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Optics and optical instruments — Vocabulary for microscopy —

Part 2: Advanced techniques in light microscopy

Optique et instruments d'optique — Vocabulaire relatif à la microscopie —

Partie 2: Techniques avancées en microscopie optique

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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ISO 10934-2 was prepared by Technical Committee ISO/TC 172, *Optics and photonics*, Subcommittee SC 5, *Microscopes and endoscopes*.

ISO 10934 consists of the following parts, under the general title *Optics and optical instruments — Vocabulary for microscopy*:

— *Part 1: Light microscopy*

— *Part 2: Advanced techniques in light microscopy*

Optics and optical instruments — Vocabulary for microscopy —

Part 2: Advanced techniques in light microscopy

1 Scope

This part of ISO 10934 specifies terms and definitions to be used in the field of advanced light microscopy.

2 Terms and definitions

NOTE Terms shown in **bold** within a definition or a note are defined elsewhere within this part of ISO 10934.

2.1
acousto-optical modulator
electronically-tunable device used to control the direction and/or intensity of a laser by an acoustically-induced diffraction grating in a crystal

2.2
acousto-optical tunable filter
AOTF
electronically-tunable filter for selection of wavelengths by an acoustically-induced diffraction grating in a crystal

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

2.4
auto-focus
method of bringing an object automatically into focus, controlled by an imaging software algorithm and/or a hardware device that detects the object position

2.5
axial resolution
resolution in the direction of the optical axis

2.6
background subtraction
removal of that part of the signal that is present in the absence of the object, to reveal underlying image information

2.7
binning
mode of operation of an image sensor where the charge of adjacent pixels is accumulated and is read out as a single value

2.8
confocal
microscopy state in which, ideally, a point in the object field is illuminated by a diffraction-limited spot of light, and light emanating from this point is focused upon and detected from an area smaller than the central area of the diffraction disc situated in the corresponding position in a subsequent field plane

2.9
channel
particular signal path containing one type of image information

2.10
co-localization
overlay of images with coincidence of pixels corresponding to the same object points

2.11
confocal microscope
microscope in which, ideally, a point in the object plane is illuminated by a diffraction-limited spot of light, and light emanating from this point is focused upon and detected from an area smaller than the central area of the diffraction disc situated in the corresponding position in a subsequent field plane

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

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

2.11.1
laser-scanning confocal microscope
confocal microscope (2.11) in which the light source is a laser

2.11.2
multiple-beam confocal microscope
confocal microscope (2.11) using more than one illuminated and detected spot simultaneously

2.11.3
Nipkow disc confocal microscope
confocal microscope (2.11) in which the scanning of the illuminated and detected spots is performed using a **Nipkow disc** (2.11.3.1)

2.11.3.1
Nipkow disc
opaque disc with many ideally identical small holes arranged in Archimedean spirals

2.11.3.2
Tandem-scanning confocal microscope
Nipkow disc confocal microscope (2.11.3) in which the illuminating light and the detected light pass through separate holes

2.11.4
spectral confocal microscope
confocal microscope (2.11) in which a spectrum is recorded corresponding to spatial positions in an object

2.11.5
theta confocal microscope
confocal microscope (2.11) in which two objectives positioned at an angle, θ, with respect to one another, and with focal points coincident in the object, are used for excitation and collection respectively

2.11.6
white-light confocal microscope
confocal microscope (2.11) using an illumination source and a detector operating throughout the visible spectrum

2.11.7
confocal point spread function
product of the **point spread functions** (2.35) of the illuminating and detecting optical systems in a **confocal microscope** (2.11)

2.11.8
confocal volume
effective volume around each point in the object which gives rise to the image in a **confocal microscope** (2.11)

2.11.9
4 Pi confocal microscope
confocal microscope (2.11) in which two opposing objective lenses with focal points coincident in the object are used to produce interference in the focal region from which an image signal is derived, and with further processing produces an image with enhanced **axial resolution** (2.5)

2.12
deconvolution
⟨microscopy⟩ mathematical method for reducing blur, performed either in the spatial domain, or in the frequency domain by inverse filtering techniques

NOTE If the deconvolution is based solely on theoretical as opposed to measured values it is known as blind deconvolution.

2.13
digitally enhanced contrast
contrast enhanced by manipulation of the intensity and/or colour values in a **digital image** (2.14)

2.14
digital image
image in which the information is in the form of binary or other machine code

2.15
electronic image
image in which the information is in the form of electrical signals

2.16
extended depth of field microscopy
microscopy in which the **point spread function** (2.35) is modified in a known fashion such that it becomes substantially invariant over an extended focal range, and by further processing results in an image with extended depth of field

2.17
extended focus image
⟨image processing⟩ two-dimensional image derived by summing the pixel intensity values in a projection through an **image stack** (2.28.1)

2.18
fluorescence correlation microscopy
microscopy in which time-dependant intensity fluctuations occurring within a **confocal volume** (2.11.8) are used to calculate the mobility of fluorescent molecules

2.19
fluorescence *in-situ* hybridisation microscopy
FISH
microscopy in which chromosomes or specific positions within chromosomes can be fluorescently labelled by *in-situ* hybridisation

2.20
fluorescence life-time imaging
FLIM
imaging method based on discriminating characteristic fluorescence decay rates

2.21
fluorescence recovery after photobleaching
FRAP
technique in which a region in the object is irradiated to deplete its fluorescence, the subsequent recovery of fluorescence in the irradiated region being measured

2.22
fluorescence resonance energy transfer
Förster resonance energy transfer
FRET
non-radiative transfer of energy between two fluorophores in close proximity

2.23
frame averaging
averaging the pixel values from sequential images recorded under identical conditions

NOTE Used to increase signal-to-noise ratio.

2.24
image intensifier
device which increases the dynamic range of a signal to match the range of the detector

2.25
linear array sensor
detector in the form of a line of sensitive elements

2.26
maximum intensity image
〈image processing〉 two-dimensional image derived from the maximum pixel intensity values in a projection through an **image stack** (2.28.1)

2.27
microchannel plate
device positioned in front of a detector array to multiply incoming photon flux by secondary emission

2.28
multidimensional image data set
image data generated by recording data from a sample using several parameters, e.g., three-space dimensions, wavelength, time, polarization

2.28.1
image stack
multidimensional image data set (2.28) acquired from a three-dimensional region of an object

2.28.2
focus series
Z stack
image stack (2.28.1) acquired at different focal positions

2.29
multi-mode fibre
optical fibre that can sustain more than one transverse electromagnetic mode

2.30
multi-photon fluorescence
fluorescence excitation by the simultaneous absorption of multiple coherent photons

2.30.1
multi-photon fluorescence microscopy
microscopy in which the image is formed by **multi-photon fluorescence** (2.30)

NOTE 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.

2.30.1.1
two-photon fluorescence
multi-photon fluorescence (2.30) excited by pairs of coherent photons

2.31
optical section
image from a thin region whose thickness within a thick object is defined by the **axial resolution** (2.5) of the optical system

2.32
photobleaching
destruction of fluorescing properties of molecules by light, resulting in reduced fluorescence of the sample

2.33
pinhole
⟨confocal microscopy⟩ diaphragm situated in a plane conjugate with the object, which restricts the area in the object plane that is illuminated and/or from which light is collected

2.34
point detection
detection of light collected from a restricted point-like area of an image

2.35
point spread function
⟨lens system⟩ mathematical expression of the distribution of the light amplitude or intensity in the image of a point source

2.36
Raman microscopy
microscopy utilizing Raman scattering as the source of image information

2.37
ratio imaging
forming an image in which the pixel values are obtained by dividing the corresponding pixel values of two images

2.38
real time imaging
displaying or analysing images at the same rate as that at which they are collected

NOTE 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.

2.39
region of interest
ROI
parts of an image to which discrete observations are applied

2.40
scan rate
number of scan cycles completed per unit time

2.41
scanned field
dimensions of the scanned area in object space

2.42
scanning
sequential illumination of or detection from regions in an object

NOTE Scanning may be accomplished by moving the object, illuminating beam(s), objective or detector(s).

2.42.1
descanning
process by which an imaging beam retraces the path of the illuminating beam through the **scanning** (2.42) mechanism to produce a stationary beam

2.42.2
line scanning
scanning (2.42) along a single line

2.42.3
non-descanned detection
NDD
method of obtaining an image signal in a scanning microscope without **descanning** (2.42.1)

NOTE Widely used in multiphoton fluorescence microscopy.

2.42.4
point scanning
scanning (2.42) an area using a spot of light

2.42.5
raster scanning
scanning (2.42) an area by a pattern of lines

2.42.6
slit scanning
scanning (2.42) an area with a bar of light

2.42.7
stage scanning
scanning (2.42) performed by moving the stage and hence the object

2.43
scanning microscope
microscope in which the image is formed by **scanning** (2.42)

2.43.1
disc scanning microscope
scanning microscope (2.43) in which scanning is achieved by means of a perforated disc rotated in the illumination and/or observation paths

2.43.2
laser-scanning microscope
scanning microscope (2.43) in which the object is scanned by a laser beam

2.43.3
scanning near-field microscope
scanning microscope (2.43) in which a small light-emitting probe is scanned across the object (or *vice versa*) at a close distance

NOTE When the probe diameter is smaller than the Airy disc, **super-resolution** (2.49) may be achieved.

2.44
second harmonic generation microscopy
microscopy in which the second harmonic of the excitation light is used to provide additional image information

2.45
shading correction
method for adjusting intensity levels in an image, caused by non-uniformities in the illuminating or detecting systems

2.46
single-mode fibre
optical fibre that can sustain only a single transverse electromagnetic mode

2.47
spectral imaging microscopy
microscopy in which a spectrum is recorded corresponding to spatial positions in an object

2.48
structured illumination microscope
microscope in which the object is illuminated by a spatially varying pattern so as to produce a composite image from which images with enhanced lateral and axial resolution may be produced

2.48.1
grating image microscope
structured illumination microscope (2.48) in which the object is illuminated by the pattern of a grating

2.49
super-resolution
resolution higher than that given by the diffraction limit

2.50
time course imaging
imaging based on acquiring images at intervals over a period of time

2.51
total internal reflection fluorescence microscopy
TIRFM
microscopy in which fluorescence is excited in a thin layer by an evanescent wave produced by total internal reflection

2.52
white balancing
adjustment of colour levels in an electronic image in order to display white regions of the object as white in the image

2.53
3D reconstruction
processing and display of two-dimensional images to represent the three-dimensional structure of an object

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