3.1.106) (with or without the *tube* (3.1.144)) or the *stage* (3.1.136).

3.1.69
free working distance
working distance
WD
distance in air, or in the specified *immersion liquid* (3.1.78), between the front of the *objective* (3.1.106) and the surface of the *cover glass* (3.1.34), or of the *object* (3.1.104) if uncovered, under normal operating conditions

3.1.70
graticule
reticle
pattern such as a scale or grid, together with its support, placed in an *object plane* (3.1.117.5) or an *image plane* (3.1.117.3) which is used for measurement, reference, alignment, location, counting or stereological analysis

3.1.71
ground glass
glass whose surface is roughened by mechanical or chemical means, used in microscopy to provide scattering or diffusion of the *light* (3.1.88) passing through or falling on it

Note 1 to entry: It may be used as a screen for the visualization of a real image.

3.1.72
halo
phenomenon in *phase contrast* (3.1.32.4) microscopy by which a feature in the *image* (3.1.75) is surrounded by a dark or light rim

3.1.73
illumination
application of *light* (3.1.88) to an *object* (3.1.104)

3.1.73.1
axial illumination
illumination (3.1.73) with a ray bundle whose axis coincides with the *optical axis* (3.1.107) of the *microscope* (3.1.99)

3.1.73.2
epi-illumination
coaxial light
reflected light
incident light
vertical illumination
illumination (3.1.73) which falls on the *object field* (3.1.54.5) from the same side as that from which the object field is observed

3.1.73.3
Köhler illumination
method of illuminating microscopical *objects* (3.1.104), providing a uniformly *illuminated field* (3.1.54.3) from a non-uniform *source* (3.1.135)

Note 1 to entry: An image of the source is projected by a collector into the plane of the aperture diaphragm in the front focal plane of the condenser. The condenser, in turn, projects an image of an illuminated field diaphragm at the opening of the collector into the object plane. In the reflected-light microscope, where the objective serves as its own condenser, an aperture diaphragm is imaged by a relay lens into the back focal plane of the objective and the illuminated field diaphragm is arranged to be in a plane conjugate with that of the collector.

3.1.73.4
oblique illumination
illumination (3.1.73) using a ray bundle whose axis makes an angle with the *optical axis* (3.1.107) of the *microscope* (3.1.99)

3.1.73.5
source-focused illumination
critical illumination
method of *illumination* (3.1.73) in which an *image* (3.1.75) of the *source* (3.1.135), which may carry an *illuminated field diaphragm* (3.1.38.5), is projected by the *condenser* (3.1.28) into the *object plane* (3.1.117.5)

Note 1 to entry: Even illumination is obtained from a homogeneous source.

3.1.73.6
transmitted-light illumination
trans-illumination
diascopic illumination
illumination (3.1.73) which passes through the *object field* (3.1.54.5) from the opposite side to that from which the object field is observed

3.1.74
illuminator
device designed to provide *illumination* (3.1.73)

3.1.74.1
epi-illuminator
part of the illuminating system of the *reflected-light microscope* (3.1.99.11) placed between the *objective* (3.1.106) [which serves as its own *condenser* (3.1.28)] and the lamp fitting

Note 1 to entry: The epi-illuminator is attached to or is inserted into the body tube, thus forming a section of this tube. A reflector, or a set of interchangeable reflectors, is included in the illuminator.

3.1.74.2
fibre optic illuminator
illuminating system in which the *light* (3.1.88) is delivered by a fibre optic

3.1.75
image
collection of points formed by a *lens* (3.1.87) or other imaging system, corresponding to points in the *object* (3.1.104)

Note 1 to entry: The image is a structural representation of those properties of the object which cause modulation of light. All parameters which describe the spatial and the temporal state of light can be modulated. Because of these modulations, the light in an encoded form carries the information about the object. In microscopy with the compound microscope, a primary image and a secondary image are formed, the latter being produced on the retina of the observer's eye, on photographic material or on another surface.

3.1.75.1
aerial image
real image (3.1.75.3) existing in a *plane* (3.1.117) in space and not normally visible to the naked eye

3.1.75.2
primary image
usually magnified *real image* (3.1.75.3) of the *object* (3.1.104) formed by the *objective* (3.1.106) or, in infinity-corrected systems, by the objective together with its *tube lens* (3.1.87.10)

Note 1 to entry: The "primary image" is not to be confused with the "primary interference image" as described by Abbe.

3.1.75.3
real image
image (3.1.75) which can be received on a surface

EXAMPLE The surface can be a *screen* (3.1.132).

3.1.75.4
virtual image
image (3.1.75) which cannot be received on a surface but which may be converted into a *real image* (3.1.75.3) by the optical system of the eye or other converging *lens* (3.1.87) system

3.1.76
image space
space on that side of an optical system where the *image* (3.1.75) is located

Note 1 to entry: In reflection or formation of a virtual image, this space may coincide with the object space.

3.1.77
immersion
use of an *immersion liquid* (3.1.78)

3.1.77.1
homogeneous immersion
immersion (3.1.77) in which the *immersion liquid* (3.1.78) and the adjacent optical components have the same *refractive index* (3.1.125) and *dispersion* (3.1.47) (or Abbe number) so that neither refraction nor reflection occurs between the liquid and the optical components

Note 1 to entry: In modern microscope design, refractive index differences between objective front lens, the immersion liquid and the cover glass may be deliberately introduced in order to assist in the correction of the system, so that the immersion is not truly homogeneous.

3.1.77.2
oil immersion
immersion (3.1.77) in which the *immersion liquid* (3.1.78) is *immersion oil* (3.1.78.1)

3.1.78
immersion liquid
liquid specified as suitable for use in the space between the front of an *immersion lens* (3.1.87.6) and the *object* (3.1.104)

Note 1 to entry: Because the immersion liquid is considered in the computing of corrections to be part of the lens, its refractive index and dispersion (or Abbe number) are critical.

EXAMPLE Commonly used immersion liquids are *immersion oil* (3.1.78.1), water, glycerol or silicone.

3.1.78.1
immersion oil
synthetic *immersion liquid* (3.1.78)

Note 1 to entry: The term was formerly applied to naturally occurring immersion liquids such as cedar-wood oil.

Note 2 to entry: Immersion oil is according to ISO 8036.

3.1.79
intensity
general term for the strength of a *radiation* (3.1.123), which is proportional to the square of the amplitude of the electromagnetic wave

Note 1 to entry: For measurement, this term should be replaced by the most suitable photometric or radiometric quantity.

3.1.80
interfacing dimensions
mechanical or opto-mechanical distances measured from *reference planes* (3.1.117.6), on which the calculation of *microscope* (3.1.99) *lenses* (3.1.87) and the design of microscopes are based, and which facilitate the interchange of certain components

Note 1 to entry: There are two categories of dimensions: those which are standardized internationally and others which are taken as internal standards by individual manufacturers.

3.1.80.1
mechanical interfacing dimensions of the microscope
distances between several mechanical *locating surfaces* (3.1.89) or flanges

3.1.80.2
optical interfacing dimensions of the microscope
distances of *focal points* (3.1.63), or *object planes* (3.1.117.5) or *image planes* (3.1.117.3), from mechanical *locating surfaces* (3.1.89) or flanges

3.1.80.2.1
objective to primary image distance
distance in air between the *objective locating surface of the nosepiece* (3.1.89.3) and the *primary image plane* (3.1.117.4)

Note 1 to entry: The objective to primary image distance is one of the optical interfacing dimensions and commonly has a value of either 150 mm or infinity. The latter is a hypothetical value applied to microscopes designed for infinity-corrected objectives.

3.1.80.2.2
object to primary image distance
distance in air between the *object plane* (3.1.117.5) and the *primary image plane* (3.1.117.4)

Note 1 to entry: The object to primary image distance is the fundamental optical interfacing dimension used in microscope design and commonly has a value of either 195 mm or infinity. The latter is a hypothetical value applied to microscopes designed for infinity-corrected objectives.

3.1.80.2.3
parfocalizing distance of the eyepiece
distance between the *locating flange of the eyepiece* (3.1.89.1) and the *plane* (3.1.117) upon which the *eyepiece* (3.1.52) is focused

Note 1 to entry: The plane upon which the eyepiece is focused is coincident with the plane of the final real image of the microscope when the eyepiece is mounted in the viewing tube. The parfocalizing distance of the eyepiece is one of the optical interfacing dimensions, and is commonly 10 mm.

3.1.80.2.4
parfocalizing distance of the objective
distance in air between the *object plane* (3.1.117.5) [i.e. the uncovered surface of the *object* (3.1.104)] and the *locating flange of the objective* (3.1.89.4), when the *microscope* (3.1.99) is in its working position

Note 1 to entry: The parfocalizing distance of the objective is one of the optical interfacing dimensions.

3.1.81
interference
mutual interaction between two or more coherent wave trains

Note 1 to entry: The phenomenon may be used to convert optical path length differences in the object into intensity variations in the image so providing contrast.

3.1.81.1
double-beam interference
interference between wave trains from two coherent *light* (3.1.88) beams

3.1.81.2
double-focus interference
double-beam interference (3.1.81.1) in which the two *light* (3.1.88) beams have different levels of *focus* (3.1.65) and in which one beam is focused in the *object plane* (3.1.117.5), the other above or below that plane

3.1.81.3
multiple-beam interference
interference between wave trains from more than two coherent *light* (3.1.88) beams

3.1.81.4
polarizing interference
double-beam interference (3.1.81.1) in which the two *light* (3.1.88) beams arise from one *plane-polarized* (3.1.88.1.2) beam as the result of *double refraction* (3.1.48), are plane-polarized in mutually perpendicular vibration directions and are recombined by the *analyser* (3.1.118.1)

3.1.81.5
shearing interference
double-beam interference (3.1.81.1) in which the two *light* (3.1.88) beams falling upon the *object plane* (3.1.117.5) or *image plane* (3.1.117.3) are separated laterally from one another

Note 1 to entry: This separation limits the size of features which can be studied.

3.1.82
interference colour
mixed colour resulting from *extinction* (3.1.51) or partial extinction, caused by interference, of one or several parts of the spectrum

3.1.83
interferometry
interference phenomena applied to the measurement of *optical path length differences* (3.1.108.1), from which refractive indices and thickness can also be derived

3.1.84
interpupillary distance
distance in millimetres between the centres of the pupils of a person's eyes when viewing with parallel fixation lines

Note 1 to entry: Binocular microscopes and binocular tubes are provided with an adjustment to allow for the variable interpupillary distances of different people.

3.1.85
lamp
source of *radiation* (3.1.123)

3.1.85.1
filament lamp
lamp (3.1.85) from which *radiation* (3.1.123) is emitted from a filament, usually of tungsten, heated by the passage of an electric current

Note 1 to entry: The emitted spectrum is continuous and approximates to that of a black-body radiator.

3.1.85.2
halogen lamp
filament lamp (3.1.85.1) whose envelope contains halogen vapour

Note 1 to entry: A cyclical process in which the halogen reduces loss of tungsten from the filament and its deposition on the envelope. This permits a high filament temperature and consequent higher luminance, higher colour temperature and longer operating life than a conventional filament lamp of the same input power.

3.1.85.3
mercury arc lamp
discharge *lamp* (3.1.85) containing mercury vapour, often at a high pressure when the lamp is operating

Note 1 to entry: At low pressure the lamp emits a characteristic line spectrum but when it heats up there is a strong continuous spectrum forming the background. This type of lamp is frequently used in fluorescence microscopy and also, with a suitable filter, as a source of monochromatic radiation or ultraviolet radiation.

3.1.85.4
microscope lamp
lamp (3.1.85) together with its *collector* (3.1.26), mirror, housing and fittings

Note 1 to entry: The microscope lamp may be incorporated into the microscope stand itself, or be a separate unit.

3.1.85.5
xenon arc lamp
discharge *lamp* (3.1.85) containing xenon, often at a high pressure when the lamp is operating

Note 1 to entry: The lamp emits light of high luminance, high colour temperature and with an almost continuous spectrum distributed from the ultraviolet to the infrared.

3.1.86
laser
source which emits coherent *radiation* (3.1.123) of high spectral concentration of radiance and an extremely small solid angle

Note 1 to entry: Acronym for Light Amplification by Stimulated Emission of Radiation.

3.1.87
lens
piece of transparent material with one or more curved surfaces, which is used to alter systematically the direction of rays of *light* (3.1.88)

Note 1 to entry: The term may also be used for a system of lenses which, in principle, acts as a single lens.

3.1.87.1
aspherical lens
lens (3.1.87) made with an *aspherical* (3.1.14) surface

3.1.87.2
Bertrand lens
Amici-Bertrand lens
intermediate lens (3.1.87.7) which transfers an *image* (3.1.75) of the *back focal plane* (3.1.62.1) of the *objective* (3.1.106) into the *primary image plane* (3.1.117.4)

Note 1 to entry: The Bertrand lens is used for conoscopic observation in polarized-light microscopy and for adjustment of the microscope illuminating system, especially in phase-contrast and modulation-contrast microscopy.

3.1.87.3
eye lens
lens (3.1.87) or group of lenses of an *eyepiece* (3.1.52) nearest to the observer's eye

3.1.87.4
field lens
lens (3.1.87) positioned in or close to a *field plane* (3.1.117.2) in order to adapt the exit *pupil* (3.1.122) of the preceding lenses to the entrance pupil of subsequent lenses

Note 1 to entry: Use of field lenses suppresses vignetting in the image and, more generally, provides homogeneous illumination of the field to which it relates. The term is often used without qualification to describe the field lens of the eyepiece.

3.1.87.5
gradient-index lens
lens (3.1.87) in which some or all of the refractive power results from axial, radial or spherical variation in *refractive index* (3.1.125)

3.1.87.6
immersion lens
objective (3.1.106) or *condenser* (3.1.28) designed to work with an *immersion liquid* (3.1.78)

3.1.87.7
intermediate lens
lens (3.1.87) located between the *objective* (3.1.106) and the *primary image* (3.1.75.2) which serves to control the position and/or *lateral magnification* (3.1.90.8) of the primary image, and/or to ensure the conditions for correct optical imaging if the actual *optical interfacing dimensions* (3.1.80.2) are different from the standard ones

3.1.87.8
photographic projection lens
projection lens (3.1.121) specially designed for *photomicrography* (3.1.115)

3.1.87.9
relay lens
lens (3.1.87) for transferring an *image* (3.1.75) into another *plane* (3.1.117)

3.1.87.10
tube lens
intermediate lens (3.1.87.7) designed to operate as an essential component of *infinity-corrected objectives* (3.1.106.3), and which should be regarded as part of the objective lens system when magnification and *correction* (3.1.33) are considered

3.1.87.10.1
normal tube lens
particular *tube lens* (3.1.87.10) with which an *infinity-corrected objective* (3.1.106.3) is designed to operate

3.1.87.10.2
focal length of the normal tube lens
f_{NTL}
focal length(3.1.61) related to the *magnification* (3.1.90) and the focal length of the *objectives* (3.1.106) which are designed to operate with this *tube lens* (3.1.87.10)

3.1.87.11
tubelength correction lens
intermediate lens (3.1.87.7) used to correct optically any deviation of mechanical tubelength from its nominal value

3.1.88
light
electromagnetic *radiation* (3.1.123) directly capable of causing a visual sensation

3.1.88.1
polarized light
light (3.1.88) in which the vibrations are partially or completely suppressed in certain directions at any given instant

Note 1 to entry: The vector of vibration may describe a linear, circular or elliptical shape.

3.1.88.1.1
elliptically-polarized light
polarized light (3.1.88.1) in which the vector of vibration describes an elliptical shape

3.1.88.1.2
plane-polarized light
linear-polarized light
polarized light (3.1.88.1) in which the vector of vibration describes a linear shape

3.1.88.2
stray light
light (3.1.88) which arises from scatter or reflection by the *object* (3.1.104), in *lenses* (3.1.87), or by obstacles in the light path, and which does not contribute to *image* (3.1.75) formation and reduces the *contrast* (3.1.32) in the image

3.1.89
locating surface
locating flange
surface at which two interchangeable components fit together

Note 1 to entry: These surfaces are perpendicular to the optical axis and are responsible for setting the correct axial location and centration of the optical and mechanical elements. They may coincide with reference planes for optical interfacing dimensions.

3.1.89.1
locating flange of eyepiece
flange on the *eyepiece* (3.1.52) which locates it at a given level which is the *eyepiece-locating surface of viewing tube* (3.1.89.2)

Note 1 to entry: The locating flange of eyepiece is one of the reference planes for the parfocalizing distance of the eyepiece.

3.1.89.2
eyepiece-locating surface of viewing tube
surface at the upper end of the *viewing tube* (3.1.144.6) which sets the level of the *locating flange of eyepiece* (3.1.89.1)

Note 1 to entry: The eyepiece-locating surface of viewing tube is one of the reference planes which determines the mechanical tube length.

3.1.89.3
objective-locating surface of the nosepiece
surface of the *nosepiece* (3.1.103) which locates the *objective* (3.1.106) at a given level and is coincident with *the locating flange of the objective* (3.1.89.4)

Note 1 to entry: The objective-locating surface is one of the reference planes determining the mechanical tube length, the parfocalizing distance of the objective, and the objective to primary image distance.

3.1.89.4
locating flange of the objective
objective shoulder
surface of an *objective* (3.1.106) which locates it at a given level which is the *objective-locating surface of the nosepiece* (3.1.89.3)

Note 1 to entry: The locating flange of the objective is one of the reference planes determining the mechanical tube length and parfocalizing distance of the objective.

3.1.90
magnification
process of changing the apparent dimensions of an *object* (3.1.104) by optical techniques, or the numerical expression of the result of this

Note 1 to entry: The type of magnification such as visual or lateral should always be specified.

Note 2 to entry: The more general term magnifying power as a measure of the ability of an optical system to produce visual magnification and lateral magnification under specified operating conditions has been replaced in this document by “magnification”, due to the more established use of this term in practical work.

3.1.90.1
magnification of an eyepiece
M_E
visual magnification (3.1.90.10) at the *virtual image* (3.1.75.4) formed from the *primary image* (3.1.75.2) by the *eyepiece* (3.1.52)

Note 1 to entry: The value of the magnification of an eyepiece is the ratio of the reference viewing distance to the *focal length* of the eyepiece, i.e.

$$M_E = 250 / f_E$$

where

M_E is the visual magnification of the eyepiece;
f_E is the focal length of the eyepiece in millimetres;
250 is the reference viewing distance in millimetres.

3.1.90.2
total magnification of a microscope used to produce a real image
$M_{TOT\ PROJ}$
lateral magnification (3.1.90.8) at the *real image* (3.1.75.3)

Note 1 to entry: The value of the total magnification of a microscope used to produce a real image using a normal eyepiece intended for visual observation, or a photographic projection lens, whose projection factor has been calculated, is given by the product of the magnification of the objective, the total tube factor, the magnification of the eyepiece and the projection factor, i.e.

$$M_{TOT\ PROJ} = M_O \times q \times M_E \times p$$

where

$M_{TOT\ PROJ}$ is the total (lateral) magnification of the microscope;
M_O is the magnification of the objective;
Q is the total tube factor;
M_E is the (visual) magnification of the eyepiece;
P is the projection factor.

Note 2 to entry: The value of the total magnification of a microscope used to produce a real image using a specially designed photographic lens is given by the product of the magnification of the objective, the total tube factor and the magnification of the photographic projection lens, i.e.

$$M_{TOT\ PROJ} = M_O \times q \times M_{PHOT}$$

where

$M_{TOT\ PROJ}$ is the total (lateral) magnification of the microscope;
M_O is the magnification of the objective;
Q is the total tube factor;
M_{PHOT} is the (lateral) magnification of the photographic projection lens.

3.1.90.3
total visual magnification of a microscope used for visual observation
$M_{TOT\ VIS}$
visual magnification (3.1.90.10) at the *virtual image* (3.1.75.4) formed by the *microscope* (3.1.99)

Note 1 to entry: The value of the visual magnification of a microscope is given by the product of the magnification of the objective, the total tube factor and the visual magnification of the eyepiece, i.e.

$$M_{TOT\ VIS} = M_O \times q \times M_E$$

where

$M_{TOT\ VIS}$ is the total (visual) magnification of the microscope;
M_O is the magnification of the objective;

q is the total tube factor;

M_E is the (visual) magnification of the eyepiece.

3.1.90.4
magnification of an objective with finite primary image distance
M_O
lateral magnification (3.1.90.8) at the *primary image* (3.1.75.2) formed at the distance from the *objective* (3.1.106) specified in the design of the objective

Note 1 to entry: M_O should be expressed in proportional form, e.g. 10:1.

3.1.90.5
magnification of an objective with infinite primary image distance, in combination with the normal tube lens
$M_{O\infty}$
lateral magnification (3.1.90.8) at the *real image* (3.1.75.3) produced by the combination of the *objective* (3.1.106) and the *normal tube lens* (3.1.87.10.1) (the tube lens with which the objective is designed to operate)

Note 1 to entry: The value of the magnification of an objective corrected for an infinite primary image distance is given by the ratio of the focal length of the normal tube lens to that of the objective, i.e.

$$M_{O\infty} = f_{NTL} / f_{O\infty}$$

where

$M_{O\infty}$ is the magnification of the objective corrected for an infinite primary image distance;

f_{NTL} is the focal length of the normal tube lens in millimetres;

$f_{O\infty}$ is the focal length of the objective in millimetres;

$M_{O\infty}$ should be expressed in numerical form with the multiplication sign, e.g. ×10.

3.1.90.6
axial magnification
ratio between a given axial distance in *image space* (3.1.76) and the corresponding distance in *object space* (3.1.105)

3.1.90.7
empty magnification
magnification (3.1.90) greater than the *useful range of magnification* (3.1.90.9)

Note 1 to entry: Exceeding the range of useful magnification gives no further information about the object, but sharpness and contrast appear to decrease.

3.1.90.8
lateral magnification
ratio of a given distance in the *real image* (3.1.75.3) normal to the *optical axis* (3.1.107) to the corresponding distance in the *object* (3.1.104)

Note 1 to entry: This ratio should be expressed in proportional form, e.g. 10:1.

3.1.90.9
useful range of magnification for visual observation
range of *total visual magnifications* (3.1.90.3) within which details in the *object* (3.1.104) are clearly seen in the *image* (3.1.75)

Note 1 to entry: The value of this range is usually taken to lie between 500 and 1 000 times the numerical aperture of the objective. When the total visual magnification is less than the lower limit, the resolving power of the objective cannot be fully utilized; when the total visual magnification exceeds the upper limit, empty magnification occurs. This phenomenon is due to the resolving properties of the eye, generally assumed to be between 2 and 4 minutes of arc.

3.1.90.10
visual magnification
ratio of the tangent of the *viewing angle* (3.1.147) of the *object* (3.1.104) when observed through a magnifying system with the *image* (3.1.75) at infinity, to that of the object when observed by the naked eye at the *reference viewing distance* (3.1.124) (250 mm)

Note 1 to entry: This ratio should be expressed in numerical form with the multiplication sign, e.g. ×10.

3.1.91
magnification changer
intermediate lens (3.1.87.7) for changing the *lateral magnification* (3.1.90.8) of the *primary image* (3.1.75.2)

Note 1 to entry: The effect of a magnification changer is expressed as a tube factor, which may be varied step by step or continuously. In the case of an infinity-corrected objective, the same effect may be achieved by exchanging the tube lens for another of different focal length.

3.1.92
magnifier
converging *lens* (3.1.87) used between an *object* (3.1.104) and the eye to increase the *viewing angle* (3.1.147) and hence to provide a magnified *image* (3.1.75) on the retina of the eye

3.1.92.1
focusing magnifier
adjustable *magnifier* (3.1.92) used to help in the precise *focusing* (3.1.67) of an *image* (3.1.75) in photography and *photomicrography* (3.1.115)

3.1.93
marking of optical components
inscribing of data in the form of characters or colour bands onto optical components in order to indicate their optical properties, values of certain properties and the origin of a component

Note 1 to entry: Details are given in ISO 8578.

3.1.93.1
colour marking of objectives
marking (3.1.93) of *objectives* (3.1.106) by means of coloured rings and/or engraving to denote properties according to a *colour code of objectives* (3.1.93.1.1)

3.1.93.1.1
colour code of objectives
system of *colour marking of objectives* (3.1.93.1) by means of coloured bands applied to the mount of an *objective* (3.1.106) indicating a range of *magnification* (3.1.90) and other properties

Note 1 to entry: The colours may be assigned from black, through the spectrum from red to violet, and white to denote increasing magnification, as detailed in ISO 8578.

3.1.94
micrograph
record of an *image* (3.1.75) formed by a *microscope* (3.1.99)

3.1.95
micromanipulator
instrument which allows fine manipulation of components of a preparation by means of mechanical reduction of the movements of the hand whilst they are observed with a *microscope* (3.1.99)

3.1.96
micrometer
device for measuring small lengths

3.1.96.1
stage micrometer
special *graticule* (3.1.70) in the form of a scale carried at natural size on a *microscope* (3.1.99) *slide* (3.1.134) which is used as an absolute standard of length for calibrating microscope measuring systems

3.1.97
microphotography
photography, especially of documents, arranged to produce small *images* (3.1.75) which cannot be studied without *magnification* (3.1.90)

Note 1 to entry: Not to be confused with photomicrography.

3.1.98
microprojector
microscope (3.1.99) designed or adapted to project a magnified *image* (3.1.75) onto a *screen* (3.1.132) for demonstration or drawing

3.1.99
microscope
instrument designed to extend visual capability, i.e. to make visible minute detail that is not seen with the unaided eye

Note 1 to entry: The word is qualified by prefixes (electron, X-ray, acoustic, field-ion, etc.) unless it is clear from the context that the imaging involved is by means of light.

3.1.99.1
binocular microscope
compound microscope (3.1.99.3) in which a separate *image* (3.1.75) is presented to each of the observer's eyes simultaneously

Note 1 to entry: There are two types of binocular microscope: those in which, by the use of a special viewing tube and beam splitter, both eyes are presented with identical images, and stereomicroscopes.

3.1.99.2
comparison microscope
system of two *microscopes* (3.1.99), optically linked to present their *images* (3.1.75) into one *field* (3.1.54)

Note 1 to entry: The image field is usually split so that the image from each microscope is seen in the corresponding half of the field enabling, for example, the fine details of two similar specimens to be compared.

3.1.99.3
compound microscope
microscope (3.1.99) in which the *primary image* (3.1.75.2) is generated by an *objective* (3.1.106), or an objective and a *tube lens* (3.1.87.10), and is observed through an *eyepiece* (3.1.52)

3.1.99.4
dissecting microscope
low-power *microscope* (3.1.99) of long *free working distance* (3.1.69) used for dissecting

Note 1 to entry: This is nowadays generally a stereomicroscope.

3.1.99.5
fluorescence microscope
microscope (3.1.99) in which the *image* (3.1.75) is formed by *light* (3.1.88) emitted by *fluorescence* (3.1.58) from the *object* (3.1.104) itself, and/or from a *fluorochrome* (3.1.60)

Note 1 to entry: The object may be regarded as self-luminous and the light emitted is not coherent.

3.1.99.6
infrared microscope
microscope (3.1.99) in which the *image* (3.1.75) is formed with *infrared radiation* (3.1.123.1) and is displayed by means of a photographic or electronic device

Note 1 to entry: Microscopy using near infrared radiation may be performed with a conventional microscope; microscopy in the far infrared requires special equipment.

3.1.99.7
inverted microscope
microscope (3.1.99) in which the *object* (3.1.104) is observed from beneath the *stage* (3.1.136)

3.1.99.8
light microscope
microscope (3.1.99) which uses *light* (3.1.88) as the illuminating agent

Note 1 to entry: This term is often loosely used to include ultraviolet microscopes and infrared microscopes.

3.1.99.9
monocular microscope
microscope (3.1.99) which presents the *image* (3.1.75) to only one eye

3.1.99.10
polarized-light microscope
microscope (3.1.99) specially designed or additionally equipped for *polarized-light* (3.1.88.1) microscopy

Note 1 to entry: In its fullest form it has a polarizer, analyser, strain-free lenses between the polars, a rotating stage equipped with a scale to measure rotation angles, a mechanism for centration of the stage and/or the objectives and a focusable eyepiece with centred and oriented cross lines. There is also a Bertrand lens and a tube slot for the insertion of retardation plates and compensators. A polarized-light microscope for reflected light is sometimes known as an “ore microscope”.

3.1.99.11
reflected-light microscope
microscope (3.1.99) which uses *epi-illumination* (3.1.73.2)

3.1.99.12
scanning optical microscope
microscope (3.1.99) specially designed to scan the *object plane* (3.1.117.5) or *image plane* (3.1.117.3) in a raster pattern

Note 1 to entry: Light signals at discrete and uniform intervals are received from the object by a photoelectric sensor and displayed on a screen or stored for further processing. The image is thus built up serially. There are two techniques of scanning: one is based on movement of the illuminating beam with the object remaining stationary, the other on the movement of the object, the beam remaining stationary. The instrument may be operated in the confocal imaging mode.

3.1.99.13
simple microscope
microscope (3.1.99) consisting of only one *lens* (3.1.87), the *objective* (3.1.106)

3.1.99.14
stereomicroscope
binocular microscope (3.1.99.1) in which the *object* (3.1.104) is observed by each eye from a slightly different angle, such that disparate *image* (3.1.75) points will be imaged on corresponding points of the retina and thus cause stereoscopic perception

Note 1 to entry: The Greenough microscope has two completely separate optical systems inclined at a particular convergence angle with respect to each other with prisms and/or mirrors to give an erect image. More recent systems use a common main objective whereby the convergence angle of both paths of rays is achieved by dividing the pupil in the back focal plane of the objective.

3.1.99.14.1
Greenough microscope
original design of low-power *stereomicroscope* (3.1.99.14) due to Greenough, consisting of two separate *compound microscope* (3.1.99.3) systems mounted with their axes converging at an angle of between 10° and 15°, so that they observe a common *object field* (3.1.54.4) and in which *prisms* (3.1.119) and/or mirrors are fitted to erect the *image* (3.1.75) and usually to incline the *viewing tubes* (3.1.144.6)

3.1.99.15
ultraviolet microscope
microscope (3.1.99) in which the *image* (3.1.75) is formed with *ultraviolet radiation* (3.1.123.3) and is displayed and recorded by means of a photographic or electronic device

Note 1 to entry: The well-corrected conventional microscope with high transmittance in ultraviolet region may perform microscopy using the near ultraviolet; microscopy in the far ultraviolet requires special equipment.

3.1.100
microscope base
part of the *microscope* (3.1.99) stand which rests on the work table and to which the other parts of the instrument are attached

Note 1 to entry: In modern instruments, the base may contain parts of the illuminating system.

3.1.101
monochromat
lens (3.1.87) in which the change of *focal length* (3.1.61) with wavelength is uncorrected, and *aberrations* (3.1.4) are minimized for only one wavelength

Note 1 to entry: The term is usually used to describe an objective made of fused silica and designed to operate at a specific wavelength in the ultraviolet.

3.1.102
mounting medium
liquid, synthetic resin or other medium in which the *object* (3.1.104) or objects are placed for investigation with the *microscope* (3.1.99)

Note 1 to entry: For transmitted-light microscopy, this medium is transparent, colourless and of specified refractive index, enclosed between the slide and the cover glass. For reflected-light microscopy, the mounting medium is normally a resin with which the sample is impregnated so that a polished section may be made.

3.1.103
nosepiece
part of the *body tube* (3.1.144.2) which carries the *objective* (3.1.106)

3.1.103.1
centring nosepiece
nosepiece (3.1.103) equipped with a centring mechanism which allows the position of the *objective* (3.1.106) to be adjusted laterally until its *optical axis* (3.1.107) coincides with the rotation axis of a *rotating stage* (3.1.136.7)

3.1.103.2
revolving nosepiece
nosepiece (3.1.103) with a rotating turret which facilitates changing *objectives* (3.1.106)

3.1.104
object
anything from which an *image* (3.1.75) is formed

3.1.104.1
object marker
accessory which may be fitted to the *nosepiece* (3.1.103) which, when moved to replace the *objective* (3.1.106), will mark an area of interest on an *object* (3.1.104) or preparation

3.1.105
object space
space on that side of an optical system where the *object* (3.1.104) is located

Note 1 to entry: In reflection or formation of a virtual image, this space may coincide with the image space.

3.1.106
objective
first part of the imaging system, consisting of a *lens* (3.1.87), its mount and any associated parts, which forms a *primary image* (3.1.75.2) of the *object* (3.1.104), either alone or in conjunction with a *tube lens* (3.1.87.10)

3.1.106.1
dry objective
objective (3.1.106) where the medium between the front *lens* (3.1.87) and the *cover glass* (3.1.34), or an uncovered *object* (3.1.104), is air

3.1.106.2
finite primary image distance objective
objective (3.1.106) corrected for a finite *object to primary image distance* (3.1.80.2.2) and which alone is capable of forming the *primary image* (3.1.75.2)

3.1.106.3
infinity-corrected objective
objective (3.1.106) corrected for an infinite *object to primary image distance* (3.1.80.2.2) and which, therefore is used with a *tube lens* (3.1.87.10)

Note 1 to entry: When combined with its normal tube lens of appropriate focal length, such an objective obtains its nominal magnification.

3.1.106.4
long-working-distance objective
objective (3.1.106) designed to have a longer *free working distance* (3.1.69) than a conventional objective of the same *magnification* (3.1.90)

3.1.106.5
plan objective
flat-field objective
objective (3.1.106) so corrected that the flattening of the *curvature of the image field* (3.1.4.4) in the *primary image plane* (3.1.117.4) is emphasized in addition to the *correction* (3.1.33) of other *aberrations* (3.1.4)

Note 1 to entry: This term does not imply any degree of correction for other aberrations.

3.1.106.6
spring-loaded objective
objective (3.1.106) so constructed that the front *lens* (3.1.87) and its mount will retract against a spring when brought into contact with the *object* (3.1.104) or an obstruction, thus preventing damage to either object or objective

3.1.106.7
screw thread for objective
screw thread for connecting a *microscope* (3.1.99) *objective* (3.1.106) to the *nosepiece* (3.1.103)

Note 1 to entry: Dimensions are given in ISO 9345.

3.1.106.7.1
RMS thread
screw thread for objective (3.1.106.7), originally standardized by the Royal Microscopical Society

3.1.106.8
objective spectral transmittance by design
OSTD
spectral transmittance calculated under the following conditions:

a) on-axis *light* (3.1.88) path;

b) internal absorption of transparent materials according to specifications by the materials manufacturer is included;

c) reflectance of thin film *coatings on optical surfaces* (3.1.25) according to their nominal value is included;

d) internal absorption and surface reflectance of immersion media and specimen covering is neglected

Note 1 to entry: For more information on spectral transmittance see ISO 19012-3.

3.1.107
optical axis
imaginary line joining the centres of curvature of *lens* (3.1.87) surfaces of an optical system or sub-system

3.1.108
optical path length
optical distance
product of the geometrical length of an optical path in a homogeneous medium and the *refractive index* (3.1.125) of the medium containing that path

Note 1 to entry: The optical path length is expressed either in length units or as a fraction or multiple of a given wavelength. When the medium is inhomogeneous it is the sum or integral of the product of the geometrical lengths and refractive indices of the parts.

3.1.108.1
optical path length difference
OPD
difference in *optical path length* (3.1.108) between two optical paths due to differences in geometrical length, *refractive index* (3.1.125), or both. The OPD is expressed in length units or wavelengths

3.1.109
parfocal
having the state that once any *lens* (3.1.87) of a set which can be an *objective* (3.1.106), a *tube lens* (3.1.87.10) or an *eyepiece* (3.1.52), has been focused on to the *object* (3.1.104) or adjusted so that the *image* (3.1.75) lies at its correct level, if this lens is exchanged for any others in that set at a constant setting of the *microscope* (3.1.99), only minimal readjustment of *focus* (3.1.65) may be necessary to restore sharpness

Note 1 to entry: A small readjustment may be needed, however, because of the accommodation which may take place in the eyes of an observer. Tolerances for parfocality are given in ISO 9345.

3.1.110
phase
relative position in a cyclical or wave motion which is expressed as an angle, one cycle corresponding to 2π radians or 360°

Note 1 to entry: The term “in phase” corresponds to phase angles between the two occurrences of 0 and 2π radians (360°) or a whole number multiple of these.

3.1.110.1
phase difference
phase (3.1.110) angle or fraction or number of wavelengths by which one periodic disturbance or wave lags behind or precedes another in time or space

Note 1 to entry: The phase difference is related to the optical path length difference OPD and the wavelength, λ, by the formula:

$$\text{phase difference} = 2\pi L_{OPD}/\lambda$$

where

L_{OPD} is the *optical path length difference (OPD)* (3.1.108.1);
λ is the wavelength

3.1.111
phase object
object (3.1.104) which produces a *phase difference* (3.1.110.1) between the *direct light* (3.1.45) and the *diffracted light* (3.1.40), but has a small or negligible effect on the amplitude

3.1.112
phase plate
optical device used in *phase contrast* (3.1.32.4) microscopy which influences differently the *phase* (3.1.110) and amplitude of the *direct light* (3.1.45) and *diffracted light* (3.1.40)

Note 1 to entry: The phase plate is placed in the back focal plane of the objective (or in the plane of a succeeding image of it), where it receives an image of a diaphragm (usually annular) positioned in the front focal plane of the condenser.

3.1.113
photomacrography
production of a photographic *image* (3.1.75) of an *object* (3.1.104) with a reproduction ratio in the image between 1:1 to about 15:1

3.1.114
photomicrograph
photographic record of an *image* (3.1.75) formed by a *microscope* (3.1.99)

3.1.115
photomicrography
recording by photography of an *image* (3.1.75) formed by a *microscope* (3.1.99); i.e. photography through a microscope

Note 1 to entry: Not to be confused with microphotography.

3.1.116
pixel
smallest element of the *digital image* (3.2.13) to which attributes are assigned

3.1.116.1
pixel size
shortest distance from the centre of one *pixel* (3.1.116) to the centre of an adjacent pixel measured in *object space* (3.1.105)

3.1.117
plane
imaginary surface normal to the *optical axis* (3.1.107)

3.1.117.1
aperture plane
pupil plane
plane (3.1.117) containing the *pupil* (3.1.122) of an optical system and any *plane conjugate* (3.1.29) with it

Note 1 to entry: A diaphragm inserted in an aperture plane will act as an aperture diaphragm.

3.1.117.2
field plane
object plane (3.1.117.5) and any *plane conjugate* (3.1.29) with it

Note 1 to entry: A diaphragm inserted in a field plane will act as a field diaphragm.

3.1.117.3
image plane
any *field plane* (3.1.117.2) in which an *image* (3.1.75) is situated

3.1.117.4
primary image plane
image plane (3.1.117.3) in which the *primary image* (3.1.75.2) is formed

Note 1 to entry: The primary image plane is important as one of the reference planes for the optical interfacing dimensions.

3.1.117.5
object plane
that *field plane* (3.1.117.2) in which the *object* (3.1.104) is situated

Note 1 to entry: The object plane is important as one of the reference planes for the optical interfacing dimensions.

3.1.117.6
reference plane
surface of a *microscope* (3.1.99) component or a *plane* (3.1.117) in the *light* (3.1.88) path of the microscope, used as a limit for one of the *optical interfacing dimensions* (3.1.80.2)

3.1.118
polar
device which selects *plane-polarized light* (3.1.88.1.2) from natural *light* (3.1.88)

3.1.118.1
analyser
polar (3.1.118) used after the *object* (3.1.104) to determine optical effects produced by the object on the *light* (3.1.88), polarized or otherwise, with which it is illuminated

Note 1 to entry: It is usually positioned between the objective and the primary image plane.

3.1.118.2
crossed polars
state in which the polarization directions of the *polars* (3.1.118) (*polarizer* (3.1.118.4) and *analyser* (3.1.118.1)) are mutually perpendicular

3.1.118.3
parallel polars
state in which the polarization directions of the *polars* (3.1.118) [*polarizer* (3.1.118.4) and *analyser* (3.1.118.1)] are parallel

3.1.118.4
polarizer
polar (3.1.118) placed in the *light* (3.1.88) path before the *object* (3.1.104)

3.1.119
prism
block of transparent material limited by at least two intersecting planes, used to disperse *light* (3.1.88) or deviate it through an angle

3.1.119.1
Nicol prism
type of *polarizing prism* (3.1.119.3)

3.1.119.2
Nomarski prism
form of *Wollaston prism* (3.1.119.4), introduced by Nomarski, in which the crystal axis of one of the wedges is tilted

Note 1 to entry: The Nomarski prism has the effect of shifting the point of intersection of the two beams so that it lies outside the prism. In effect this enables recombination of the beams to occur at the back focal plane of the objective, while the prism itself lies beyond this plane. Prisms of similar design may also be used in the condenser.

3.1.119.3
polarizing prism
double *prism* (3.1.119) formed from two pieces of double-refracting material, which acts by refraction and total internal reflection or by refraction only

EXAMPLE The double refracting material can be calcite, quartz or one of these plus a piece of glass, cemented together.

Note 1 to entry: The polarizing prism splits a beam of natural light into two beams of plane-polarized light having mutually perpendicular vibration directions and being propagated in two different directions. When one of these beams is removed, e.g. by absorption, the prism acts as a polar, otherwise it may be used as a beam-splitter. Many types of polarizing prism exist, most known by the name of their originator, e.g. Glan-Thompson, Nicol.

3.1.119.4
Wollaston prism
double *prism* (3.1.119) formed from two pieces of double-refracting material, which can be calcite or quartz, which acts by refraction and splits one beam of *plane-polarized light* (3.1.88.1.2) into two beams of plane-polarized light having mutually perpendicular vibration directions, propagated in two different directions

3.1.120
projection factor
p
factor by which the *total magnification of a microscope* (3.1.90.2) is changed when forming a *real image* (3.1.75.3) of the *object* (3.1.104) onto a detecting device such as a photographic emulsion in a camera

Note 1 to entry: The image can be formed in different ways:

a) Using a normal eyepiece intended for visual observation, together with an infinity-corrected camera lens focused at infinity, the value of the projection factor is given by:

$$p = f_{\mathrm{PROJ}} / 250$$

where

p is the projection factor;
f_{PROJ} is the focal length of the camera lens in millimetres;
250 is the reference viewing distance.

b) Using only a normal eyepiece intended for visual observation, the value of the projection factor is given by:

$$p = a/250$$

where

p is the projection factor;

a is the distance from the back focal plane of the eyepiece to the projected image in millimetres;

250 is the reference viewing distance.

c) Using a projection lens. A projection lens can be assigned a magnification for producing a real image in a given plane. The value of the magnification of the projection lens, M_{PHOT}, is used to calculate the total magnification of the microscope used to produce the real image.

3.1.121
projection lens
lens (3.1.87) which forms a *real image* (3.1.75.3), at a finite distance, of the *microscope's* (3.1.99) *primary image* (3.1.75.2) and which is used for projection, drawing, *photomicrography* (3.1.115) and video purposes

Note 1 to entry: A projection lens may take the form of a

— positive or converging lens positioned between the primary image and the projected image,

— positive or converging lens positioned in front of both images,

— negative or diverging lens positioned in front of both images.

3.1.122
pupil
minimum common cross-section of all ray bundles both in *object space* (3.1.105) (the entrance pupil) and in *image space* (3.1.76) (the exit pupil) of a *lens* (3.1.87)

Note 1 to entry: This term may indicate an aperture or the image of an aperture.

3.1.122.1
entrance pupil of the microscope
image (3.1.75) of the *objective's* (3.1.106) *aperture diaphragm* (3.1.38.1) at infinity (except in the case of certain low-magnification objectives) in *object space* (3.1.105)

Note 1 to entry: If the microscope has an illuminating system, any plane conjugate with the microscope entrance pupil can also be called the entrance pupil of the entire microscope.

3.1.122.2
exit pupil of the microscope
eyepoint
area lying in a *plane* (3.1.117) several millimetres after the *eyepiece* (3.1.52) on the observer's side where an *image* (3.1.75) of the *objective's* (3.1.106) exit *pupil* (3.1.122) is formed by the *eyepiece* together with any *intermediate lenses* (3.1.87.7)

Note 1 to entry: The exit pupil of the microscope is important because its position and size dictate the position of the pupil of the observer's eye and the nature of other succeeding optical systems such as cameras.

3.1.123
radiation
energy in the form of electromagnetic waves or particles

3.1.123.1
infrared radiation
radiation (3.1.123) in which the wavelengths of its components are longer than those for visible *light* (3.1.88) and less than about 1 mm

3.1.123.2
monochromatic radiation
radiation (3.1.123) consisting of only a single wavelength, or of only a very narrow band of wavelengths of which the central wavelength is quoted

3.1.123.3
ultraviolet radiation
radiation (3.1.123) in which the wavelengths of its components are shorter than those of visible *light* (3.1.88) and longer than about 100 nm

3.1.124
reference viewing distance
internationally agreed standardized distance of 250 mm between an *object* (3.1.104) and the vertex of the cornea of the eye

Note 1 to entry: This term supersedes the older "nearest distance of distinct vision" in optical calculations.

3.1.125
refractive index
n or n'
ratio of the speed of *light* (3.1.88) (more exactly, the phase velocity) in a vacuum to that in a given medium

3.1.126
relief
<surface> differences in height of a surface, e.g. of a flat sculpture

Note 1 to entry: When illuminated from one side, such an object shows a characteristic distribution of light and shadow which enables the observer to recognize the three-dimensional form of the object.

3.1.127
relief
<contrast techniques> *light* (3.1.88) distribution appearing in microscopy using azimuthal methods like *oblique illumination* (3.1.73.4), *relief contrast* (3.1.32.5), *differential interference contrast* (3.1.32.2.1) or *modulation contrast* (3.1.32.3) at the interfaces of *object* (3.1.104) elements with different *optical path length* (3.1.108), even when no geometrical *relief* (3.1.126) exists

Note 1 to entry: In this case, the light distribution appears similar to that produced by a genuine relief. Take care to avoid misinterpreting optical path length differences as being geometrical ones. A further misinterpretation of relief may be caused by inversion.

3.1.128
resolution
result of displaying fine details in an *image* (3.1.75)

Note 1 to entry: The term "resolution" sometimes refers to its quantitative expression, the resolved distance.

Note 2 to entry: When used without any qualification, this term refers to distances at right angles to the optical axis.

3.1.128.1
lateral resolution
resolution (3.1.128) perpendicular to the *optical axis* (3.1.107)

3.1.128.2
minimum resolvable distance
smallest separation of points in an *object* (3.1.104) which can be recognized as distinct in an *image* (3.1.75)

Note 1 to entry: In microscopy this is normally expressed in units of length (µm or nm).

3.1.128.3
resolved distance
distance equal to or greater than the *minimum resolvable distance* (3.1.128.2)

3.1.128.4
resolving power
ability to make points or lines which are closely adjacent in an *object* (3.1.104) distinguishable in an *image* (3.1.75)

Note 1 to entry: High resolving power implies that the resolved distance is small.

3.1.128.5
axial resolution
resolution (3.1.128) in the direction of the *optical axis* (3.1.107)

3.1.128.4.1
diffraction limit of resolving power
diffraction limit
fundamental limitation imposed upon the *resolving power* (3.1.128.4) of a system by the phenomenon of *diffraction* (3.1.41) alone and not by *aberrations* (3.1.4)

3.1.129
retardation
difference in *optical path length* (3.1.108) expressed in wavelengths, length units or phase angles between two mutually perpendicular *plane-polarized light* (3.1.88.1.2) waves

3.1.130
retardation plate
compensator
piece, or pieces, of optically *anisotropic* (3.1.8) material with plane faces, inserted between *crossed polars* (3.1.118.2) in a diagonal position to produce a specific *optical path length difference* (3.1.108.1) between mutually perpendicular *plane-polarized light* (3.1.88.1.2) waves

3.1.131
scale bar
line of calculated length drawn on a *micrograph* (3.1.94) to indicate the length in the micrograph of a stated length in the *object* (3.1.104)

3.1.132
screen
reflecting or translucent surface on which a *real image* (3.1.75.3) may be formed and observed

3.1.133
semi-apochromat
fluorite objective
objective (3.1.106) intermediate in its *correction* (3.1.33) and complexity of construction between *achromats* (3.1.6) and *apochromats* (3.1.13)

3.1.134
slide
flat rectangular plate of glass on which an *object* (3.1.104) is mounted for microscopical examination

Note 1 to entry: For calculation and correction of the condenser, it is regarded as part of the condenser, so that its thickness, refractive index and dispersion must be adapted to the demands of the condenser. These parameters together with its length and width are defined by ISO 8037-1.

3.1.135
source
source of *radiation* (3.1.123)

3.1.135.1
point source
source whose dimensions are sufficiently small to cause the emitted *radiation* (3.1.123) to have a very high degree of coherence

3.1.136
stage
microscope stage
platform, at right angles to the *optical axis* (3.1.107) of the *microscope* (3.1.99), which carries the *object* (3.1.104) and which is often fitted with mechanical movements [as in a *mechanical stage* (3.1.136.6)] to allow easy positioning of the object in the *x*- and *y*-axes, and movement along, and rotation about, the *z*-axis

3.1.136.1
centring stage
rotating stage (3.1.136.7) fitted with provision for bringing its axis of rotation into coincidence with the *optical axis* (3.1.107) of the *microscope* (3.1.99)

3.1.136.2
cooling stage
stage (3.1.136) fitted with means for lowering the temperature of the *object* (3.1.104)

3.1.136.3
gliding stage
movable *stage* (3.1.136) consisting of two flat plates, the upper of which can be moved smoothly in all directions in the *x*-*y* plane over the lower one, which is fixed to the *stand* (3.1.138)

Note 1 to entry: The ease of movement is regulated by the viscosity of the layer of grease which is used to connect the two plates.

3.1.136.4
heating stage
stage (3.1.136) fitted with means for raising the temperature of the *object* (3.1.104)

3.1.136.5
levelling stage
stage (3.1.136) designed to hold a polished section so that its surface is normal to the *optical axis* (3.1.107) of the *microscope* (3.1.99)

3.1.136.6
mechanical stage
stage (3.1.136) fitted with screw or rack mechanisms to assist in precise translational movement of the *object* (3.1.104) in the *x* and *y* directions

Note 1 to entry: The stage may be manually or motor operated, attachable, or built into the microscope stand. In computer-controlled microscopes, motors are used to drive the stage.

3.1.136.7
rotating stage
stage (3.1.136) fitted with means for rotating the *object* (3.1.104) with respect to the *optical axis* (3.1.107) of the *microscope* (3.1.99) which may or may not be centrable and/or calibrated for measuring angles of rotation

3.1.136.8
scanning stage
mechanical stage (3.1.136.6) electronically or electrically controlled to move the *object* (3.1.104) in steps or continuously in a raster fashion

3.1.136.9
universal stage
device mounted on a *rotating stage* (3.1.136.7) and equipped with a gimbal mechanism for movement of the *object* (3.1.104), which enables the effect of optical anisotropy to be investigated at any direction of *illumination* (3.1.73) or observation

Note 1 to entry: The movements comprise calibrated tilting and rotation around three or four axes (according to the type of stage) in addition to the usual movements of the microscope stage.

3.1.137
stage clip
flat spring used to hold a *slide* (3.1.134) in contact with the *microscope* (3.1.99) *stage* (3.1.136)

Note 1 to entry: The use of stage clips facilitates the precise movement of a slide with the fingers.

3.1.138
stand
microscope stand
chassis on which the mechanical and optical parts of the *microscope* (3.1.99) are carried

3.1.139
stop
diaphragm (3.1.38), usually of fixed size

Note 1 to entry: This term is often used loosely.

3.1.140
strain-free
property of a *lens* (3.1.87) intended for use with the *polarized-light microscope* (3.1.99.10), manufactured by careful selection and mounting of its component parts so that *double refraction* (3.1.48) due to strain is minimized

3.1.141
substage
assembly of mechanical and opto-mechanical parts attached to the *stand* (3.1.138) of a transmitted-light *microscope* (3.1.99) before the *stage* (3.1.136), consisting of the *condenser* (3.1.28) with its carrier and, optionally, a *filter* (3.1.55) tray, a *polarizer* (3.1.118.4) with its carrier and/or auxiliary *lenses* (3.1.87) with their carriers

3.1.142
temporal resolution
smallest time interval between sequential *digital* (3.2.13) or *electronic images* (3.2.14) from which independent information can be obtained

3.1.143
test object
object (3.1.104) designed to assess the performance of a *microscope* (3.1.99) system, e.g. *Abbe test plate* (3.1.1), diatom preparation

3.1.144
tube
part of the *microscope* (3.1.99) which connects the *objective* (3.1.106) and the *eyepiece* (3.1.52)

Note 1 to entry: In early microscopes, the tube was in the form of a hollow cylinder carrying at one end the objective-locating surface and at the other the *eyepiece* -locating surface. In microscopes of more recent design, the tube may be divided into two or more sections or housings, one or more being attached to the stand. The housings may not be cylindrical but shaped to offer the most convenient manipulation of the included opto-mechanical elements. In the case of a reflected-light microscope, that part of the epi-illuminator, which is situated between the nosepiece and the primary image plane, is considered to be part of the tube.

3.1.144.1
binocular tube
viewing tube (3.1.144.6) designed to accept two *eyepiece* (3.1.52) for binocular viewing

3.1.144.2
body tube
part of the *tube* (3.1.144), fixed to or incorporated into the *stand* (3.1.138), containing the *nosepiece* (3.1.103) on one side and carrying the *intermediate tube* (3.1.144.3) or *viewing tube* (3.1.144.6) on the other

Note 1 to entry: For certain purposes, the body tube may contain optical or opto-mechanical elements, e.g. intermediate lenses, beamsplitter, magnification changer, reflector or epi-illuminator, Bertrand lens, mechanisms for operating filters, retardation plates, etc. For work with infinity-corrected objectives, it may contain the tube lens.

3.1.144.3
intermediate tube
optional part of the *tube* (3.1.144) which is a housing, either integral with the *stand* (3.1.138) or exchangeable, forming part of the tube and containing some opto-mechanical elements

EXAMPLE Magnification changer, filter tray, Bertrand lens, analyser, slots for holding retardation plates, beamsplitter, etc.

3.1.144.4
monocular tube
viewing tube (3.1.144.6) designed to accept only one *eyepiece* (3.1.52)

3.1.144.5
trinocular tube
viewing tube (3.1.144.6) designed to accept two *eyepieces* (3.1.52) for binocular viewing, together with a third eyepiece or other *lens* (3.1.87) to enable simultaneous and/or alternate viewing and other use of the *image* (3.1.75)

3.1.144.6
viewing tube
part of the *tube* (3.1.144) equipped to carry one or more *eyepieces* (3.1.52), limited at one end by the *eyepiece-locating surface* (3.1.89.2) and at the other by the *body tube* (3.1.144.2) *locating surface* (3.1.89)

Note 1 to entry: For use with infinity-corrected objectives, it may contain the tube lens.

3.1.145
tube length
distance between mechanical and/or optical surfaces or *planes* (3.1.117) in the *tube* (3.1.144) of a *microscope* (3.1.99)

3.1.145.1
mechanical tube length
distance in air between the objective-locating surface of the nosepiece and the eyepiece-locating surface of the viewing tube

Note 1 to entry: It is the length of the tube in its simplest form without any intermediate lenses for objectives corrected for a finite primary image distance.

Note 2 to entry: It commonly has a value of 160 mm. See ISO 9345.

Note 3 to entry: For infinity-corrected objectives, the mechanical tube length is hypothetically considered to be infinite.

3.1.145.2
optical tube length
distance between the *back focal plane* (3.1.62.1) of the *objective* (3.1.106) and the *primary image plane* (3.1.117.4)

Note 1 to entry: This distance is not one of the optical interfacing dimensions of the microscope and is relevant only to tubes fitted with finite primary image distance objectives.

3.1.146
tube factor
q
factor by which the *lateral magnification* (3.1.90.8) at the *primary image* (3.1.75.2) is changed by an *intermediate lens* (3.1.87.7) or *lens* (3.1.87) system inserted between the *objective* (3.1.106) and the primary image

Note 1 to entry: Intermediate lenses can be fixed, interchangeable, or associated with accessories having their own tube factors. The total tube factor is the product of individual factors of the intermediate lenses. In the case of objectives corrected for infinite primary image distance, the value of the tube factor of a tube lens used instead of the normal tube lens, with which the objective is designed to operate, is given by the ratio of its focal length to that of the normal tube lens, i.e.

$$q = f_{TL} / f_{NTL}$$

where

q is the total tube factor;
f_{TL} is the focal length of the tube lens in millimetres;
f_{NTL} is the focal length of the normal tube lens in millimetres.

3.1.147
viewing angle
angle subtended by an *object* (3.1.104) or a *field* (3.1.54) at the eye

3.1.147.1
angle of view
<eyepiece> angle between two principal rays coming from opposite points on the margin of the *visual field diaphragm* (3.1.38.8) of the *eyepiece* (3.1.52) and passing through the centre of the *exit pupil of the microscope* (3.1.122.2)

Note 1 to entry: The extent of the retina covered by the image is governed by this angle.

3.1.148
zoom
property of an optical system signifying that its *focal length* (3.1.61) and *magnification* (3.1.90) can be changed continuously between limits by moving a single *lens* (3.1.87) or group of lenses without altering the positions of the *object planes* (3.1.117.5) or *image planes* (3.1.117.3)

3.2 Terms and definitions relating to advanced techniques in light microscopy

3.2.1
acousto-optical modulator
electronically-tunable device used to control the direction and/or *intensity* (3.1.79) of a *laser* (3.1.86) by an acoustically-induced *diffraction grating* (3.1.42) in a crystal

3.2.2
acousto-optical tunable filter
AOTF
electronically-tunable *filter* (3.1.55) for selection of wavelengths by an acoustically-induced *diffraction grating* (3.1.42) in a crystal

3.2.3
aliasing
phenomenon caused by sampling at too low a frequency (i.e. lower than the Nyquist frequency) resulting in the loss of information and/or the creation of spurious information

3.2.4
autofocus
method of bringing an *object* (3.1.104) automatically into *focus* (3.1.65), controlled by an imaging software algorithm and/or a hardware device that detects the object position

3.2.5
background subtraction
removal of that part of the signal that is present in the absence of the *object* (3.1.104), to reveal underlying *image* (3.1.75) information

3.2.6
binning
mode of operation of an image sensor where the charge of adjacent *pixels* (3.1.116) is accumulated and is read out as a single value

3.2.7
confocal
microscopy state in which, ideally, a point in the *object field* (3.1.54.5) is illuminated by a diffraction-limited spot of *light* (3.1.88), and light emanating from this point is focused upon and detected from an area smaller than the central area of the *diffraction disc* (3.1.7.1) situated in the corresponding position in a subsequent *field plane* (3.1.117.2)

3.2.8
channel
particular signal path containing one type of *image* (3.1.75) information

3.2.9
co-localization
overlay of *digital images* (3.2.13) with coincidence of *pixels* (3.1.116) corresponding to the same *object* (3.1.104) points containing different information

3.2.10
confocal microscopy
microscopic technique in which, ideally, a point in the *object plane* (3.1.117.5) is illuminated by a diffraction-limited spot of *light* (3.1.88), and light emanating from this point is focused upon and detected from an area smaller than the central area of the *diffraction disc* (3.1.7.1) situated in the corresponding position in a subsequent *field plane* (3.1.117.2)

Note 1 to entry: An image of an extended area is formed either by scanning the object, or by scanning the illuminated and detected spots simultaneously.

Note 2 to entry: The confocal principle leads to improved axial resolution by suppression of light from out-of-focus planes.

3.2.10.1
laser-scanning confocal microscopy
confocal microscopy (3.2.10) in which the *light* (3.1.88) source is a *laser* (3.1.86)

3.2.10.2
multiple-beam confocal microscopy
confocal microscopy (3.2.10) using more than one illuminated and detected spot simultaneously

3.2.10.3
Nipkow disc confocal microscopy
confocal microscopy (3.2.10) in which the *scanning* (3.2.41) of the illuminated and detected spots is performed using a *Nipkow disc* (3.2.10.3.1)

3.2.10.3.1
Nipkow disc
opaque disc with many small holes, ideally identical, arranged in Archimedean spirals

3.2.10.3.2
tandem-scanning confocal microscopy
Nipkow disc confocal microscopy (3.2.10.3) in which the illuminating *light* (3.1.88) and the detected light pass through separate holes

3.2.10.4
spectral confocal microscopy
confocal microscopy (3.2.10) in which a spectrum is recorded corresponding to spatial positions in an *object* (3.1.104)

3.2.10.5
theta confocal microscopy
confocal microscopy (3.2.10) in which two *objectives* (3.1.106) positioned at an angle, θ, with respect to one another, and with *focal points* (3.1.63) coincident in the *object* (3.1.104), are used for *excitation* (3.1.49) and collection respectively

3.2.10.6
white-light confocal microscopy
confocal microscopy (3.2.10) using an *illumination* (3.1.73) *source* (3.1.135) and a detector operating throughout the visible spectrum

3.2.10.7
confocal point spread function
product of the *point spread functions* (3.2.34) of the illuminating and detecting optical systems in *confocal microscopy* (3.2.10)

3.2.10.8
confocal volume
effective volume around each point in the *object* (3.1.104) which gives rise to the *image* (3.1.75) in *confocal microscopy* (3.2.10)

3.2.10.9
4 pi confocal microscopy
confocal microscopy (3.2.10) in which two opposing *objective* (3.1.106) lenses with *focal points* (3.1.63) coincident in the *object* (3.1.104) are used to produce interference in the focal region from which an *image* (3.1.75) signal is derived, and with further processing produces an *digital* (3.2.13) or *electronic image* (3.2.15) with enhanced *axial resolution* (3.1.128.5)

3.2.11
deconvolution
<microscopy> mathematical technique for reducing blur, performed either in the spatial domain, or in the frequency domain by inverse filtering techniques

Note 1 to entry: If the deconvolution is based solely on theoretical as opposed to measured values it is known as blind deconvolution.

3.2.12
digitally enhanced contrast
contrast (3.1.32) enhanced by manipulation of the intensity and/or colour values in a *digital image* (3.2.13)

3.2.13
digital image
image (3.1.75) in which the information is in the form of binary or another machine code

3.2.14
electronic image
image (3.1.75) in which the information is in the form of electrical signals

3.2.15
extended depth of field microscopy
microscopy in which the *point spread function* (3.2.34) is modified in a known fashion such that it becomes substantially invariant over an extended focal range, and by further processing results in an *digital* (3.2.13) or *electronic image* (3.2.14) with extended *depth of field* (3.1.36)

3.2.16
extended focus image
<image processing> two-dimensional *digital image* (3.2.13) derived by summing the *pixel* (3.1.116) intensity values in a projection through an *image stack* (3.2.27.1)

3.2.17
fluorescence correlation microscopy
microscopy in which time-dependant *intensity* (3.1.79) fluctuations occurring within a *confocal volume* (3.2.10.8) are used to calculate the mobility of fluorescent molecules

3.2.18
fluorescence *in-situ* hybridisation microscopy
FISH
microscopy in which chromosomes or specific positions within chromosomes can be fluorescently labelled by *in-situ* hybridisation

3.2.19
fluorescence life-time imaging microscopy
FLIM
imaging technique based on discriminating characteristic *fluorescence* (3.1.58) decay rates

3.2.20
fluorescence recovery after photobleaching
FRAP
technique in which a region in the *object* (3.1.104) is irradiated to deplete its *fluorescence* (3.1.58), the subsequent recovery of fluorescence in the irradiated region being measured

3.2.21
fluorescence resonance energy transfer
Förster resonance energy transfer
FRET
non-radiative transfer of energy between two fluorophores in close proximity

3.2.22
frame averaging
averaging the *pixel* (3.1.116) values from sequential *digital* (3.2.13) or *electronic images* (3.2.14) recorded under identical conditions

Note 1 to entry: Used to increase signal-to-noise ratio.

3.2.23
image intensifier
device which increases the dynamic range of a signal to match the range of the detector

3.2.24
linear array sensor
detector in the form of a line of sensitive elements

3.2.25
maximum intensity image
<image processing> two-dimensional *digital* (3.2.13) or *electronic image* (3.2.14) derived from the maximum *pixel* (3.1.116) intensity values in a projection through an *image stack* (3.2.27.1)

3.2.26
microchannel plate
device positioned in front of a detector array to multiply incoming photon flux by secondary emission

3.2.27
multidimensional image data set
digital (3.2.13) or *electronic image* (3.2.14) data generated by recording data from a sample using several parameters, e.g., three-space dimensions, wavelength, time, polarization

3.2.27.1
image stack
multidimensional image data set (3.2.27) acquired from a three-dimensional region of an *object* (3.1.104)

3.2.27.2
focus series
Z stack
image stack (3.2.27.1) acquired at different focal positions

3.2.28
multi-mode fibre
optical fibre that can sustain more than one transverse electromagnetic mode

3.2.29
multi-photon fluorescence
fluorescence (3.1.58) *excitation* (3.1.49) by the simultaneous absorption of multiple coherent photons

3.2.29.1
multi-photon fluorescence microscopy
microscopy in which the *image* (3.1.75) is formed by *multi-photon fluorescence* (3.2.29)

Note 1 to entry: Since sufficient excitation intensity is achieved only in a limited focal volume, multi-photon fluorescence results in optical sectioning without the need for a confocal pinhole. It also permits excitation by longer wavelengths.

3.2.29.1.1
two-photon fluorescence
fluorescence excited by pairs of coherent photons

3.2.30
optical section
image (3.1.75) from a thin region whose thickness within a thick *object* (3.1.104) is defined by the *axial resolution* (3.1.128.5) of the optical system

3.2.31
photobleaching
destruction of fluorescing properties of molecules by *light* (3.1.88), resulting in reduced *fluorescence* (3.1.58) of the sample

3.2.32
pinhole
<confocal microscopy> *diaphragm* (3.1.38) situated in a *plane conjugate* (3.1.29) with the *object* (3.1.104), which restricts the area in the *object plane* (3.1.117.5) that is illuminated and/or from which *light* (3.1.88) is collected

3.2.33
point detection
detection of *light* (3.1.88) collected from a restricted point-like area of an *image* (3.1.75)

3.2.34
point spread function
PSF
<microscope system> mathematical expression of the distribution of the *light* (3.1.88) amplitude or *intensity* (3.1.79) in the *image* (3.1.75) of a *point source* (3.1.135.1)

3.2.34.1
intensity point spread function
mathematical expression of the distribution of the *light* (3.1.88) *intensity* (3.1.79) in the *image* (3.1.75) of a *point source* (3.1.135.1)

3.2.34.2
amplitude point spread function
mathematical expression of the distribution of the *light* (3.1.88) amplitude in the *image* (3.1.75) of a *point source* (3.1.135.1)

3.2.35
Raman microscopy
microscopy utilizing Raman scattering as the source of *image* (3.1.75) information

3.2.35.1
coherent anti-stokes Raman scattering microscopy
CARS microscopy
microscopy technique that employs multiple photons to induce the molecular vibrations and produces a coherent signal to form an *image* (3.1.75)

Note 1 to entry: One of the attributes is that the signal is orders of magnitude stronger than spontaneous Raman emission.

3.2.35.2
stimulated Raman scattering microscopy
SRS microscopy
microscopy technique that detects *intensity* (3.1.79) modulation of an *excitation* (3.1.49) beam caused by stimulated Raman excitation of the vibrational transition

Note 1 to entry: The key attribute of SRS microscopy is that it provides higher contrast imaging without non-resonant background signals.

3.2.36
ratio imaging
forming a *digital image* (3.2.13) in which the *pixel* (3.1.116) values are obtained by dividing the corresponding pixel values of two *images* (3.1.75)

3.2.37
real time imaging
displaying or analysing images at the same rate as that at which they are collected

Note 1 to entry: This rate is normally also commensurate with the dynamics of the processes to be observed within the specimen and perceived to be continuous by the eye.

3.2.38
region of interest
ROI
parts of an *image* (3.1.75) to which discrete observations are applied

3.2.39
scan rate
number of scan cycles completed per unit time

3.2.40
scanned field
dimensions of the scanned area in *object space* (3.1.105)

3.2.41
scanning
sequential *illumination* (3.1.73) of or detection from regions in an *object* (3.1.104)

Note 1 to entry: Scanning may be accomplished by moving the object, illuminating beam(s), objective or detector(s).

3.2.41.1
descanning
process by which an imaging beam retraces the path of the illuminating beam through the *scanning* (3.2.41) mechanism to produce a stationary beam

3.2.41.2
line scanning
scanning (3.2.41) along a single line

3.2.41.3
non-descanned detection
NDD
technique of obtaining an *image* (3.1.75) signal in a *scanning microscope* (3.2.42) without *descanning* (3.2.41.1)

Note 1 to entry: Widely used in multiphoton fluorescence microscopy.

3.2.41.4
point scanning
scanning (3.2.41) an area using a spot of *light* (3.1.88)

3.2.41.5
raster scanning
scanning (3.2.41) an area by a pattern of lines

3.2.41.6
slit scanning
scanning (3.2.41) an area with a bar of *light* (3.1.88)

3.2.41.7
stage scanning
scanning (3.2.41) performed by moving the *stage* (3.1.136) and hence the *object* (3.1.104)

3.2.42
scanning microscope
microscope (3.1.99) in which the *image* (3.1.75) is formed by *scanning* (3.2.41)

3.2.42.1
disc scanning microscope
scanning microscope (3.2.42) in which scanning is achieved by means of a perforated disc rotated in the *illumination* (3.1.73) and/or observation paths

3.2.42.2
laser-scanning microscope
scanning microscope (3.2.42) in which the *object* (3.1.104) is scanned by a *laser* (3.1.86) beam

3.2.42.3
scanning near-field microscope
scanning microscope (3.2.42) in which a small light-emitting probe is scanned across the *object* (3.1.104) (or *vice versa*) at a close distance

Note 1 to entry: When the probe diameter is smaller than the Airy disc, super-resolution may be achieved.

3.2.43
second harmonic generation microscopy
SHG-microscopy
microscopy in which the second harmonic of the *excitation* (3.1.49) *light* (3.1.88) is used to provide additional *image* (3.1.75) information

3.2.44
shading correction
method for adjusting intensity levels in a *digital* (3.2.13) or *electronic image* (3.2.14), caused by non-uniformities in the illuminating or detecting systems

3.2.45
single-mode fibre
optical fibre that can sustain only a single transverse electromagnetic mode

3.2.46
spectral imaging microscopy
microscopy in which a spectrum is recorded corresponding to spatial positions in an *object* (3.1.104)

3.2.47
structured illumination microscopy
SIM
microscopic technique in which the *object* (3.1.104) is illuminated by a spatially varying pattern so as to produce a composite *image* (3.1.75) from which *digital images* (3.2.13) with enhanced lateral and *axial resolution* (3.1.128.5) may be produced

3.2.47.1
grating image microscopy
structured illumination microscopy (3.2.47) in which the *object* (3.1.104) is illuminated by the pattern of a grating

3.2.48
super-resolution microscopy
microscopy technique achieving resolving power higher than given by the *diffraction limit* (3.1.128.1)

3.2.48.1
localization microscopy
technique to create a *super-resolution microscopy* (3.2.48) *digital* (3.2.13) or *electronic image* (3.2.14) of a marked specimen by localizing the positions of individual markers and generating a graphical representation of their determined spatial distribution

Note 1 to entry: The lateral resolution of this image is only limited by the precision of the localization process for each marker.

Note 2 to entry: Markers are typically photoactivatable or photoswitchable fluorescent probes. In order to generate the graphical representation, typically multiple images are captured sequentially.

3.2.48.2
stimulated emission depletion microscopy
STED-microscopy
super-resolution technique in fluorescence microscopy, where the *resolution* (3.1.128) enhancement is achieved by stimulated emission of excited molecules in the outer region of the spatial distribution of the *excitation* (3.1.49) *focus* (3.1.65) reducing the fluorescent volume

Note 1 to entry: In order to achieve higher resolutions, the stimulated emission process needs to be as complete as possible; i.e. the stimulated emission needs to be saturated.

3.2.48.3
super-resolution structured illumination microscopy
super resolution
super-resolution-SIM
structured illumination microscopy (3.2.47) in which the pattern resolution is close to the *diffraction limit* (3.1.128.1) of the optical system in order to compute *digital images* (3.2.13) with resolving power higher than given by the *diffraction limit* (3.1.128.1)

Note 1 to entry: SIM using linear responses may achieve twice the resolution limit at maximum where using non-linear responses may achieve more than twice the resolution limit.

3.2.49
time lapse imaging
imaging based on acquiring *images* (3.1.75) at intervals over a period of time

3.2.50
total internal reflection fluorescence microscopy
TIRFM
microscopy in which *fluorescence* (3.1.58) is excited in a thin layer by an evanescent wave produced by total internal reflection

3.2.51
white balancing
adjustment of colour levels in a *digital* (3.2.13) or *electronic image* (3.2.14) in order to display white regions of the *object* (3.1.104) as white in the image

3.2.52
3D reconstruction
processing and display of two-dimensional *digital* (3.2.13) or *electronic images* (3.2.14) to represent the three-dimensional structure of an *object* (3.1.104)

3.2.53
light sheet microscopy
microscopy technique where *illumination* (3.1.73) is only realized within the plane of observation

Note 1 to entry: Light sheet microscopy has the capability to produce 3D images with intrinsic optical sectioning by moving the light sheet and the specimen relative to each other. An advantage is the reduced light exposure of the specimen.

3.2.54
digital holographic microscopy
DHM
microscopy technique where the wavefront information originating from the *object* (3.1.104) is digitally recorded as a hologram, from which the object image is calculated by using a numerical reconstruction algorithm

Note 1 to entry: Usually, interference between the signal wave and a reference wave is used to obtain amplitude and phase information and thus a hologram.

3.2.55
optical coherence microscopy
OCM
optical interferometric measurement technique for obtaining cross-sectional images of a target *object* (3.1.104), using a partially coherent narrow *scanning* (3.2.41) beam to determine the relative depths of reflective surfaces within the object

Note 1 to entry: The lateral resolution is a property of the optics in the scanning beam path. It is decoupled from the axial resolution.

Note 2 to entry: Cross sectional images are generally acquired by scanning a focused beam laterally across the sample. Volumetric images are generally acquired by a series of cross sectional images.

[SOURCE: ISO 16971:2015, 3.1, modified to add Notes 1 and 2 to entry and add the abbreviation OCM.]

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