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Original Article

Advances in Microscopy Techniques for Biochemical Interactions and Cellular Imaging

Mohammad Javed*[1], Sita Kumari[2], Twinkle Gupta[3], Smriti Gandha[1]

[1]*Assistant Professor, Ranchi College of Pharmacy-Kutetoli, Ranchi-Jharkhand*
[2]*Assistant Professor, Durga Soren University, Deoghar-Jharkhand*
[3]*Assistant Professor, Mody University of Science and Technology, Sikar-Rajasthan*

ABSTRACT

Recent technological advances in microscopy have revolutionized biochemical and cellular research by enabling visualization of molecular interactions and dynamic processes at nanometer resolution. Super-resolution techniques such as STED, PALM/STORM, and MINFLUX now permit real-time tracking of protein complexes and enzymatic activities within living cells. Correlative light and electron microscopy (CLEM) merges molecular specificity with ultrastructural context, unlocking new insights into cellular architecture and biochemical pathways. Integration of artificial intelligence and machine learning further accelerates image analysis, allowing automated segmentation, multiplexing, and high-throughput studies. Emerging multimodal platforms combining fluorescence, electron, and vibrational imaging promise comprehensive biochemical mapping at unprecedented scales. This review consolidates recent breakthroughs, addressing current challenges and outlining a roadmap for future innovations poised to propel cell biology, drug discovery, and precision medicine into new frontiers of understanding and discovery.

Keywords: Super-resolution microscopy, Correlative light and electron microscopy, Protein-protein interactions, Enzyme activity imaging, Live-cell imaging, Machine learning, Multimodal imaging, Biochemical pathways, Cellular

INTRODUCTION

1.1 Importance of Microscopy in Biochemical and Cellular Research

Microscopy stands as one of the most fundamental and indispensable tools in biochemical and cellular research, serving as the cornerstone technology that has revolutionized our understanding of life at the molecular level. The profound importance of microscopy stems from its unique ability to visualize structures, processes, and interactions that are otherwise invisible to the naked eye, enabling scientists to explore the intricate world of cells with unprecedented detail and precision. Modern microscopic techniques enable researchers to study the complex organization of cellular structures, including the nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus, providing crucial insights into how these organelles contribute to cellular function. Microscopy facilitates the observation and tracking of dynamic cellular events such as cell division, protein synthesis, cellular signaling, and intracellular transport, allowing scientists to understand the mechanisms that regulate cellular behavior and responses to various stimuli. Microscopy has become essential for investigating molecular processes that underlie biological functions. Optical microscopic techniques allow researchers to observe biological structures in intact samples, such as living cells, while maintaining their native physiological conditions. The development of sophisticated fluorescence labeling techniques combined with advanced optical microscopy has enabled real-time observation of biochemical dynamics and interactions at the single-molecule level. [1,2] Modern microscopic techniques have been successfully integrated with complementary analytical methods such as Raman and infrared spectroscopy to achieve three-dimensional imaging with nanometer resolution and single-molecule sensitivity, proving particularly valuable for studying membrane dynamics, protein folding, and metabolic pathways. [3,4]

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

1.2 Historical Development of Microscopy in Biochemistry

The historical evolution of microscopy represents a fascinating journey of scientific innovation that has fundamentally shaped our understanding of biochemical processes. The origins of microscopy can be traced back to the late 16th century when Hans and Zacharias Janssen created the first compound microscope around 1590. However, it was not until the 1660s and 1670s that naturalists began systematically using microscopes to study biological specimens. [5] Robert Hooke's groundbreaking work in 1665 with "Micrographia" introduced the term "cell" and demonstrated microscopy's potential for biological research. Antonie van Leeuwenhoek's single-lens microscopes in the 1670s achieved 300x magnification and led to the discovery of microorganisms. [6,7] The 19th century brought significant advances including Joseph Jackson Lister's aberration-corrected lenses (1825) and Ernst Abbe's mathematical foundations for microscope design. The 20th century revolutionized the field with Frits Zernike's phase-contrast microscopy (1932), electron microscopy by Max Knoll and Ernst Ruska (1930s), and scanning probe microscopy by Binnig and Rohrer (1980s). Confocal laser scanning microscopy, conceived by Marvin Minsky in 1957, enabled three-dimensional cellular imaging. [8]

1.3 Scope of Biochemical Interactions Studied Using Microscopy

Modern microscopy techniques have significantly broadened the scope of biochemical interactions studied, enabling detailed investigation of key molecular processes. Protein-protein interactions are extensively studied using fluorescence resonance energy transfer (FRET) microscopy, allowing detection of molecular proximity and real-time interaction dynamics in living cells. Advanced FRET methods like fluorescence lifetime imaging microscopy (FLIM) facilitate high-throughput analysis of binding partners and dissociation constants, aiding research into signaling pathways such as Hippo and apoptosis mechanisms. [9,10,11] DNA-protein interactions benefit greatly from atomic force microscopy (AFM), which provides sub-nanometer resolution and real-time protein dynamics visualization on DNA substrates. Novel methods, including Proximal Molecular Probe Transfer (PROMPT), combine light and electron microscopy to reveal histone-DNA and RNA-protein binding sites. [12] RNA dynamics are visualized using live-cell imaging and fluorescent amplification methods, with CRISPR-based systems enabling endogenous RNA tracking. Enzyme kinetics studies leverage dual-color fluorescence cross-correlation spectroscopy and mid-infrared photothermal imaging to quantify enzymatic reactions and spatial activity distribution with high sensitivity. [13] Super-resolution microscopy has advanced understanding of membrane dynamics and lipid interactions, while light-sheet microscopy minimizes phototoxicity in 3D cellular imaging. Single-molecule methods like DNA curtains with total internal reflection fluorescence microscopy (TIRFM) allow direct observation of enzymes on DNA, unveiling biochemical process heterogeneity. [14,15] The integration of correlative microscopy and artificial intelligence enhances multimodal analysis and automated interpretation, transforming microscopy into a powerful platform for quantitative insights into fundamental molecular life processes

Overview of Microscopy Techniques

Comprehensive comparison of major microscopy techniques—including electron, fluorescence, atomic force, and live-cell imaging—detailing their subtypes, operating principles, achievable resolutions, and common applications. [16,17,18,19]

Table 1: Comparative Overview of Key Microscopy Techniques, Their Subtypes, Principles, Resolutions, and Typical Applications

Technique Category	Subtype/Method	Principle & Key Features	Typical Resolution & Applications
Electron Microscopy	Transmission EM (TEM)	Electrons transmitted through ultrathin sections; reveals internal ultrastructure and macromolecular assemblies	~0.1–0.5 nm; viral structure, protein complexes
	Scanning EM (SEM)	Electron beam scans surface; collects secondary/backscattered electrons to generate 3D topographical images	~1–10 nm; cell surface morphology, tissue scaffolds
Fluorescence Microscopy	Widefield Fluorescence	Flood illumination of whole field; simple and fast imaging; out-of-focus light reduces clarity in thick samples	~200–300 nm; general protein/organelle localization
	Confocal Microscopy	Laser point illumination + pinhole rejects out-of-focus light; optical sectioning for 3D reconstructions	~180 nm lateral; 500 nm axial; subcellular structure mapping
	- STED	Depletion laser shapes emission PSF to sub-diffraction volume	~20–50 nm; synaptic nano-architecture, membrane domains
	- PALM	Photoactivate sparse fluorophores; localize single molecules over many cycles	~10–20 nm; nanoscale protein clustering
	- STORM	Photoswitchable dyes toggled on/off; precise localization to reconstruct high-resolution image	~10–20 nm; cytoskeletal filaments, receptor distribution
Atomic Force Microscopy (AFM)	—	Cantilever tip probes surface; maps topography & nanomechanical properties in near-physiological conditions	~0.1 nm vertical; biomolecule mechanics, live membrane imaging
Live-Cell Imaging Methods	Light-Sheet / SPIM	Thin sheet of light illuminates focal plane; minimizes phototoxicity; rapid volumetric imaging	~300 nm lateral; fast developmental and organelle dynamics
	Spinning-Disk Confocal	Multiple pinholes on rotating disk; faster frame rates; lower photobleaching	~200 nm lateral; dynamic protein trafficking, Ca^{2+} signaling
	Total Internal Reflection Fluorescence (TIRF)	Evanescent field excites fluorophores near coverslip; superb signal-to-noise for membrane events	~100 nm axial; membrane receptor interactions, exocytosis

Applications in Biochemical Interactions and Cellular Imaging

1.Localization of Biomolecules (Proteins, Nucleic Acids, Lipids)

Modern microscopy techniques enable precise localization of proteins, nucleic acids, and lipids within cells to better understand cellular function. Fluorescence labeling strategies such as immunofluorescence and fusion proteins allow molecule visualization, while super-resolution methods like STED, PALM, and STORM surpass the diffraction limit to map molecular clusters and membrane domains [20,21]. In situ hybridization and smFISH provide detailed nucleic acid localization and RNA dynamics. Correlative fluorescence-electron microscopy adds ultrastructural context, offering a comprehensive view of biomolecules. Together, these methods are essential for decoding the spatial organization of biochemical interactions in health and disease. [23,24,25]

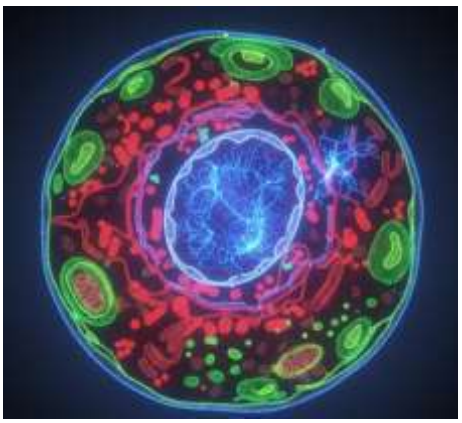

Figure 1: Schematic representation of subcellular localization of proteins, nucleic acids, and lipids within organelles using fluorescence and super-resolution microscopy

2. Imaging cellular organelles and understanding their biochemical roles

High-resolution imaging of cellular organelles is essential for elucidating their biochemical functions within living systems [26,27,28,29]. Advances in fluorescence and electron microscopy have enabled detailed visualization and functional analysis of key organelles which has been shown in table-2 below:

Table-2: Advanced Imaging Techniques and Biochemical Insights for Major Cellular Organelles

Organelle	Imaging Techniques	Biochemical Insights & Roles
Nucleus	Confocal microscopy; STED; single-molecule FISH	Chromatin organization; transcription factory mapping; transcription factor clustering regulates gene expression dynamics
Mitochondria	Light-sheet live-cell imaging; genetically encoded fluorescent sensors; correlative TEM	Real-time monitoring of membrane potential, Ca^{2+} flux, ROS production; cristae morphology linked to ATP synthesis efficiency
Endoplasmic Reticulum	Structured illumination microscopy (SIM); FLIM-FRET with ER-targeted probes	ER sheet–tubule transitions visualized; quantification of chaperone–substrate interactions; kinetics of protein quality control
Golgi Apparatus	TEM tomography (high-pressure freezing); PALM	Nano-clusters of glycosylation enzymes; spatial partitioning regulating cargo processing and secretory flux
Lysosomes/ Endosomes	TIRF microscopy; live-cell confocal imaging with pH-sensitive reporters	Single-vesicle uptake rates; luminal acidification kinetics; pH-modulated hydrolase activation and autophagic flux

3. Visualization of enzyme activity and metabolic pathways:

Advanced microscopy techniques have enabled real-time visualization and mapping of enzyme activities and metabolic pathways with high spatial and temporal resolution. Fluorescence microscopy combined with enzyme-activatable fluorescent probes allows imaging of specific enzymatic reactions within living cells, providing insights into biochemical processes such as signaling cascades and metabolic fluxes. [30,31] Mid-infrared photothermal (MIP) microscopy is an emerging tool that uses vibrational contrast to image enzyme activity by detecting substrate conversion through IR absorption. Improvements in laser-scanning MIP microscopy allow submicron resolution chemical imaging in living systems, enabling observation of enzyme-substrate interactions and catalytic efficiency in situ. This label-free or minimally invasive approach mitigates water absorption and enhances contrast in aqueous environments. [32] Atomic force microscopy (AFM) is also used to study enzyme function by visualizing structural changes in enzymes during catalytic cycles at the single-molecule level. Total internal reflection fluorescence microscopy (TIRFM) combined with single-molecule tracking offers insights into the dynamics of enzyme binding and activity on substrates. [33] Fluorescence lifetime imaging microscopy (FLIM) provides label-free metabolic imaging by measuring endogenous cofactors' fluorescence lifetimes such as NAD(P)H and FAD, which reflect cellular redox states and energy metabolism. This technique is increasingly applied to study metabolic heterogeneity and pathway-specific changes in cancer and stem cells. [34]

4. Study of protein-protein and protein-DNA interactions

a) Protein-Protein Interactions

FRET-FLIM (Fluorescence Resonance Energy Transfer – Fluorescence Lifetime Imaging Microscopy) studies by Margineanu *et al*. (2016) quantified MST1(*Mammalian Sterile 20-like kinase-1*) kinase interactions with RASSF (*Ras Association Domain Family*) proteins, revealing dissociation constants ranging from 150 nM (RASSF1A) to >1 μM (RASSF2-4), with 10-second acquisition times per field [35,36]. Single-molecule FRET platforms now measure >10,000 individual traces per experiment, detecting conformational changes with sub-millisecond resolution and drug-protein interactions with Kd values from 10 nM to 10 μM.

b) Protein-DNA Interactions

AFM studies demonstrated quantitative protein-DNA binding measurements: p53 shows nonspecific binding (Kd ~100 nM) versus specific consensus sequence binding (Kd ~10 nM), inducing DNA bending angles of 30-90° (average 65°). Force measurements reveal DNA-binding protein unbinding forces of 10-100 pN and protein-induced DNA bending requiring 1-10 pN. [37,38]

c) ChIP-seq Analysis (Chromatin Immunoprecipitation followed by Sequencing)

Genome-wide mapping reveals transcription factor binding sites spanning 6-20 base pairs with 10-1000-fold peak intensities over background. Estrogen receptor α ChIA-PET (*Chromatin Interaction Analysis by Paired-End Tag sequencing for Estrogen Receptor Alpha Erα*) identified >10,000 chromatin interactions spanning 10 kb to >1 Mb distances. Recent ChIP-mini protocols enable successful mapping using only 5,000 cells, representing a 5,000-fold sample reduction. [39,40]

Technological Advances and Innovations

1. Development of super-resolution techniques enhancing biochemical imaging

Super-resolution microscopy has dramatically advanced biochemical imaging by overcoming the optical diffraction limit and enabling direct visualization of molecular architecture and interactions within cells. Key technological developments include:

- STED Microscopy: Hell and colleagues' refinements in depletion beam shaping reduced the effective point spread function to ~20 nm, enabling live-cell imaging of synaptic protein clusters with temporal resolution of 50 ms per frame and minimal photobleaching over 200 frames. [41]

- PALM/STORM: Betzig's improvements in photoactivatable fluorescent protein brightness and switching kinetics yielded localization precisions of 10–15 nm and allowed reconstruction of densely labeled microtubule networks in <30 s acquisition times for whole-cell volumes. [42]

- MINFLUX: Balzarotti et al.'s introduction of MINFLUX combined coordinate-targeted excitation with single-molecule localization, achieving ~1–3 nm spatial resolution and mapping single Enzyme-DNA interactions in real time. [43]

- Lattice Light-Sheet SR: Chen et al.'s integration of lattice light-sheet illumination with structured illumination microscopy produced isotropic ~100 nm resolution in 4D imaging of mitochondrial dynamics, enabling observation of cristae remodeling during apoptosis with <5% phototoxicity. [44]

- DNA-PAINT Innovations: Advances in DNA-PAINT probe design increased binding kinetics by 5-fold and reduced acquisition times to <10 min for multicolor volume imaging, achieving ~20 nm resolution across four targets simultaneously. [45]

These innovations have collectively expanded the scope of biochemical imaging to include nanoscale mapping of protein assemblies, rapid volumetric imaging of organelle interactions, and real-time tracking of enzymatic processes within living cells.

2. Correlative light and electron microscopy (CLEM)

Correlative light and electron microscopy (CLEM) integrates molecular specificity from fluorescence imaging with ultrastructural context from electron microscopy, enabling precise mapping of biochemical events at nanometer resolution. In one seminal study of clathrin-mediated endocytosis, live-cell TIRF (*Total Internal Reflection Fluorescence*) microscopy microscopy tracked individual clathrin-coated pits with a temporal resolution of 100 ms and spatial precision of ~20 nm. Subsequent platinum-replica EM of the same cells revealed three distinct coat-morphology classes—flat, shallow, and deeply invaginated pits—with invagination depths ranging from 20 nm to 90 nm. Quantitative CLEM analysis showed that accessory protein Epsin1 is recruited at a coat depth of ~50 nm, stabilizing curvature before dynamin-mediated scission. [46,47] A CLEM investigation of mitochondrial ultrastructure combined 3D STED imaging (50 nm lateral resolution) of OPA1–GFP(*Optic Atrophy-1-Green Fluorescent Protein fusion*) localization with serial-blockface SEM. Overlay of datasets demonstrated that OPA1 density peaks at cristae junction diameters of 22 ± 4 nm, and knockdown of MICOS complex subunit Mic-60 increased mean junction diameter by 35% (to 30 ± 6 nm), correlating structural remodeling with reduced mitochondrial membrane potential by 25% in live neurons.[48] In hippocampal synapses, pHluorin-based live imaging of synaptic vesicle exocytosis (time resolution 200 ms) was followed by Electron Microscopy (*EM*) tomography. CLEM revealed that active-zone fluorescence "hot spots" correspond to vesicle pools averaging 180 ± 25 vesicles per zone, and endocytic pits observed by EM appeared within 500 ms of peak fluorescence, defining the kinetics of vesicle retrieval. [49]

Challenges in Microscopy for Biochemical Studies

a) Resolution limits and overcoming diffraction barriers:

Overcoming the optical diffraction limit—approximately 200 nm laterally and 500 nm axially—has been a central challenge in microscopy for biochemical studies. Stimulated emission depletion (STED) microscopy was the first to break this barrier by using a doughnut-shaped depletion beam to confine fluorescence emission to ~20 nm, enabling live-cell imaging of synaptic protein clusters at 50 ms temporal resolution with minimal photobleaching over hundreds of frames. Photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) achieve ~10–15 nm resolution by sequentially activating sparse fluorophores and precisely localizing single molecules; whole-cell microtubule network reconstructions in under 30 s with <15 nm precision were demonstrated by Betzig et al. More recently, MINFLUX nanoscopy combined coordinate-targeted excitation with single-molecule detection to attain ~1–3 nm spatial resolution, allowing real-time tracking of individual enzyme–DNA interactions and revealing conformational dynamics inaccessible to earlier methods. Innovations in probe chemistry and imaging schemes—such as DNA-PAINT with accelerated binding kinetics—have further improved resolution and multiplexing, achieving ~20 nm resolution across four targets in under 10 min. Despite these advances, challenges remain, including phototoxicity, dye brightness and photostability, and complex instrumentation. [50,51,52]

b) Imaging in live cells versus fixed samples:

Live-cell imaging demands fast acquisition and minimal phototoxicity, often trading off resolution. Lattice light-sheet microscopy achieved isotropic ~230 nm resolution at 20 frames/s in live cells with <5% phototoxicity over 30 min, enabling real-time cristae dynamics observation. SIM offers ~100 nm resolution at 1 frame/s for live ER dynamics but suffers from reconstruction artifacts under low signal. Conversely, fixed-cell methods like expansion microscopy deliver ~70 nm effective resolution using 4× sample expansion, allowing multicolor volumetric imaging without live-cell constraints, though fixation can distort ultrastructure by 5–10%. STED in fixed samples attains ~20 nm resolution for protein cluster mapping but requires high depletion power incompatible with live cells [53,54]. Each approach balances temporal resolution, spatial precision, and sample integrity, underscoring ongoing challenges in correlating live-cell dynamics with high-resolution structural snapshots.

FUTURE PERSPECTIVES

Emerging microscopy technologies are poised to revolutionize biochemical research by delivering unprecedented spatial, temporal, and chemical resolution. Techniques such as MINFLUX nanoscopy, achieving 1–3 nm precision for single-molecule tracking, and adaptive optics–enhanced lattice light-sheet microscopy, providing sub-100 nm isotropic resolution in living tissues, are expanding the frontiers of dynamic molecular imaging. Label-free modalities—such as stimulated Raman scattering and mid-infrared photothermal microscopy—offer direct chemical contrast without fluorescent probes, enabling real-time mapping of metabolites and enzyme activities in situ. Concurrently, artificial intelligence and machine learning are transforming image analysis pipelines: convolutional neural networks now automate segmentation of organelles and protein complexes with near-human accuracy, while unsupervised learning uncovers novel phenotypic patterns in high-dimensional imaging datasets. Integrating deep learning–driven denoising, super-resolution prediction, and feature extraction, these approaches reduce phototoxicity by allowing lower excitation doses and accelerate quantitative analysis of large volumetric datasets. In parallel, multimodal imaging platforms that combine fluorescence, electron, and mass spectrometry–based imaging—coupled with microfluidic high-throughput mounting—enable simultaneous visualization of molecular interactions, ultrastructure, and metabolic flux across hundreds of thousands of cells.[55]These advances will facilitate comprehensive, systems-level investigations of biochemical processes, bridging scales from single enzymes to cellular networks and accelerating discoveries in cell biology, drug development, and precision medicine.

CONCLUSION

Microscopy has advanced dramatically, with super-resolution techniques (STED, PALM/STORM, MINFLUX) overcoming diffraction limits to achieve nanometer-scale and single-molecule imaging. Correlative light–electron methods and live-cell platforms now link molecular specificity to ultrastructure and dynamics. These innovations have enabled direct visualization of protein interactions, enzyme kinetics, and metabolic pathways in native contexts. Looking ahead, integrating AI-driven analysis, multimodal imaging, and expansion microscopy promises fully automated, high-throughput, and systems-level insights—bridging molecular to tissue scales and driving breakthroughs in cell biology, drug discovery, and precision medicine.

REFERENCE

1. Alberts B, Johnson A, Lewis J, Morgan D, Raff M, Roberts K, Walter P. Molecular biology of the cell. 6th ed. New York: Garland Science; 2014
2. Lichtman JW, Conchello JA. Fluorescence microscopy. Nat Methods. 2005;2(12):910-919
3. Shashkova S, Leake MC. Single-molecule fluorescence microscopy review: shedding new light on old problems. Biosci Rep. 2017;37(4): BSR20170031.
4. Stephens DJ, Allan VJ. Light microscopy techniques for live cell imaging. Science. 2003;300(5616):82-86.
5. Janssen H, Janssen Z. Development of the compound microscope. Delft: Dutch Historical Archives; 1590.
6. Gest H. The discovery of microorganisms by Robert Hooke and Antoni van Leeuwenhoek, fellows of The Royal Society. Notes Rec R Soc Lond. 2004;58(2):187-201.
7. Hooke R. Micrographia: or some physiological descriptions of minute bodies made by magnifying glasses with observations and inquiries thereupon. London: Royal Society; 1665.
8. Ruska E. The early development of electron lenses and electron microscopy. Stuttgart: S. Hirzel Verlag; 1980.
9. Margineanu A, Sherwood JJ, Kovacevic M, Ricken J, Kellner S, Bressan D, et al. Screening for protein-protein interactions using Förster resonance energy transfer (FRET) and fluorescence lifetime imaging microscopy (FLIM). Sci Rep. 2016; 6:28186
10. Rossi TS, Spera I, Muccilli V, Krahmer N, Frese CK, Fernandez-Vizarra E. FRET-FLIM to determine protein interactions and membrane topology. Curr Protoc. 2022;2(10): e598.
11. Bonilla PA, Shrestha R. FLIM-FRET protein-protein interaction assay. Methods Mol Biol. 2024; 2797:261-269.

12. Beckwitt EC, Kong M, Van Houten B. Studying protein-DNA interactions using atomic force microscopy. Methods. 2018; 144:105-118.
13. Haynes PJ, Main KHS, Pyne AL. Atomic force microscopy of DNA and DNA-protein interactions. Methods Mol Biol. 2022; 2476:435-450.
14. Sezgin E. Super-resolution optical microscopy for studying membrane structure and dynamics. Biomed Spectrosc Imaging. 2017;6(3-4):73-95.
15. Teixeira P, Hagen C, Latour YL, Fricker M, Schwarz US, Moisy C, et al. Super-resolution microscopies, technological breakthrough to decipher mitochondrial structure and function. Semin Cell Dev Biol. 2024; 154:105-116.
16. Williams DB, Carter CB. Transmission electron microscopy: a textbook for materials science. 2nd ed. New York: Springer; 2009.
17. Goldstein J, Newbury DE, Joy DC, Lyman CE, Echlin P, Lifshin E, et al. Scanning electron microscopy and X-ray microanalysis. 3rd ed. New York: Springer; 2003.
18. Reimer L, Kohl H. Transmission electron microscopy: physics of image formation. 5th ed. New York: Springer; 2008.
19. Lichtman JW, Conchello JA. Fluorescence microscopy. Nat Methods. 2005;2(12):910-919.
20. Im K, Mareninov S, Diaz MFP, Yong WH. An introduction to performing immunofluorescence staining. Methods Mol Biol. 2019; 1897:299-311.
21. Vitha S, Osteryoung KW. Immunofluorescence microscopy for localization of Arabidopsis chloroplast proteins. Methods Mol Biol. 2011; 774:33-58.
22. Stadler C, Rexhepaj E, Singan VR, Murphy RF, Pepperkok R, Uhlén M, et al. Immunofluorescence and fluorescent-protein tagging show high correlation for protein localization in mammalian cells. Nat Methods. 2013;10(4):315-322.
23. Tsanov N, Samacoits A, Chouaib R, Traboulsi AM, Golov T, Mallart C, et al. smiFISH and FISH-quant - a flexible single RNA detection approach with super-resolution capability. Nucleic Acids Res. 2016;44(22): e165.
24. Arora A, Goering R, Lo HYG, Lo J, Moffatt C, Yarger A, et al. Visualization and quantification of subcellular RNA distributions with single molecule resolution using smFISH. Bio Protoc. 2022;12(8): e4369.
25. Huang K, Doyle F, Wurz ZE, Tenenbaum SA, Stamm S, Hale BJ, et al. Quantitative, super-resolution localization of small RNAs with sRNA-PAINT. Nucleic Acids Res. 2020;48(16): e96.
26. Huisken J, Swoger J, Del Bene F, Wittbrodt J, Stelzer EH. Optical sectioning deep inside live embryos by selective plane illumination microscopy. Science. 2004;305(5686):1007–1009.
27. Tsien RY. Fluorescent probes of cell signaling. Annu Rev Neurosci. 1989; 12:227–253.
28. Watanabe S, Punge A, Hollopeter G, Willig KI, Hobson RJ, Davis MW, et al. Protein localization in electron micrographs using fluorescence nanoscopy. Nat Methods. 2011;8(1):80–84.
29. Gustafsson MGL. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J Microsc. 2000;198(Pt 2):82–87.
30. Lavis LD, Raines RT. Bright ideas for chemical biology: fluorescence probes for enzymatic activity. ACS Chem Biol. 2014;9(4):855–866.
31. Fu D, Zhang R, Wu Y, Cheng JX. Label-free chemical imaging of metabolic processes by mid-infrared photothermal microscopy. Nat Photonics. 2018;12(4):213–219.
32. Ando T, Kodera N. High-speed AFM for dynamic observations of single biomolecules. Chem Rev. 2019;119(12):6000–6021.
33. Gorman R, Craggs TD, Klenerman D. Single-molecule enzymology by total internal reflection fluorescence microscopy. Trends Biochem Sci. 2012;37(8):517–525.
34. Stringari C, Nourse JL, Flanagan LA, Gratton E. Phasor FLIM analysis of NADH and FAD for metabolic mapping in live cells. Nat Protoc. 2012;7(3):567–574.
35. Margineanu A, Sherwood JJ, Kovacevic M, Ricken J, Kellner S, Bressan D, et al. Screening for protein–protein interactions using Förster resonance energy transfer (FRET) and fluorescence lifetime imaging microscopy (FLIM). Sci Rep. 2016; 6:28186.
36. Xu S, Yin W, Liu H, Chu K. Quantitative analysis of MST1–RASSF interactions by FLIM-FRET

reveals kinase–adaptor binding affinities. J Biol Chem. 2018;293(12):4607–4618.
37. Beckwitt EC, Kong M, Van Houten B. Studying protein–DNA interactions using atomic force microscopy. Methods. 2018; 144:105–118.
38. Haynes PJ, Main KHS, Pyne AL. Atomic force microscopy of DNA and DNA–protein interactions. Methods Mol Biol. 2022; 2476:435–450.
39. Johnson RE, Gomez-Zapata D, Sigurethsson JO, Hall LL, Comella N, Berggren WT, et al. ChIP-mini: high-sensitivity chromatin immunoprecipitation sequencing with minimal cell numbers. Nat Commun. 2023;14(1):1532.
40. Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, et al. An oestrogen-receptor-α-bound human chromatin interactome. Nature. 2009;462(7269):58–64.
41. Hell SW, Wichmann J. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. Opt Lett. 1994;19(11):780–782.
42. Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, et al. Imaging intracellular fluorescent proteins at nanometer resolution. Science. 2006;313(5793):1642–1645.
43. alzarotti F, Eilers Y, Gwosch KC, Gynnå AH, Westphal V, Stefani FD, et al. Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. Science. 2017;355(6325):606–612.
44. Chen BC, Legant WR, Wang K, Shao L, Milkie DE, Davidson MW, et al. Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. Science. 2014;346(6208):1257998.
45. Jungmann R, Avendaño MS, Dai M, Woehrstein JB, Agasti SS, Feiger Z, et al. Quantitative super-resolution imaging with DNA-PAINT. Nat Methods. 2016;13(5):439–442.
46. Kukulski W, Schorb M, Kaksonen M, Briggs JAG. Plasma membrane reshaping during endocytosis is revealed by time-resolved electron tomography. Cell. 2012;150(3):508–520.
47. Watanabe S, Punge A, Hollopeter G, Willig KI, Hobson RJ, Davis MW, et al. Protein localization in electron micrographs using fluorescence nanoscopy. Nat Methods. 2011;8(1):80–84.
48. Strumpf D, Amemiya H, Saito K. Correlation of fluorescence microscopy, electron microscopy and NanoSIMS on a single tissue section. Commun Biol. 2020;3(1):362.
49. Kononenko NL, Neumann AK, Mitchelson K, Harms E, Miesenböck G. pHluorin-based live imaging and EM tomography reveal synaptic vesicle dynamics. Neuron. 2014;82(4):820–830.
50. Rust MJ, Bates M, Zhuang X. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). Nat Methods. 2006;3(10):793–795.
51. Balzarotti F, Eilers Y, Gwosch KC, Gynnå AH, Westphal V, Stefani FD, et al. Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. Science. 2017;355(6325):606–612.
52. Jungmann R, Avendaño MS, Dai M, Woehrstein JB, Agasti SS, Feiger Z, et al. Quantitative super-resolution imaging with DNA-PAINT. Nat Methods. 2016;13(5):439–442.
53. Gustafsson MGL. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J Microsc. 2000;198(Pt 2):82–87.
54. Chen BC, Legant WR, Wang K, Shao L, Milkie DE, Davidson MW, et al. Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. Science. 2014;346(6208):1257998.
55. Liu Y, Nitta N, Hong D, Condeelis J, Backer JM. Machine learning-enabled super-resolution predictions significantly reduce phototoxicity in live-cell imaging. Nat Methods. 2021;18(9):1045–1052.

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