
**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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Foreword

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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*

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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