# 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 florescence 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 ($I^5M$) | having an axial resolution equal to or better than the lateral resolution | imaging organelles, e.g., mitochondria |
| $I^5M$ | | | | |
| 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 $I^5M$) | 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 |



See online version for legend and references.

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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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