

# SnapShot: Light Microscopy

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Light Microscopy Techniques	Contrast Source	Basic Principles	Key Characteristics	Application Examples	
Epi-fluorescence microscopy	fluorescence	wide-field illumination and detection through the same objective lens, separating the fluorescence emission from excitation light using dichroic mirrors and band-pass filters	specific visualization of biomolecules with low background (compared to label-free methods)	imaging fluorescent protein (FP)-labeled, dye-stained, or immunostained sample	
Total internal reflection fluorescence (TIRF) microscopy	fluorescence—optical sectioning	limiting the excitation volume to the close vicinity of the interface by sending the excitation light at an incident angle higher than the critical angle; usually implemented using a high numerical aperture (NA) objective.	high signal-to-background for near-surface structures	monitoring plasma membrane processes single-molecule detection	
Deconvolution microscopy		capturing a series of wide-field fluorescence images at different focal planes; computing the full three-dimensional (3D) structure using prior knowledge of the point spread function (PSF)	optical sectioning in wide-field imaging; resolution enhancement (limited by the signal-to-noise ratio)	general 3D imaging of cells and tissue samples	
Confocal microscopy		using a focused laser for excitation and a pinhole in the detection path to reject the out-of-focus fluorescence;	optical sectioning of a thick sample; diffraction-limited resolution (~250 nm lateral, ~600 nm axial)	general 3D imaging of cells and tissue samples; monitoring intracellular processes	
Multiphoton excitation microscopy		using high-intensity, focused infrared light to excite fluorophores by simultaneous absorption of multiple (usually two) photons;	infrared excitation and pinhole-free detection make it less affected by scattering in deep tissue imaging	in vivo microscopy such as monitoring neuron behavior in live animals	
Single-plane illumination microscopy (SPIM)/light-sheet fluorescence microscopy		using a sheet of light to excite the sample from the direction perpendicular to the direction to detect fluorescence emission; wide-field images are captured with the depth-of-view confined by the thickness of the excitation light sheet	reduced photo damage because the out-of-focus region is not illuminated during image acquisition	imaging the development of a whole embryo	
Light-field microscopy		inserting a microlens array in front of the camera so that not only the intensities but also the directions of light rays are recorded (at the cost of reduced lateral resolution)	capturing a full 3D structure with a single snapshot	3D imaging of tissue samples	
4Pi microscopy		fluorescence-enhanced resolution	using two opposing objectives and the interference of light through these two objectives to enhance the axial resolution; implemented in either point scanning format (4Pi microscopy) or wide-field format (I <sup>2</sup> M)	having an axial resolution equal to or better than the lateral resolution	imaging organelles, e.g., mitochondria
I <sup>2</sup> M					
Structured illumination microscopy (SIM)	illuminating the sample with a sinusoidal light pattern; the spatial information encoded in this pattern extends the range of spatial frequency that can be detected		doubling the spatial resolution: ~120 nm lateral, ~250 nm axial (~120 nm when combined with I <sup>2</sup> M)	tracking live cell microtubule and mitochondria dynamics	
Stimulated emission depletion (STED) microscopy	fluorescence—super-resolution	using a longer wavelength laser to suppress fluorescence emission from molecules at the periphery of the excitation focal spot; saturation of this suppression causes the fluorescence signal only to originate from the center of the focal spot	typical resolution 30 nm, depending on depletion light intensity; isotropic 30 nm 3D resolution with 4Pi configuration	resolving ultrastructures of both FP-labeled and immunostained samples	
RESOLFT microscopy		a generalization of the STED concept to other reversible photoswitching processes; requiring fluorophores that can endure many switching cycles	can use much lower depletion light intensity compared to STED		
Saturated SIM		using the saturation of fluorescence emission at high excitation intensity to create subdiffraction features in the effective illumination pattern	photobleaching at high excitation intensity is so far the major limitation		
Saturated excitation (SAX) microscopy					
STORM/PALM/FPALM		randomly switching fluorophores from a nonfluorescent state to the fluorescent state; imaging individual molecules and determining their positions with a high precision	typically 20–30 nm lateral resolution, 50–70 nm axial resolution (as high as 10 nm with 4Pi configuration)		
Near-field scanning optical microscopy (NSOM)	fluorescence—near-field	using a sharp probe (either an optical fiber or a metal tip) physically close to the sample to locally excite fluorescence signal; limited to imaging surface structures	resolution set by the probe size, typically 20–50 nm	imaging plasma membrane protein distribution	
(Spontaneous) Raman microscopy	Raman scattering	detecting spontaneous Raman scattering from the vibration of chemical bonds	inherent chemical contrast; long acquisition time due to weak signal	imaging lipids in cells and tissue samples (Raman signal from C-H bonds)	
Coherent anti-Stokes Raman scattering (CARS) microscopy		detecting anti-Stokes Raman scattering signal induced by two pump lasers with the frequency difference matching the Raman Stokes shift	fast Raman imaging		
Stimulated Raman scattering (SRS) microscopy		using the aforementioned two pump lasers, detecting the change in laser intensity after passing through the sample due to stimulated Raman scattering	higher sensitivity than CARS microscopy		
Second harmonic generation (SHG) microscopy	nonlinear scattering	detecting the generation of light with one-half the wavelength as the incident light when strong incident light passes through the sample	detects highly ordered structures	imaging collagen fibers in tissue	
Differential interference contrast (DIC) microscopy	refractive index	splitting illumination light into two beams with slight spatial offset; recombining them to create contrast from the optical path length difference between these two beams	generating contrast from transparent samples	imaging transparent samples	
Phase-contrast microscopy		generating contrast from the phase variation of light passing through the sample			
Quantitative phase microscopy		using interferometry with reference light to determine the path length when light passes through the sample, which reflects the sample refractive index	quantitatively imaging the refractive index of a sample		measuring the total protein mass of a cell
Bright-field microscopy	absorption	observing the light transmitted through a sample	the simplest light microscopy method; low sensitivity to absorption	imaging stained and unstained samples	
Stimulated emission microscopy		similar to SRS, but detecting the change in laser intensity due to chromophore absorption and stimulated emission	as high as single-molecule sensitivity in absorption measurement	imaging blood vessels through heme absorption	
Photoacoustic microscopy		illuminating the sample with pulsed light; detecting the ultrasound generated from the thermo-expansion due to light absorption; determining the depth of the absorption from the sound wave arrival time	high sensitivity; large penetration depth	imaging oxygenation of hemoglobin in capillaries	
Photoacoustic tomography		illuminating with pulsed light; using an array of ultrasound transducers to triangulate the absorption sources	centimeter-scale penetration depth because sound is not susceptible to soft tissue scattering	medical diagnosis	
Optical projection tomography (OPT)		bright-field imaging of an object at multiple angles to reconstruct the 3D structure	imaging large objects.	whole-embryo imaging	
Dark-field microscopy		scattering	sending the illumination light at an angle that is blocked in the detection path; imaging the scattered light from the sample	detecting inhomogeneities not visible in bright-field microscopy	tracking scattering objects (e.g., nanoparticles).
Optical coherence tomography (OCT)			using interferometry to determine the depth of the scattering sources that generate back-scattered light from the sample	millimeter scale penetration depth	medical diagnosis, e.g., endoscope

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Over 400 hundred years of development has made light microscopy an indispensable tool for almost every life scientist. This SnapShot provides an overview of various light microscopy modalities that allow us to investigate biological structures too small or too faint to be seen by the naked eye. The chart groups different light microscopy methods according to how they obtain the image contrast. Fluorescence microscopy, not surprisingly, comprises the largest group. Numerous ways to specifically tag biomolecules with fluorescent probes have enabled us to identify these molecules from a vast number of others in a cell and track their behavior in living samples. Optical sectioning methods such as confocal microscopy allow 3D imaging of cells and tissue samples. The sensitivity of fluorescence microscopy has been pushed to the level of detecting a single molecule, and the recent emergence of super-resolution microscopy has brought the spatial resolution to the scale of molecular complexes.

On the other hand, the need for fluorescent labeling can sometimes be challenging, which can be avoided by reading out intrinsic contrasts such as absorption, refractive index, scattering, and nonlinear optical properties of the sample. Although these label-free methods generally do not have as high specificity and sensitivity compared to fluorescence microscopy, they have their unique advantages. For example, Raman microscopy, in particular CARS and SRS microscopy, can visualize molecules not easily tagged, such as lipids. Absorption-based methods, such as photoacoustic microscopy and stimulated emission microscopy, detect chromophores that absorb light but do not fluoresce, with the example of hemoglobin. Many of these microscopy techniques have large penetration depths so that they can be used for medical imaging in patients.

Despite the apparent division of these light microscopy modalities, it needs to be emphasized that many of them can be put together. A prominent example is that all super-resolution microscopy approaches can be combined with multiphoton excitation to gain deeper tissue-imaging capability.

## Abbreviations

RESOLFT: reversible saturable optically linear transitions; STORM: stochastic optical reconstruction microscopy; PALM: photoactivated localization microscopy; FPALM: fluorescence photoactivation localization microscopy.

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