

Advanced optical microscopy for skin imaging: from confocal to novel techniques

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Abstract

Timely and accurate diagnosis of dermatological diseases remains a global challenge, particularly in healthcare systems with limited access to dermatopathology services. Advanced optical microscopy techniques have emerged as powerful tools for non-invasive skin imaging, offering real-time visualization of microscopic structures with near-histological resolution. Reflectance confocal microscopy (RCM) and ex vivo confocal microscopy (EVCN) have achieved significant clinical traction, demonstrating high concordance with conventional histopathology and high diagnostic accuracy for melanocytic lesions and non-melanoma skin cancers. In parallel, developments in optical coherence tomography (OCT) and its variants—including dynamic OCT and line-field confocal OCT (LC-OCT)—provide complementary morphological and vascular information with greater penetration depth. LC-OCT further combines near-cellular resolution with simultaneous vertical and horizontal imaging, narrowing the gap between non-invasive imaging and histopathology. Emerging multimodal imaging platforms integrating OCT, Raman spectroscopy, photoacoustic tomography, and ultrasound, together with artificial intelligence-assisted image analysis, continue to expand the diagnostic capabilities of optical microscopy. Although RCM, EVCN, OCT, and LC-OCT are increasingly being incorporated into routine dermatological practice, several emerging technologies remain largely confined to specialized academic centers because of their cost, technical complexity, and limited clinical validation. This narrative review summarizes current evidence on confocal microscopy and related optical imaging technologies for skin diagnostics, emphasizing diagnostic performance, clinical utility, implementation challenges, and future directions.

Keywords: optical microscopy, confocal microscopy, reflectance confocal microscopy, line-field confocal OCT, artificial intelligence

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Introduction

Dermatological diseases encompass a broad spectrum of neoplastic, inflammatory, and infectious conditions and create a substantial global healthcare burden. Accurate diagnosis is critical for optimal patient management, but it remains challenging due to the heterogeneity of skin lesions. Although histopathology continues to serve as the diagnostic gold standard, its invasive nature, processing time, and dependence on specialized infrastructure motivate the development of complementary non-invasive diagnostic technologies.

Over the past 2 decades, advanced optical microscopy techniques have emerged as a cornerstone of modern skin imaging, leveraging light–tissue interactions to generate high-resolution morphological and functional information in vivo and ex vivo. Unlike conventional optical microscopy relying on transmitted light and thin tissue sections, dermatological optical imaging systems are predominantly based on epi-illumination, allowing examination of intact skin without physical sectioning (1). These techniques exploit reflectance, fluorescence, scattering, and absorption properties of skin constituents to achieve contrast at the cellular and subcellular scales.

Among the modalities available, reflectance confocal microscopy (RCM), ex vivo confocal microscopy (EVCN), optical coherence tomography (OCT), and, more recently, line-field confocal OCT (LC-OCT) have already entered routine clinical practice for selected dermatological indications, whereas techniques such

as multiphoton tomography, Raman spectroscopy, optoacoustic imaging, and multimodal artificial intelligence (AI) platforms remain largely confined to specialized research or tertiary referral centers because of their technical complexity, cost, and limited availability (2). However, these emerging technologies provide complementary structural, functional, and molecular information that may further improve diagnostic precision and support the development of personalized dermatological care (2).

This narrative review summarizes the current evidence regarding the physical principles, diagnostic performance, clinical applications, limitations, and future potential of advanced optical microscopy techniques in dermatology. Particular emphasis is placed on their current level of clinical implementation, practical barriers to widespread adoption, and their potential role in improving diagnostic pathways across various healthcare settings.

Methods

This narrative review summarizes current evidence about advanced optical microscopy techniques for skin imaging, with a focus on confocal microscopy and emerging photonic modalities. A literature search was performed in PubMed/MEDLINE, supplemented by targeted searches in Web of Science and Google Scholar, using combinations of keywords such as *confocal microscopy*, *reflectance confocal microscopy*, *ex vivo confocal microscopy*, *optical coherence tomography*, *line-field confocal OCT*, *multiphoton microscopy*, *Raman spectroscopy*, *photoacoustic imaging*, and *skin*

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imaging. The search was limited to publications in English and German, with priority given to peer-reviewed original articles, reviews, and guidelines published between 2015 and 2025.

The initial search identified approximately 85 publications. After title and abstract screening, around 28 articles underwent full-text evaluation. Following assessment for relevance, methodological quality, and overlap of information, the most representative and up-to-date publications were selected for inclusion in this narrative review.

Studies were selected based on relevance to dermatological *in vivo* or *ex vivo* imaging, diagnostic performance, clinical utility, and technological innovation. Non-dermatological applications and publications without accessible full text were excluded. The selected evidence was synthesized narratively, with particular emphasis on imaging principles, clinical applications, technological characteristics, diagnostic performance, and future developments. Given the narrative design of this review and the methodological heterogeneity of the available literature, neither a formal systematic review process nor a meta-analysis was performed, and a Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) flow diagram was not considered applicable. Whenever available, priority was given to systematic reviews, meta-analyses, clinical guidelines, and prospective clinical studies.

Results

Confocal laser microscopy: optical principles and dermatological implementation

RCM, also referred to as confocal laser scanning microscopy, is a non-invasive optical imaging technique that achieves high lateral resolution through spatial filtering of out-of-focus light. In dermatological systems, monochromatic laser illumination of a selected wavelength is focused onto a defined horizontal plane within the skin (3). Light reflected or emitted from refractive index mismatches is directed through a pinhole aperture conjugated to the focal plane, thereby suppressing out-of-focus signals and allowing optical sectioning (3).

Image contrast in reflectance-based confocal microscopy primarily arises from endogenous chromophores and structural interfaces, including keratin, melanin, collagen, and cellular membranes. The technique provides lateral resolution on the order of 1 to 3 μm and a penetration depth of approximately 200 to 300 μm , allowing visualization of individual keratinocytes, melanocytes, dermal papillae, and superficial dermal structures with near-histological resolution (4).

Beyond its optical characteristics, RCM has become one of the most extensively validated non-invasive imaging techniques in dermatology. A recent systematic review and meta-analysis demonstrated pooled diagnostic sensitivities exceeding 90% and specificities approaching 80% to 90% for melanoma detection, and prospective real-world studies have shown that RCM substantially reduces unnecessary skin biopsies without compromising diagnostic safety (5). These findings have established RCM as a valuable adjunct to dermoscopy, particularly for the evaluation of equivocal melanocytic lesions and the diagnosis of basal cell carcinoma (4).

Beyond *in vivo* imaging, confocal microscopy can be applied *ex vivo* to freshly excised tissue. EVCM allows rapid microscopic evaluation of surgical specimens without formal fixation or sectioning, preserving tissue for subsequent histopathological

processing. When combined with fluorescence contrast agents, cellular and nuclear detail is further enhanced, facilitating intraoperative margin assessment in skin cancer surgery (6).

Several studies have demonstrated excellent agreement between EVCM and conventional histopathology, with reported concordance rates exceeding 90% for basal cell carcinoma margin assessment (7). This allows rapid intraoperative decision-making, shortens diagnostic turnaround time, and preserves tissue integrity for subsequent routine histopathological examination. Consequently, EVCM has become increasingly integrated into Mohs micrographic surgery and other dermatological surgical workflows at specialized centers (6).

Optical coherence tomography and hybrid confocal–optical coherence tomography approaches

OCT is a non-invasive interferometric imaging modality that provides cross-sectional and three-dimensional visualization of tissue microstructure based on backscattered near-infrared light. OCT offers greater imaging depth than confocal microscopy—typically up to 1 to 2 mm—while maintaining axial resolutions in the range of 3 to 15 μm . This depth advantage allows visualization of the full epidermis, dermis, and superficial subcutis (8).

Compared with RCM, OCT provides lower cellular resolution but substantially greater penetration depth, making it particularly useful for evaluating lesion thickness, tumor architecture, and deeper dermal involvement (9). Clinical studies have demonstrated good diagnostic accuracy for basal cell carcinoma and actinic keratosis, supporting its role as a complementary imaging modality in routine dermatological practice (10).

Dynamic OCT extends conventional OCT by detecting motion-induced speckle variance, thereby visualizing microvascular networks and perfusion patterns. Vascular morphology captured by dynamic OCT has demonstrated relevance for tumor biology, inflammatory activity, and treatment monitoring (11).

Recent developments in LC-OCT integrate the advantages of RCM and OCT, achieving near-cellular resolution with increased penetration depth while allowing simultaneous acquisition of vertical and horizontal optical sections. LC-OCT effectively bridges the resolution gap between confocal microscopy and conventional OCT, providing three-dimensional reconstructions that closely resemble histological architecture (12).

Several studies have reported excellent agreement between LC-OCT findings and histopathology for epithelial skin tumors, particularly basal cell carcinoma and squamous cell carcinoma, with diagnostic performance approaching that of RCM while offering additional vertical imaging capability. Owing to these advantages, LC-OCT is increasingly being incorporated into specialized dermatology clinics for the evaluation of non-melanoma skin cancer and selected inflammatory skin disorders (13).

More recently, LC-OCT has also shown promising applications in the assessment of selected inherited and genodermatoses, including disorders of keratinization and epidermolysis bullosa, for which simultaneous horizontal and vertical visualization improves characterization of epidermal structural abnormalities (14).

Multiphoton tomography and nonlinear optical microscopy

Multiphoton tomography (MPT) employs nonlinear excitation processes to generate intrinsic fluorescence from endogenous chromophores such as NADH, FAD, melanin, elastin, and collagen. In ad-

dition, second harmonic generation provides label-free contrast from non-centrosymmetric structures, particularly collagen fibers. These properties allow high-resolution imaging of epidermal and dermal architecture without exogenous contrast agents (2).

The incorporation of fluorescence lifetime imaging microscopy (FLIM) adds a functional dimension by capturing metabolic signatures associated with cellular redox states. Although penetration depth remains limited to approximately 200 μm and instrumentation costs are high, multiphoton techniques offer unique insights into tissue metabolism and extracellular matrix organization, positioning them as powerful research tools with potential future clinical applications (15).

Recent studies suggest that MPT combined with FLIM may improve the differentiation between benign and malignant skin lesions by detecting metabolic alterations before morphological changes become evident. However, despite its excellent spatial resolution and label-free imaging capability, the widespread clinical implementation of MPT remains limited by high equipment costs, prolonged image acquisition times, and the need for specialized expertise. Consequently, MPT is currently predominantly used in academic research centers rather than routine dermatological practice (15).

Raman spectroscopy and molecular optical imaging

Raman spectroscopy is based on inelastic scattering of light and provides molecular-level information reflecting biochemical composition. In dermatology, Raman-based techniques allow identification of spectral fingerprints associated with malignant transformation, inflammation, or metabolic alterations (16).

Recent systematic investigations have demonstrated diagnostic sensitivities of approximately 90% and specificities exceeding 85% for melanoma detection, highlighting the ability of Raman spectroscopy to differentiate benign from malignant pigmented lesions based on their biochemical composition rather than morphological appearance (17).

Despite high specificity, clinical translation remains constrained by acquisition time, signal-to-noise limitations, and technical complexity. Ongoing developments in fiber-optic probes, surface-enhanced Raman spectroscopy, and machine learning-assisted spectral classification are expected to improve feasibility and diagnostic performance (18).

Optoacoustic and hybrid imaging modalities

Optoacoustic imaging combines optical excitation with ultrasonic detection, allowing visualization of optical absorption contrast at clinically relevant depths. Multispectral optoacoustic tomography allows differentiation of chromophores such as hemoglobin, melanin, and lipids, providing functional and vascular information. Raster-scan optoacoustic mesoscopy further enhances spatial resolution and has shown potential for non-invasive assessment of tumor-associated vascular patterns (19).

Hybrid platforms integrating optoacoustic imaging with OCT, ultrasound, or Raman spectroscopy show a growing trend toward multimodal skin imaging, capturing complementary structural, functional, and molecular information within a single system (20).

The combination of structural and functional imaging improves visualization of tumor vascularization, oxygenation, and tissue composition, thereby offering additional information beyond conventional morphological assessment. Nevertheless, op-

toacoustic imaging is currently available mainly in specialized research institutions, and its routine clinical adoption remains limited by equipment availability, standardization challenges, and the need for further large-scale validation studies (20).

Computational enhancements and artificial intelligence

The increasing complexity and volume of optical imaging data have driven the integration of computational methods, including digital staining algorithms and AI-based image analysis. Machine learning models trained on large datasets can assist in pattern recognition, lesion classification, and margin detection, potentially reducing interobserver variability and improving diagnostic consistency. Computational imaging is a critical interface between hardware innovation and clinical usability, allowing translation of advanced optical techniques into routine dermatological workflows (21).

More recently, multimodal AI systems integrating dermoscopy, RCM, OCT, and clinical images have shown encouraging results in lesion classification and diagnostic support. However, current evidence is still limited, and most available data originate from retrospective studies conducted in highly specialized centers. Therefore, although early findings suggest improved diagnostic performance, prospective multicenter validation and external testing remain essential before these systems can be routinely implemented in clinical practice (21).

Emerging optical techniques and future research directions

Despite considerable progress in established optical imaging modalities such as RCM and OCT, ongoing research continues to expand the landscape of advanced skin imaging technologies with the potential to further improve non-invasive dermatological diagnostics.

Building upon its established clinical applications discussed above, LC-OCT continues to evolve through the integration of dermoscopic guidance, automated image analysis, and AI-assisted interpretation. These developments are expected to improve diagnostic confidence, workflow efficiency, and the reproducibility of image interpretation in routine clinical practice (22).

In addition, another emerging direction is holotomography (HT), an advanced optical imaging modality capable of reconstructing three-dimensional refractive index distributions of tissue. HT generates high-resolution volumetric images with minimal phototoxicity and is being integrated with deep learning approaches for virtual staining, automated analysis, and structural phenotyping beyond superficial layers, potentially allowing more sophisticated morphological and biophysical profiling of skin *in vivo* (23).

Although early evidence suggests that these multimodal AI systems may improve performance in complex diagnostic tasks, their current evidence base remains limited, and prospective multicenter validation studies are still required before widespread clinical implementation can be recommended (24).

Recent work also underscores the synergistic potential of combining structural and functional imaging. For example, OCT paired with photoacoustic imaging facilitates simultaneous visualization of tissue microstructure and chromophore distribution, offering a more comprehensive assessment of skin physiology and pathology. This multimodal strategy may improve early disease detection, vascular characterization, and treatment monitoring (25).

Current limitations and barriers to clinical implementation

Despite remarkable technological advances, several important barriers continue to limit the widespread implementation of advanced optical imaging techniques in routine dermatological practice.

First, the availability of these technologies varies considerably. RCM, EVCM, OCT, and LC-OCT have already been incorporated into clinical practice at specialized dermatology centers and university hospitals, whereas multiphoton tomography, Raman spectroscopy, optoacoustic imaging, and holotomography remain largely research-based technologies because of their high acquisition costs and limited commercial availability (2).

Second, image acquisition and interpretation require dedicated training and substantial operator experience. Diagnostic accuracy is highly dependent on examiner expertise, particularly for confocal microscopy and LC-OCT, emphasizing the need for standardized training programs and consensus-based interpretation criteria (6).

Another important challenge is the lack of standardized reimbursement policies and unequal access to advanced imaging technologies across different healthcare systems. Although these modalities have the potential to reduce unnecessary biopsies and improve diagnostic efficiency, their initial implementation costs remain considerable and may limit accessibility, particularly in resource-constrained healthcare settings (26).

Furthermore, regulatory approval and clinical implementation differ among imaging modalities. RCM, OCT, and LC-OCT are supported by growing clinical evidence and are increasingly integrated into routine dermatological practice, whereas several emerging techniques still require large prospective multicenter studies before broader regulatory acceptance and clinical adoption can be achieved (27).

Finally, although AI has demonstrated considerable potential for automated image analysis, current algorithms have been trained predominantly using datasets generated in highly specialized centers. External validation, standardized image acquisition protocols, robust multicenter databases, and transparent regulatory frameworks will be essential before AI-assisted optical imaging can be safely integrated into everyday clinical practice (24).

Addressing these scientific, technical, economic, and regula-

tory challenges will be essential for translating emerging optical imaging technologies from specialized research environments into routine dermatological care.

A comparative overview of the principal optical imaging modalities, including their spatial resolution, penetration depth, major clinical indications, and current level of clinical implementation, is presented in Table 1.

Conclusions

Advanced optical microscopy has substantially broadened the diagnostic and monitoring capabilities of skin imaging. Whereas RCM, EVCM, OCT, and LC-OCT have become increasingly integrated into clinical dermatological practice, emerging techniques such as multiphoton tomography, Raman spectroscopy, optoacoustic imaging, and holotomography provide complementary structural, functional, and molecular information that may further improve diagnostic precision.

Among currently available technologies, RCM and LC-OCT offer the strongest evidence supporting routine clinical application, whereas multiphoton tomography, Raman spectroscopy, optoacoustic imaging, and advanced multimodal AI platforms remain predominantly research oriented because of technical complexity, cost, and limited clinical validation.

Future progress will depend not only on technological innovation but also on standardized imaging protocols, multicenter prospective validation studies, regulatory approval, cost-effectiveness analyses, and structured training programs to facilitate broader clinical implementation.

The integration of multimodal optical imaging with AI has the potential to further improve diagnostic accuracy, reduce unnecessary biopsies, and support personalized dermatological care. Continued validation in diverse patient populations and real-world clinical settings remains essential before these technologies can be widely adopted.

The continued convergence of photonic innovation, computational imaging, and clinical dermatology is expected to accelerate the translation of these modalities into routine practice, allowing more precise non-invasive diagnostics and personalized patient management.

Table 1 | Comparison of advanced optical imaging techniques in dermatology.

Imaging modality	Resolution	Penetration depth	Main clinical indications	Clinical readiness
RCM	1–3 µm	200–300 µm	Melanoma, BCC, SCC, inflammatory dermatoses	Routine clinical practice (FDA-cleared systems available)
EVCM	≈ 1 µm	Fresh excised tissue	Mohs surgery, surgical margin assessment	Routine use in specialized dermatological surgery centers
OCT	3–15 µm	1–2 mm	BCC, AK, inflammatory diseases	Routine clinical practice
D-OCT	3–15 µm	1–2 mm	Microvasculature, treatment monitoring	Specialized clinical centers
LC-OCT	≈ 1 µm lateral	≈ 500 µm	BCC, SCC, melanoma, inflammatory dermatoses	Increasing clinical adoption (CE-marked systems)
MPT	Submicron	≈ 200 µm	Skin aging, collagen imaging, research oncology	Research
Raman spectroscopy	Molecular	Surface–superficial dermis	Melanoma, NMSC	Research / early clinical translation
MSOT/RSOM	20–50 µm	2–5 mm	Tumor vascularization, inflammatory diseases	Research / limited clinical use
HT	Cellular	Variable	Experimental tissue characterization	Research only
AI-assisted multimodal imaging	—	—	Decision support, lesion classification	Emerging clinical technology

AI = artificial intelligence, AK = actinic keratosis, BCC = basal cell carcinoma, CE = Conformité Européenne, D-OCT = dynamic optical coherence tomography, EVCM = ex vivo confocal microscopy, FDA = U.S. Food and Drug Administration, HT = holotomography, LC-OCT = line-field confocal optical coherence tomography, MPT = multiphoton tomography, MSOT = multispectral optoacoustic tomography, NMSC = nonmelanoma skin cancer, OCT = optical coherence tomography, RCM = reflectance confocal microscopy, RSOM = raster-scan optoacoustic mesoscopy, SCC = squamous cell carcinoma.

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