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Cell-resolution Optical Microscopy for Clinical Translation

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Optical microscopy remains a cornerstone of medical diagnostics, most notably in histopathology, where cell-resolution imaging of biopsied tissue defines the diagnostic gold standard. Digital pathology and AI-enabled analysis have modernized slide-based workflows, while *in vivo* confocal and related technologies have demonstrated the feasibility of cellular imaging in fields such as dermatology and ophthalmology. Nonetheless, widespread clinical adoption of high-resolution optical microscopy has been limited by various factors, including limited contrast mechanisms, trade-offs among field of view and resolution, imaging depth, sensitivity to motion, etc. To clarify the landscape of technologies capable of delivering clinically actionable cellular information, this review organizes optical microscopy by underlying contrast mechanisms, including intrinsic contrast (reflection, absorption, refraction/phase, autofluorescence, and Raman scattering) and extrinsic contrast generated by untargeted or targeted molecular probes, and evaluates how these mechanisms are implemented across emerging platforms such as slide-free histopathology, *in vivo* transmission-based microscopy, and fluorescence-based *in situ* microscopy. For each modality, we summarize representative system architectures and key preclinical or clinical validation studies. Looking ahead, advances in computational imaging and AI-assisted interpretation are expected to address current limitations and support the selective incorporation of cell-resolution optical microscopy into future diagnostic and surgical workflows.

Keywords : Advanced techniques, Image contrast, Medical applications, Optical microscopy
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I. INTRODUCTION

Optical devices, including microscopes, play a central role in modern medicine by enabling visualization of tissue structure across multiple spatial scales, from gross morphology to cellular architecture. Clinical optical instruments can be broadly classified into two functional categories: (1) Magnifying systems that enhance visualization of tissue surfaces and superficial subsurfaces, and (2) cell-resolution microscopy systems that resolve individual cells and subcellular features relevant to disease processes. These categories are complementary, rather than hierarchical. Magnifying devices—such as dermoscopes, colposcopes, otoscopes, ophthalmic slit lamps, and surgical microscopes—are indispensable for real-time mesoscopic assessment across medical specialties, whereas cell-resolution microscopy systems are employed when microscopic pathology provides additional diagnostic or prognostic value.

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Cell-resolution optical microscopy has long been the cornerstone of diagnostic histopathology [1]. Conventional bright-field microscopy of thin-sectioned, chemically stained tissue sections provides high-contrast visualization of nuclei, cytoplasm, and extracellular structures, forming the basis for diagnosing malignancy, inflammation, and degenerative disorders. Recent advances, including whole-slide imaging and digital pathology platforms, have modernized this workflow by enabling high-throughput scanning, remote consultation, and computational analysis [2]. Nevertheless, histopathology remains inherently limited to biopsied *ex vivo* specimens and requires labor-intensive sample preparation, constraining its utility for real-time clinical decision-making and longitudinal disease monitoring.

To bridge this gap, several advanced microscopy techniques, originally developed for biological research, have been translated toward clinical use, with the goal of extending cell-level imaging to living tissue. Among these, *in vivo* confocal microscopy (IVCM) has emerged as a representative modality, particularly in ophthalmology and dermatology, where tissues are optically accessible and relatively stable, enabling reliable acquisition of cell-resolution images *in vivo* [3, 4]. In these settings, IVCM has demonstrated clinical value in identifying characteristic cellular patterns associated with specific disease states, and in monitoring structural changes over time. However, because its contrast mechanism is primarily based on reflection or scattering, the information it provides is predominantly structural. As a result, while reflectance-based imaging is valuable for morphological assessment, it may be insufficient to capture disease-specific biochemical or functional signatures required for broader diagnostic generalization.

More generally, a key challenge limiting the widespread clinical adoption of cell-resolution optical microscopy is the availability of diagnostically specific contrast under practical *in vivo* conditions. Histopathology benefits from standardized staining protocols that reliably distinguish cellular and subcellular components, but such chemical labeling is generally not feasible in living patients. Label-free clinical microscopy techniques, although safe and convenient, often face trade-offs involving specificity, imaging field of view, and imaging speed. These constraints become particularly pronounced outside dermatology and ophthalmology, where tissue motion, limited accessibility, and restricted imaging time complicate stable cell-resolution imaging. Extension of optical microscopy to internal organs—for example, via endomicroscopy—typically requires prior localization guided by mesoscopic imaging modalities, and sufficiently high imaging speeds to mitigate motion artifacts [5].

To address these limitations, emerging approaches pursue two complementary strategies. First, intrinsic contrast mechanisms,such as refractive-index variations (phase contrast), endogenous fluorescence (autofluorescence), and molecular vibrational signatures (Raman scattering), are leveraged to extract additional biochemical and morphological information without exogenous labels. Second, extrinsic contrast agents, including targeted fluorescent probes and intrinsically fluorescent drugs with established clinical safety profiles, are being explored to selectively enhance disease-relevant features. In all cases practical considerations, including field of view, imaging depth, acquisition speed, motion robustness, and system complexity, play a decisive role in determining translational feasibility.

In this review, we organize recent advances in cell-resolution optical microscopy according to the contrast mechanisms that generate diagnostically meaningful information—absorption, scattering/reflection, refraction/phase, autofluorescence (AF), Raman scattering, and extrinsic fluorescence—and examine how these mechanisms are implemented in emerging slide-free and *in vivo* imaging platforms. This contrast-centric framework facilitates systematic comparison of the strengths, limitations, and degrees of clinical validation across modalities. We further highlight the increasingly important role of computational reconstruction and AI-assisted interpretation, including virtual staining, automated cell segmentation, and rapid classification, which are becoming essential for translating high-resolution optical images into actionable clinical insights. Figure 1 summarizes the major technological advances and practical constraints shaping the current landscape of cell-resolution optical microscopy.

II. CURRENT STATUS AND EMERGING TECHNOLOGIES OF CELL-RESOLUTION OPTICAL MEDICAL MICROSCOPY

Optical microscopy plays an increasingly important role in clinical medicine, supporting both diagnostic evaluation and intraoperative decision-making. This section outlines the status of cell-resolution optical microscopy in medical

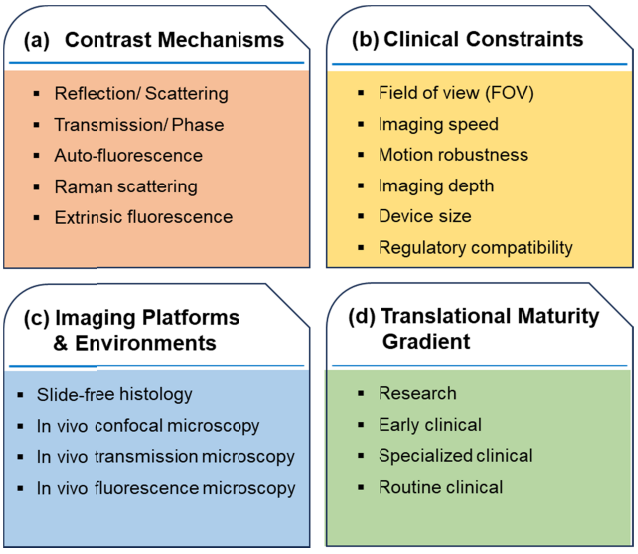

FIG. 1. Contrast mechanisms, clinical constraints, and translational pathways of cell-resolution optical microscopy.

practice, highlights emerging technologies, and describes the contrast mechanisms that enable clinically meaningful visualization. We begin with distinctions between biological and clinical environments, followed by a clinically oriented framework for understanding contrast generation. As schematically illustrated in Fig. 1, these modalities can be broadly organized by contrast mechanism, imaging configuration, and translational maturity, which together shape their clinical applicability.

2.1. Overview of Optical Medical Microscopies

Conventional bright-field microscopy and automated whole-slide scanners remain the backbone of histopathological diagnostics, providing gold-standard subcellular information from thin-sectioned, stained tissue slides. Beyond *ex vivo* slides, IVCM offers *en face* cellular information of intact tissue, primarily in ophthalmology and dermatology, but further adoption has been limited. Recent developments point toward new contrast mechanisms, faster imaging architectures, miniaturization for endoscopic access, and computational interpretation for clinical feasibility.

In biological research, optical microscopy has advanced rapidly in multiple directions [6]: (1) Higher spatial resolution (*e.g*. super-resolution microscopy), (2) faster acquisition via line or plane scanning, and (3) increased molecular specificity enabled by a large toolbox of fluorescent probes. Clinical environments, in contrast, have access to only a narrow set of approved imaging agents, most of which lack molecular targeting capability. As a result, clinical microscopy depends heavily on intrinsic contrast mechanisms of reflection, phase, autofluorescence, and Raman scattering, while a small number of recent regulatory approvals for targeted fluorophores in image-guided surgery signal growing opportunities for molecular imaging in patient care.

To provide a clinically anchored perspective, we classify contrast mechanisms by their relevance to cell-resolution imaging, summarize their physical bases, and outline their translational maturity. Table 1 summarizes the physical basis, clinical applications, and translational maturity of major contrast mechanisms used in medical optical microscopy.

TABLE 1. Contrast mechanisms used in medical optical microscopy

Contrast Mechanism	Representative Devices/ Techniques	Typical Clinical or Translational Applications	Key Advantages	Primary Limitations	Clinical Maturity
Reflectance/ Scattering	*In Vivo* Confocal Microscopy (IVCM) [3, 4], Optical Coherence Microscopy (OCM) [9]	Ophthalmology, Dermatology	Label-free; Robust; Compatible with *in Vivo* Imaging	Optical Contrast; Speckle Noise; Subtle Cellular Contrast	Routine
Absorption	Bright-field Microscopy (Histopathology), Microscopy with UV Surface Excitation (*ex Vivo*) [12, 13]	Diagnostic Pathology, Slide-free Histology	High Subcellular Specificity with Stains or UV Absorption	Requires *ex Vivo* Tissue; UV Photodamage	Routine (*ex Vivo*)
Transmission (Phase)	Oblique Back-illumination Microscopy (OBM) [16, 17], Transscleral Retina Imaging [45]	Early Clinical Studies (Brain, Retina)	Sensitive to Weak Refractive-index Variations; Label-free Cellular Morphology	Optical Contrast	Emerging/ Research
Autofluorescence	Two-photon Microscopy (TPM) [18–20], Fluorescence Lifetime Imaging [18, 22]	Ophthalmology, Dermatology, Metabolic Imaging	Endogenous Biochemical and Metabolic Contrast	Low Signal; Mixed Fluorophore Contributions	Limited/ Specialized
Raman Scattering (Spontaneous)	Point-based Raman Probes, Fiber Raman Spectroscopy [25]	Early Clinical Research (Tumor-margin Assessment)	High Molecular Specificity; Label-free Chemical Information	Extremely Weak Signal; Slow Acquisition; Limited Imaging Capability	Early Clinical/ Translational
Enhanced Raman Scattering	Stimulated Raman Scattering (SRS), CARS Microscopy [26–28]	Intraoperative Brain-tumor Imaging; Slide-free Histology Research	Video-rate Imaging; Histology-like Chemical Contrast	Complex Laser Systems; Limited Clinical Availability	Early Clinical/ Translational
Extrinsic Fluorescence (Dyes, Probes)	Surgical Microscopy, Fluorescence Endoscopy (Meso-scale)	Tumor-margin Detection, Vascular Imaging	High Signal-to-Background Ratio; Molecular Specificity	Typically Mesoscopic Resolution; Limited Cellular Labeling	Specialized
Intrinsically Fluorescent Drugs	Moxifloxacin-based Fluorescence Microscopy [29]	Ophthalmology (Goblet Cells); Epithelial Secretory Cells	Clinically Approved Agents; Selective Cellular Uptake	Limited Range of Applicable Drugs and Targets	Emerging

2.2. Contrast Mechanisms in Optical Medical Microscopy

Image contrast is essential for visualizing diagnostically meaningful microstructures. Whereas biological research often relies on exogenous labeling and controlled sample preparation, clinical applications demand contrast that is safe, fast, robust to motion, and compatible with workflow constraints. Below we summarize intrinsic and extrinsic contrast mechanisms that currently support, or are poised to support, cell-resolution clinical microscopy.

2.2.1. Intrinsic Contrast Mechanisms

Reflection contrast is the most widely adopted intrinsic optical mechanism capable of providing cellular information *in vivo*. IVCM detects backscattered light arising from refractive-index variations within tissue microstructures, including cell nuclei, membranes, and extracellular interfaces, and is now well established in ophthalmology and dermatology [3, 7, 8]. Optical coherence microscopy (OCM) extends coherence-gated reflection imaging to the cellular scale, offering improved optical sectioning compared to confocal approaches [9]. Although optical coherence tomography (OCT) [10] is ubiquitous in clinical practice, its axial and lateral resolution generally do not reach the cellular regime, so it is not discussed further in this review. Reflection-based microscopies are robust, label-free, and well suited for visualizing cellular structure *in vivo*. However, they are inherently sensitive to speckle noise, and often provide relatively limited contrast for subtle cellular morphology.

Absorption contrast underlies conventional bright-field histopathology, where chemical stains such as hematoxylin and eosin (H&E) provide high-contrast visualization of cellular and subcellular morphology. *In vivo* endogenous absorbers, including hemoglobin and melanin, provide useful intrinsic contrast for mesoscopic assessment of vasculature or pigmented lesions [11], but intrinsic absorption rarely yields label-free, cell-resolution contrast in living tissue. At shorter wavelengths, intrinsic absorption in the deep ultraviolet can provide nuclear and cellular information in *ex vivo* tissue specimens, where strong absorption by nucleic acids and proteins enables high-contrast imaging [12, 13]. However, these approaches are currently restricted to non-living samples, due to phototoxicity. Deep-UV absorption is therefore primarily used for slide-free histopathology of biopsied tissue specimens.

Phase and transmission contrast arise from refractive-index variations within cells, and are therefore inherently well suited for label-free cellular imaging. While both reflection and phase contrasts originate from refractive-index heterogeneity, phase-based methods are more sensitive to weak refractive-index variations and provide enhanced morphological contrast. In transparent specimens such as cell cultures, phase information is commonly captured using quantitative phase imaging (QPI) [14] or differential phase contrast (DPC) imaging [15], enabling visualization of subcellular structures. For intact tissue, however, conventional phase microscopy is generally not feasible due to the requirement for transillumination geometry. To address this limitation, oblique back-illumination microscopy (OBM) enables detection of phase-gradient contrast in scattering tissue by introducing illumination light outside the imaging field of view and converting it into an effective transmissive, obliquely illuminating field through multiple scattering within tissue [16, 17]. OBM and related approaches therefore represent promising strategies for label-free cellular imaging *in vivo*, and are discussed in detail in Section 2.3.2.

Autofluorescence (AF) originates from endogenous fluorophores naturally present in biological tissue, and provides intrinsic biochemical contrast at the cellular level. Major fluorophores include reduced nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), aromatic amino acids such as tryptophan, and vitamin A derivatives such as retinol. NADH and FAD are central to cellular metabolism and form the basis of metabolic imaging via redox ratio analysis [18], whereas tryptophan fluorescence reflects protein content and conformation, and retinol contributes prominently in epithelial and ocular tissues. Research-grade AF microscopy, including two-photon microscopy [19, 20] and fluorescence-lifetime imaging microscopy (FLIM), can achieve cell-resolution metabolic and biochemical imaging in experimental and translational settings [21, 22]. In clinical practice, however, AF signals often represent mixed contributions from multiple fluorophores. Retinal AF imaging, for example, primarily reflects lipofuscin and melanin, and is typically performed at mesoscopic scales for detecting pathological deposition [23]. Although only a limited number of AF implementations currently achieve cellular resolution in patients, ongoing improvements in detector sensitivity, excitation strategies, and computational denoising are expanding the feasibility of AF-based *in vivo* cellular microscopy (Section 2.3.3).

Raman scattering encodes molecular vibrational signatures and enables label-free biochemical fingerprinting with high chemical specificity. Owing to the inherently weak Raman signal, most clinical Raman systems are currently point-based rather than imaging-based [24–26]. Recent advances in coherent Raman techniques have demonstrated video-rate imaging speeds and substantially improved throughput [27]. Enhanced Raman microscopy has shown particular promise for intraoperative tissue characterization and molecular diagnostics [28, 29].

2.2.2. Extrinsic Contrast Mechanisms

Extrinsic contrast relies on externally administered agents that enhance signal strength or provide molecular specificity beyond what is accessible through intrinsic tissue properties alone. Most clinically approved contrast agents and fluorophores have been optimized for vascular, tissue-level, or lesion-level assessment, rather than for selective cellular labeling at microscopic resolution. Conven-

tional clinical fluorophores include fluorescein, indocyanine green (ICG), methylene blue, and 5-aminolevulinic acid (5-ALA). Fluorescein and ICG generate high-signal contrast following intravenous administration and are widely used to assess vascular perfusion, leakage, or barrier integrity. However, they do not selectively label specific cell populations and are typically imaged using slit lamps, fundus cameras, or surgical microscopes at mesoscopic spatial scales. Methylene blue and 5-ALA exhibit greater cellular affinity—through nuclear or cytoplasmic uptake in the case of methylene blue, and selective accumulation of protoporphyrin IX (PpIX) in tumor cells for 5-ALA—but in current clinical practice these agents are likewise visualized predominantly with surgical or endoscopic systems rather than with cell-resolution microscopy. As a result, while these agents provide molecular or functional specificity, they are only infrequently used for true cellular-level examination.

A notable exception is a class of clinically approved drugs that exhibit intrinsic fluorescence and can function as practical cellular labels. Among these, moxifloxacin demonstrates strong intrinsic fluorescence, rapid intracellular uptake, and preferential labeling of specific secretory cell types, including conjunctival goblet cells [30] and intestinal Paneth cells. When combined with high-resolution fluorescence microscopy, moxifloxacin enables cell-resolution imaging of labeled cells in both *ex vivo* and *in vivo* settings (Section 2.3.3), providing a rare example of a clinically approved agent compatible with *in vivo* cellular microscopy.

Commonly used nuclear and cytoplasmic fluorescent dyes, such as acridine orange, DAPI, Hoechst, and related probes, produce strong and well-defined cellular contrast, and are integral to slide-free histopathology systems based on high-speed three-dimensional fluorescence microscopy and virtual staining workflows [31]. Due to safety and regulatory considerations, these dyes are restricted to *ex vivo* applications; nevertheless, they remain essential for the development and validation of translational imaging platforms that aim to replicate or augment conventional histopathology.

Overall, while most clinically approved extrinsic contrast agents are designed for mesoscopic imaging and examination, only a limited subset—including intrinsically fluorescent drugs and selectively targeted dyes—currently provide molecular specificity compatible with cell-resolution optical microscopy. Expanding this repertoire remains a key challenge for translating cellular-resolution imaging into broader clinical practice.

2.3. Advances in Cell-resolution Optical Microscopy for Medicine

Optical microscopy in medicine has traditionally been used in conventional slide-based histopathology, where high-quality cellular information becomes available only after fixation, thin-sectioning, and staining. Recent advances in optical engineering and computational analysis have expanded the role of microscopy by enabling cell-resolution imaging directly from fresh tissue or *in vivo*, reducing preparation time and supporting real-time clinical assessment. These innovations address various limitations of conventional histology—slow processing, sampling artifacts, and inability to image intact tissue—and expand cellular visualization into anatomical regions where biopsy is difficult or impractical. This section summarizes three major technology classes that form the foundation of modern clinical cell-resolution microscopy: (1) Slide-free microscopy for excised or biopsied tissue, (2) *in vivo* transmission microscopy for label-free cellular imaging, and (3) *in vivo* fluorescence microscopy for sensitive cellular and molecular imaging.

2.3.1. Slide-free Microscopy for Histopathological Examination

Conventional histopathology remains the diagnostic gold standard, with H&E–stained tissue sections providing high-contrast visualization of cellular and subcellular morphology. However, the conventional workflow requires chemical fixation, microtome sectioning, and staining, which delay diagnosis and may introduce processing-related artifacts. Slide-free microscopy seeks to acquire subcellular-resolution images directly from fresh, unsectioned tissue specimens, thereby enabling rapid biopsy triage, intraoperative consultation, and streamlined pathology workflows [32]. Representative slide-free optical microscopy platforms that are being explored for rapid assessment of excised tissue, along with their imaging characteristics and translational status, are summarized in Table 2.

Microscopy with ultraviolet surface excitation (MUSE) employs deep-ultraviolet illumination, which confines excitation to a shallow surface layer due to strong UV absorption in biological tissue [12]. This intrinsic optical sectioning enables surface-specific imaging with minimal sample preparation. MUSE can be operated with exogenous fluorescent dyes (such as DAPI) for strong nuclear contrast, or without dyes by exploiting intrinsic absorption and AF to generate diagnostically informative images. As a result, MUSE is well suited for rapid, low-preparation evaluation of subcellular morphology on the surface of a fresh tissue specimen. Similarly, ultraviolet absorption–based photoacoustic microscopy [33] can provide subcellular information by detecting acoustic waves generated from absorbed UV light. This hybrid approach has shown utility in tissues such as bone, where optical access to cellular structures is limited.

Beyond surface-confined methods, several high-speed optical sectioning techniques enable high-throughput extraction of subcellular information from superficial tissue depths on the order of several tens of micrometers. Structured illumination microscopy (SIM) achieves optically sectioned, subcellular-resolution imaging through patterned illumination and computational reconstruction [34]. Line-scanning confocal microscopy supports rapid mosaic imaging of large tissue areas, with throughput primarily limited

TABLE 2. Slide-free microscopy techniques

Modality	Imaging Geometry/Contrast	Key Strengths	Primary Limitations/Notes
Microscopy with Ultraviolet Surface Excitation (MUSE) [31]	UV Absorption and Autofluorescence (Surface-confined)	Simple Wide-field Implementation; High-Speed; Minimal Sample Preparation; Compatible with Intraoperative Workflows	Limited Axial Sectioning (>10 μm)
UV Photoacoustic Microscopy (UV-PAM) [32]	UV Absorption & Ultrasonic Detection (Nuclear Contrast)	Label-free, Hematoxylin-like Nuclear Contrast; Compatible with Thick, Unsectioned Tissue	Acoustic Coupling Required; System Complexity; Point Scanning & Relatively Slow
Structured Illumination Microscopy (SIM) [33]	Fluorescence with Optical Sectioning	High Imaging Speed Close to Wide-field Imaging; Optical Sectioning; Compatible with Virtual Staining Pipelines	Sensitive to Surface Roughness; Volumetric Imaging Requires Axial Scanning
Open-top Light-sheet Fluorescence Microscopy (OT-LSFM) [34, 35]	Selective Planar Fluorescence (Single- or Two-photon Excitation)	Surface-roughness Tolerant; High-speed; Scalable Imaging Depth with Two-photon Excitation	Imaging Depth Limited (<50 μm native, ~100 μm with Two-photon); System Complexity
Enhanced Raman Microscopy [26–28]	Stimulated Raman Scattering (SRS), CARS Microscopy	Label-free Chemical Contrast; Histology-like Imaging without Staining; Specialized for Brain-tumor Detection (Myelination)	Complex Laser Systems; Limited Penetration Depth; Brain-tumor Detection Only

by camera speed and scanning efficiency. Open-top light-sheet fluorescence microscopy (OT-LSFM) adapts selective planar illumination to an open imaging geometry, accommodating large or irregularly shaped specimens while providing fast optical sectioning near the tissue surface, with minimal sample handling [35]. Two-photon OT-LSFM further extends imaging depth to approximately 100 μm by employing near-infrared excitation, while preserving the same open-top configuration [36].

Across these three-dimensional fluorescence-based modalities, including SIM, LSFM, and confocal microscopy, standard nuclear and cytoplasmic dyes commonly used in biological research (*e.g*. DAPI, Hoechst, acridine orange, and cytoplasmic markers) enable rapid, high-contrast *ex vivo* subcellular imaging. These dyes form the foundation for virtual staining pipelines that computationally transform fluorescence images into H&E-like representations compatible with established histopathological interpretation.

To image thicker tissue volumes, optical clearing strategies reduce light scattering through refractive-index homogenization, enabling millimeter-scale volumetric imaging of intact specimens [37]. While optical clearing substantially enhances imaging depth and three-dimensional-reconstruction fidelity, the required chemical processing and incubation times currently limit its applicability to research settings or postoperative analysis, rather than real-time clinical pathology.

Finally, artificial intelligence–assisted tools—including virtual staining, automated cell and structure segmentation, margin-probability mapping, and artifact suppression—are increasingly integrated into slide-free microscopy platforms [38]. These computational approaches facilitate alignment with digital pathology workflows and play a critical role in accelerating interpretation, improving reproducibility, and enabling scalable clinical deployment.

Despite the demonstrated ability of slide-free microscopy to enable rapid examination of biopsied tissue specimens, further advances are required to match the diagnostic fidelity of conventional H&E histology. In particular, accurately resolving fine subcellular features, such as nuclear morphology and chromatin organization, remains challenging in fresh, unsectioned tissue [39–41]. These features are central to pathological grading and diagnosis, and are routinely visualized with high reliability in stained thin sections. Continued improvements in optical resolution, contrast mechanisms, volumetric sampling strategies, and computational reconstruction will be essential for slide-free microscopy to consistently deliver H&E–quality information suitable for routine clinical pathology.

2.3.2. Transmission Microscopy for *in Vivo* Examination

Transmission-based *in vivo* microscopy exploits refractive-index variations and associated phase shifts to visualize cellular structures that generate weak reflection contrast. Compared to reflection-based modalities such as IVCM, transmission geometries are intrinsically more sensitive to subtle intracellular refractive-index variations, and therefore provide enhanced morphological contrast of cells. OBM represents a leading approach for enabling transmission-like phase contrast in intact, scattering tissue [16, 42, 43]. In OBM, illumination is introduced from the periphery of the field of view. Through multiple scattering within tissue, this illumination is converted into an effective oblique transillumination at the focal plane. Local refractive-index variations refract the oblique light back toward the objective, producing differential phase-contrast (DPC) images with shadowlike enhancement of cellular morphology and fine microarchitecture.

OBM has been investigated for imaging superficial brain structures, including tumor margins and cortical micro-

architecture [42]. Miniaturized OBM probes have further demonstrated feasibility for endoscopic and intraoperative applications [44]. In ocular surface imaging, OBM enables visualization of secretory goblet-cell populations and functions in the conjunctiva [45]. When applied to superficial vasculature, OBM can also resolve individual blood cells in real time [46, 47], supporting label-free analysis of microcirculatory dynamics and inflammation-associated cellular responses.

A related transmission-based strategy for retinal imaging is trans-scleral illumination, in which light is delivered through the sclera while image collection occurs through the pupil [48]. This configuration generates highly oblique illumination of the retina, suppressing specular reflections and enhancing phase-gradient contrast from deeper retinal layers. Trans-scleral imaging enables visualization of structures such as the retinal pigment epithelium (RPE), whose contrast is difficult to achieve using conventional transpupillary illumination alone. Computational approaches including machine-learning-based denoising, morphological enhancement, and phase-to-histology translation further improve image interpretability, and enable automated quantification of microcirculatory metrics such as cell velocity and flux [49].

Together, OBM and trans-scleral illumination extend label-free, phase-gradient cellular imaging to intact living tissue. These approaches provide contrast mechanisms that complement reflection- and fluorescence-based modalities, and expand the range of anatomical sites accessible to *in vivo* cellular microscopy.

2.3.3. Fluorescence Microscopy for *in Vivo* Examination

Fluorescence microscopy provides strong and specific contrast derived from endogenous AF or externally administered fluorophores. Because fluorescence originates from defined biochemical constituents, it enables high sensitivity, excellent signal-to-background ratios, and visualization of structural, metabolic, and molecular features at subcellular resolution. The practical implementation and clinical impact of fluorescence microscopy, however, are largely determined by the underlying imaging modality, rather than by contrast alone.

Two-photon microscopy (TPM) is a central technique for *in vivo* cell-resolution AF imaging [20]. Near-infrared femtosecond excitation confines fluorescence generation to the focal volume, reducing out-of-focus background, minimizing photodamage, and improving imaging depth. By tuning the excitation wavelength, TPM selectively excites metabolic cofactors such as NAD(P)H and FAD, enabling simultaneous visualization of tissue microarchitecture and intrinsic metabolic state. TPM has been applied to skin and ocular tissues [50, 51], demonstrating sensitivity to cellular and metabolic abnormalities. Its broader clinical translation, however, is constrained by a limited field of view and relatively slow acquisition speeds associated with weak endogenous fluorescence signals.

Single-photon wide-field AF imaging is widely used clinically, particularly in ophthalmology, where it provides mesoscopic functional assessment rather than cellular detail. Techniques such as fundus autofluorescence (FAF) and fluorescence-lifetime imaging ophthalmoscopy (FLIO) capture signals from lipofuscin, melanin, and flavins to assess retinal integrity and metabolic status [23, 52]. Although these modalities do not achieve cellular resolution, they illustrate the clinical value of fluorescence-based techniques for functional imaging and longitudinal monitoring.

Light-sheet fluorescence microscopy (LSFM) enables high-speed volumetric imaging by selectively illuminating and acquiring fluorescence from a thin optical plane. While conventional LSFM has been primarily used in biological research, recent miniaturized implementations have demonstrated *in vivo* cellular imaging of superficial epithelial tissues [53]. These developments highlight the feasibility of compact light-sheet architectures for rapid AF imaging, and suggest potential pathways toward clinical translation, where speed and volumetric coverage are critical.

Externally administered fluorophores can extend *in vivo* fluorescence microscopy by enabling selective cellular contrast, when paired with suitable imaging architectures. The intrinsically fluorescent antibiotic moxifloxacin provides a practical example, allowing cell-resolution visualization of secretory epithelial populations such as conjunctival goblet cells. Combined with high-resolution, extended-depth-of-field surface fluorescence microscopy, this approach supports rapid, noncontact *in vivo* examination in human subjects [54].

At a broader clinical scale, fluorescence-guided surgery (FGS) highlights the expanding role of molecular fluorescence in medicine. FGS typically employs mesoscopic surgical microscopes or endoscopes and uses agents such as 5-ALA, fluorescein sodium, and ICG for intraoperative visualization of tumor margins or vasculature. More recently, targeted fluorophores such as pafolacianine (Cytalux) have demonstrated rapid progress in molecularly selective probes. Although these approaches generally operate at mesoscopic resolution, they underscore the growing clinical demand for molecular fluorescence as a complement to cell-resolution imaging.

Taken together, fluorescence microscopy, transmission-based phase microscopy, and slide-free histopathology constitute a complementary set of technologies for modern cell-resolution clinical imaging. Each modality offers distinct strengths of molecular specificity, label-free cellular contrast, real-time imaging capability, or compatibility with intact tissue. Their continued convergence is expanding the range of clinical scenarios in which cellular-scale optical imaging can be practically deployed. An overview of *in vivo* cell-resolution optical microscopy applications across different organ systems, including imaging context and current translational status, is provided in Table 3.

TABLE 3. *In vivo* cell-resolution optical microscopy techniques and clinical applications

Primary Contrast Source	Representative Techniques & Target Tissue	Clinical Role & Key Strength	Current Status
Reflectance/ Scattering	*In Vivo* Confocal Microscopy (IVCM), Optical Coherence Microscopy (OCM); Skin, Cornea	Skin-cancer Diagnosis; Optical Biopsy; Corneal Neuropathy and Infectious Keratitis; Label-free and Robust with Established Clinical Protocols	Routine (Dermatology, Ophthalmology)
Transmission (Phase)	Trans-scleral Retinal Imaging [47], Oblique Back-illumination Microscopy (OBM) [41–48]; Retina, Superficial Brain	Visualization of RPE, Brain-tumor Cells, and Blood Cells; Sensitive to Weak Refractive-index Variations; Compatible with Scattering Tissue	Early Clinical/ Translational
Autofluorescence (AF)	Two-photon Microscopy (TPM) [49, 50], Fluorescence-lifetime Imaging Microscopy (FLIM); Skin, Cornea	Skin Cancer and Metabolic Imaging using Endogenous Biochemical Contrast with Optical Sectioning	Limited/ Specialized
Intrinsically Fluorescent Drugs	Moxifloxacin-based Fluorescence Microscopy [53]; Conjunctiva	*In Vivo* Assessment of Goblet-cell Density and Morphology using a Clinically Approved Drug with Selective Cellular Uptake	Early Clinical

III. CLINICAL APPLICATIONS OF CELL-RESOLUTION OPTICAL MICROSCOPY

Cell-resolution optical microscopy is increasingly being applied to clinical medicine, offering real-time visualization of cellular and subcellular architecture, inflammatory processes, and early pathological changes. These techniques complement radiologic imaging and conventional histopathology by enabling direct assessment of epithelial surfaces and superficial tissue layers, often without biopsy or time-consuming preparation. This section highlights representative clinical applications, organized by organ system and clinical workflow.

3.1. Ophthalmological Imaging

The eye represents the most clinically advanced domain for *in vivo* cell-resolution optical microscopy, owing to its optical accessibility and the diagnostic relevance of epithelial, neural, and vascular microstructures across both the anterior and posterior segments. In the anterior segment, IVCM is routinely used for cellular-level assessment of the cornea, enabling visualization of epithelial cells, the sub-basal nerve plexus, keratocytes, and the endothelium. Quantitative metrics derived from IVCM such as nerve-fiber density and nerve tortuosity are widely used as objective biomarkers for dry-eye disease, infectious keratitis, diabetic neuropathy, and neurotrophic keratopathy. In parallel, specular microscopy remains an established clinical tool for routine evaluation of the morphology and density of corneal endothelial cells.

Despite these successes, clinically relevant limitations persist, particularly restricted field of view, imaging speed, and insufficient contrast for fine corneal nerves and weakly scattering microstructures. To address these challenges, phase-sensitive and volumetric ophthalmic imaging approaches are undergoing rapid development, to improve corneal nerve conspicuity and enable high-speed three-dimensional examination [55, 56]. These advances are expected to enhance assessment of corneal neuropathies and support more robust longitudinal monitoring, complementing existing confocal methods.

Fluorescence-based imaging further extends anterior-segment applications by enabling selective cellular contrast. AF-based multiphoton approaches have been explored for corneal and conjunctival imaging, but remain limited in routine clinical use. More recently, intrinsically fluorescent drugs have provided a practical pathway to targeted *in vivo* fluorescence imaging. Moxifloxacin-based fluorescence microscopy enables selective visualization of conjunctival goblet cells, facilitating objective assessment of mucin-related ocular surface disorders.

In the posterior segment, retinal imaging has benefited from high-resolution optical techniques that support differentiation of layered retinal structures. Scanning laser ophthalmoscopy (SLO) is routinely used in clinical practice, and provides optical sectioning capability that enables *en face* visualization of retinal layers, serving as a key workhorse for retinal-disease assessment. Beyond this, phase-sensitive retinal imaging with trans-scleral illumination strategies is emerging to improve contrast for cellular and microvascular features, extending sensitivity beyond conventional reflectance imaging [48]. Higher-resolution retinal microscopy has enabled visualization of individual photoreceptors, retinal-pigment epithelial cells, and microvascular structures *in vivo*, supporting early detection and longitudinal monitoring of retinal degeneration, vascular abnormalities, and neurodegenerative changes. Nevertheless, broader clinical adoption of true cell-resolution retinal microscopy remains constrained by system complexity, acquisition speed, and sensitivity to ocular motion.

Overall, ophthalmology continues to lead clinical translation of cell-resolution optical microscopy. Continued advances in high-speed volumetric imaging, phase-sensitive contrast, and robust clinical implementation are expected to further expand the diagnostic impact of cell-resolution im-

aging across both anterior and posterior ocular applications.

3.2. Dermatological Imaging

Dermatology represents one of the most clinically mature application domains for *in vivo* cell-resolution optical microscopy, driven primarily by the need for noninvasive evaluation of skin cancer. IVCM has been widely adopted for the assessment of suspicious cutaneous lesions, enabling cellular-level visualization of epidermal and superficial dermal structures and supporting optical biopsy–based clinical decision-making. In particular, basal-cell carcinoma and melanoma have been extensively studied, with confocal imaging shown to reduce unnecessary excisions and improve lesion triage in routine practice.

More recently, line-field confocal optical coherence tomography (LC-OCT) has emerged as a competitive modality for dermatologic imaging by providing high-resolution, three-dimensional cellular visualization over extended depths [57]. By enabling volumetric assessment of skin architecture at near-cellular resolution, LC-OCT addresses several limitations of point-scanning IVCM and has demonstrated growing potential for *in vivo* diagnosis and longitudinal monitoring of skin cancer. These developments reflect a broader shift toward high-speed volumetric cellular imaging, rather than purely *en face* examination.

Multiphoton microscopy (MPM) has also been actively investigated in dermatology, offering label-free visualization of skin morphology and endogenous biochemical contrast [58]. *In vivo* MPM has demonstrated the ability to visualize cellular and extracellular structures relevant to skin cancer, photoaging, and inflammatory disease, as well as metabolic and structural features not accessible with reflectance-based imaging. However, its clinical translation remains limited by system complexity, restricted field of view, and practical constraints on routine clinical deployment, positioning MPM primarily as a research and specialized translational tool rather than a broadly adopted diagnostic modality.

Beyond *in vivo* diagnosis, cell-resolution optical microscopy has been applied to *ex vivo* evaluation of excised tissue, particularly for rapid assessment of surgical margins during procedures such as Mohs surgery. Confocal fluorescence imaging has been explored in this context, often leveraging shared imaging platforms with alternative contrast modes [59]. While these approaches offer near-real-time feedback and may complement frozen-section histology, broader clinical adoption remains limited by workflow integration and interpretive expertise.

Cell-resolution imaging has additionally been investigated in inflammatory and degenerative skin diseases, including psoriasis and dermatitis, where cellular morphology and immune-cell infiltration can be visualized *in vivo* [59, 60]. Although these applications remain largely investigational, they demonstrate the broader potential of cellular imaging beyond oncologic use. Phase-based imaging approaches are also beginning to be explored in dermatology, with the potential to provide complementary contrast for weakly scattering cellular features, although their clinical utility has yet to be established.

Despite substantial progress, dermatologic cell-resolution microscopy continues to face challenges related to limited imaging depth, restricted field of view, cellular contrast, and operator dependence. Ongoing developments in high-speed volumetric imaging, phase-sensitive contrast, and AI-assisted interpretation are expected to further enhance diagnostic robustness and scalability in clinical dermatology.

3.3 Surgical, Perioperative, and Slide-free Tissue Assessment

Cell-resolution optical microscopy has been actively investigated across surgical and perioperative settings as a means of providing rapid tissue assessment to support clinical workflows. These efforts span *in vivo*, endomicroscopic, and *ex vivo* implementations and reflect varying requirements in terms of imaging speed, information content, and allowable processing time.

In vivo cell-resolution optical imaging has been applied to lesion assessment and decision support, particularly in dermatologic contexts such as the evaluation of skin lesions. By enabling cellular-level visualization directly at the point of care, these approaches have been used to assist in lesion selection, biopsy guidance, and longitudinal surveillance. In such applications, *in vivo* optical imaging supports clinical decisions in scenarios where immediacy and minimal disruption to workflow are prioritized.

Endomicroscopic implementations of cell-resolution optical microscopy extend *in vivo* cellular imaging to internal tissues during surgical procedures. Neurosurgical applications have received particular attention, where localized, real-time cellular visualization can be integrated into the operative workflow. In brain-tumor surgery, probe-based cellular imaging has been explored to assess tissue microstructure at the resection margin, supporting intraoperative guidance, biopsy targeting, and differentiation between tumor and surrounding brain tissue. These approaches provide focal cellular information that complements neuronavigation, fluorescence-guided surgery, and intraoperative pathology. The availability of clinically approved endomicroscopic platforms, including systems developed by Carl Zeiss Meditec, reflects the increasing translational maturity of cellular endomicroscopy in neurosurgical settings. Beyond neurosurgery, endoscopic cellular imaging has also been investigated in other organ systems, where its role continues to be evaluated in relation to existing clinical workflows and assessment needs.

Ex vivo optical microscopy has also been explored for the examination of freshly excised tissue in surgical and perioperative environments. Under these conditions, reduced motion and controlled imaging parameters enable rapid acquisition over larger tissue areas. Fluorescence-based nuclear and cytoplasmic labeling strategies are

commonly employed to generate images resembling conventional histological sections, facilitating interpretation within established diagnostic frameworks. These slide-free approaches have been investigated for applications such as margin screening, tissue triage, and rapid feedback during surgical procedures, where shortened turnaround time may offer practical advantages.

A wide range of optical microscopy platforms has been applied to slide-free tissue assessment, including fluorescence-, absorption-, photoacoustic-, and light-sheet-based techniques. As discussed in Section 2 and summarized in Table 2, many of these systems already achieve sufficient imaging speed and large-area coverage for rapid examination of excised specimens, highlighting the feasibility of optical approaches for time-sensitive workflows.

Beyond two-dimensional imaging, three-dimensional (3D) optical microscopy is increasingly being explored to provide additional structural context through volumetric visualization of cellular organization. Such approaches may complement section-based assessment by capturing tissue architecture across depth. The integration of 3D imaging with tissue-clearing or contrast-enhancement strategies has also been investigated, to enable extended-depth visualization. While the additional preparation time required for optical clearing limits its suitability for real-time intraoperative use, these methods may be valuable in perioperative or non-time-critical settings where more comprehensive tissue assessment is desired.

Overall, cell-resolution optical microscopy continues to be evaluated across a spectrum of surgical and histology-related applications. By balancing imaging speed, information content, and dimensionality, these approaches are being positioned as complementary tools that can support rapid screening, decision-making, and structural assessment within surgical and perioperative workflows, rather than as direct replacements for conventional histopathology.

IV. CONCLUSION AND FUTURE PERSPECTIVES

Cell-resolution optical microscopy represents one of the most promising frontiers in modern medical imaging. By enabling visualization of cells, subcellular structures, and microarchitectural patterns in fresh or living tissue, these technologies provide diagnostic information that bridges the longstanding gap between radiologic imaging and conventional histopathology. Despite this compelling potential, widespread clinical adoption remains limited. Most emerging modalities reviewed here (including slide-free histopathology platforms, transmission-based *in vivo* phase microscopy, and high-contrast fluorescence techniques) remain in early or intermediate stages of translation, with only a small number achieving focused clinical use. A clear understanding of both the opportunities and the persistent barriers is therefore essential for guiding future development.

A fundamental challenge lies in the optical requirements for achieving micrometer-scale subcellular or cellular resolution in clinical tissue. High numerical apertures improve lateral resolution but inherently restrict FOV and reduce tolerance to motion. In addition, biological tissues exhibit heterogeneous scattering, refractive-index variations, and physiological motion, all of which degrade image stability and contrast. Consequently, many cell-resolution modalities remain confined to superficial epithelial surfaces or require specialized access through probes, contact interfaces, or surgical exposure. FOV limitations further complicate clinical utility, as imaging anatomically or diagnostically relevant regions often necessitates mosaicking or stitching, introducing additional acquisition time and susceptibility to motion artifacts. In practice these workflow constraints, rather than insufficient spatial resolution, remain major barriers to routine clinical deployment.

Contrast specificity remains a central consideration for clinical translation. Label-free optical contrasts encompass diverse mechanisms with distinct strengths and limitations. Reflection- and phase-based imaging primarily emphasize cellular structure and morphology, offering robust structural information and limited chemical specificity. In contrast, intrinsic molecular contrasts such as AF and Raman scattering provide sensitivity to biochemical composition and metabolic state, although their clinical deployment is often constrained by acquisition speed, signal strength, or system complexity. Conventional histological staining, while not molecularly specific, continues to provide highly reproducible, high-contrast morphological visualization—particularly of nuclear and cytoplasmic features—that sets a practical benchmark for diagnostic interpretation. Bridging the gap between structural contrast, molecular information, and clinical workflow remains an active area of development in cell-resolution optical microscopy.

Despite these challenges, the field is advancing rapidly toward higher contrast, improved resolution, and greater molecular specificity. Optical engineering innovations—including miniaturized objectives, extended-depth-of-field designs, remote focusing architectures, and high-speed detectors—are improving system robustness, acquisition speed, and compatibility with clinical workflows. At the same time, multimodal nonlinear optical microscopy, combining contrasts such as multiphoton fluorescence, second- and third-harmonic generation, and coherent Raman signals, is increasingly being explored as a means of integrating structural, functional, and biochemical information within a single imaging framework [25]. While such multimodal approaches remain technically complex and are not yet routine in clinical practice, they illustrate a broader trend toward richer, multicontrast cellular imaging, rather than reliance on a single optical mechanism.

Progress in contrast development is equally important. Intrinsically fluorescent clinical drugs, exemplified by moxifloxacin, demonstrate that some approved compounds can function as practical cellular labels without introduc-

ing additional regulatory burden. In parallel, targeted peptides, activatable fluorophores, and disease-specific small molecules are advancing through early clinical evaluation. Label-free techniques are also benefiting substantially from computational enhancement. Methods such as phase retrieval, virtual staining, AF lifetime mapping, spectral unmixing, and multimodal data fusion are improving the interpretability and diagnostic relevance of intrinsic optical signals. Together, these developments are narrowing the gap between raw optical contrast and the level of cellular detail expected from histopathology.

Perhaps the most transformative momentum lies in computational imaging and AI-assisted interpretation. Learning-based algorithms now support denoising, motion correction, phase recovery, virtual staining, and quantitative feature extraction across a wide range of optical modalities. Automated segmentation, cell quantification, lesion classification, and decision-support tools are maturing rapidly. For many emerging imaging platforms, computational interpretation has become as critical as the optical hardware itself. Embedding these capabilities directly into acquisition workflows will be essential for achieving the speed, reproducibility, and interoperability required in clinical environments.

Looking forward, rigorous clinical validation will be indispensable. Prospective studies, harmonized imaging protocols, and large, well-annotated datasets are required to establish diagnostic performance and ensure reproducibility across institutions. Regulatory frameworks must also evolve to accommodate hybrid hardware-software systems in which computational reconstruction and AI-based interpretation play central roles. Education and training of clinicians and pathologists will be equally important, particularly as optical images increasingly diverge from traditional H&E appearances. Ultimately, system design must prioritize workflow integration, ensuring that emerging technologies deliver actionable clinical information, rather than raw image data.

Despite the remaining challenges, the overall trajectory of the field is positive. Miniaturized high-resolution probes are extending cellular imaging to internal organs, slide-free histopathology platforms are reducing diagnostic turnaround times, and *in vivo* phase-, fluorescence-, and nonlinear-based microscopies are revealing cellular structures and tissue properties previously inaccessible in clinical settings. As contrast strategies mature and computational workflows continue to evolve, cell-resolution optical microscopy is poised to become an increasingly valuable adjunct to existing diagnostic pathways across ophthalmology, dermatology, surgery, and pathology.

In summary, although cell-resolution optical microscopy has not yet achieved widespread clinical adoption, the foundation for translational impact is rapidly strengthening. Continued progress in optical system design, enhanced contrast generation, molecular labeling strategies, computational interpretation, and workflow integration will determine the pace at which these technologies transition from research instruments to routine clinical tools. The convergence of these advances points toward a future in which minimally invasive, real-time cellular assessment becomes an integral component of medical care—supporting earlier diagnosis, guiding intervention, and enabling more personalized, image-driven medicine.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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