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Optical Biopsy: A Non-Invasive Approach for Real-Time Disease Diagnosis

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Abstract

Contex: Optical biopsy, an innovative and interdisciplinary approach leveraging light-tissue interaction, holds significant potential to revolutionize medical diagnostics. This study explores the fundamental physical and biological principles underlying this approach, including light absorption, scattering, reflection, and fluorescence by endogenous and exogenous chromophores and fluorophores.

Evidence Acquisition: Key optical biopsy techniques, including fluorescence, Raman, and reflectance/absorbance spectroscopies, are detailed. Furthermore, advanced optical imaging methods such as confocal microscopy, optical coherence tomography (OCT), multi/hyperspectral imaging, and advanced optical endoscopy are systematically reviewed.

Results: The analysis highlights broad clinical applications in early cancer detection (skin, gastrointestinal, respiratory, cervical, and other organs), non-cancerous diseases (inflammation, infection, wound monitoring), and intraoperative guidance. Despite advantages like non-invasiveness and real-time diagnosis, limitations such as limited light penetration depth, complexity of data interpretation, and the inability to fully replace histopathology remain significant challenges.

Conclusion: The future outlook is highly promising, driven by the development of portable instruments, advancements in artificial intelligence (AI) algorithms, and multimodal approaches. This article concludes that optical biopsy will play a pivotal role as a powerful complementary tool in enhancing disease diagnosis and management in the future.

Keywords: Fluorescence Spectroscopy, Optical Coherence Tomography, Confocal Microscopy, Raman Spectroscopy**.**

1. Contex

Early and accurate diagnosis of diseases, particularly cancer, is considered one of the most critical pillars for successful treatment and improved clinical outcomes. Traditional diagnostic methods, such as invasive biopsy and pathology, while regarded as the "gold standard," are associated with limitations. These constraints include their invasive nature, pain, risk of infection, prolonged turnaround time for results, and the inability for real-time monitoring. These limitations underscore the pressing need to develop non-invasive or minimally invasive diagnostic approaches (1).

Optical biopsy, an emerging domain in both clinical practice and biomedical research, responds directly to this increasing demand for advanced diagnostic modalities. It exploits the principles of light–tissue interactions to enable in situ assessment of the biochemical and structural characteristics of tissue, thereby obviating the need for physical specimen excision (2). Optical biopsy not only facilitates the early detection of diseases but also holds the potential for more precise guidance of traditional biopsies, monitoring treatment response, and rapid differentiation of healthy from diseased tissue during surgery (3).

This approach is fundamentally based on the principle that diseased tissues exhibit distinct differences in their optical properties (such as absorption, scattering, reflection, and fluorescence) compared to healthy tissues. These differences may stem from alterations in cellular and tissue morphology, the concentration of endogenous chromophores and fluorophores (e.g., hemoglobin, collagen, NADPH, FAD), or metabolic changes associated with disease progression (4).

2. Evidence Acquisition

This review article is based on a comprehensive synthesis of existing literature regarding the physical principles, technological advancements, and clinical applications of optical biopsy. The evidence was gathered by analyzing peer-reviewed studies and fundamental texts in the fields of biophotonics, medical physics, and clinical oncology.

Search Strategy and Data Sources: A systematic review of scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar, was conducted to identify relevant research on light-tissue interactions such as absorption, scattering, reflection, and fluorescence. The search employed keywords related to specific modalities, including Fluorescence Spectroscopy, Raman Spectroscopy, Confocal Microscopy, and Optical Coherence Tomography (OCT).

Study Selection and Inclusion Criteria: Articles were selected based on their relevance to early cancer detection (skin, gastrointestinal, respiratory, and cervical), diagnosis of non-cancerous conditions (inflammatory diseases and infections), and intraoperative guidance. Both endogenous chromophores/fluorophores (e.g., hemoglobin, collagen, NADPH) and exogenous agents (e.g., 5-ALA) were considered as key optical biomarkers.

Analysis and Synthesis: The collected evidence was evaluated to contrast the strengths of optical biopsy—namely real-time diagnostic capability and non-invasive application—with its current limitations, including restricted penetration depth and the complexity of data interpretation. In addition, recent developments in Artificial Intelligence (AI) and multimodal imaging were integrated to delineate the prospective trajectory of this field.

3. Results

The present review article aims to provide a comprehensive and up-to-date perspective on optical biopsy. We will initially explore the physical and biological principles of light-tissue interaction that form the foundation of optical diagnostics. Subsequently, a review of the primary optical biopsy techniques, including fluorescence spectroscopy, Raman spectroscopy, reflectance spectroscopy, and advanced imaging techniques such as Confocal Microscopy and Optical Coherence Tomography (OCT), will be presented. Following this, the clinical applications of these techniques in the diagnosis of various cancers and other diseases, as well as their role in intraoperative applications, will be discussed. Finally, the current advantages and limitations of optical biopsy will be debated, and the challenges and future prospects of this field—including the development of new instrumentation, advancements in data analysis using artificial intelligence, and multimodal approaches—will be elucidated. The ultimate goal is to highlight the potential of optical biopsy as a powerful, complementary tool in disease diagnosis and management within the era of modern medicine.

3.1. Physical and Biological Principles of Optical Biopsy

Optical biopsy is fundamentally based on an in-depth understanding of light interactions with biological tissues. Tissues are optically complex media that absorb, scatter, reflect, and sometimes fluoresce light (5). Comprehending these processes and how pathological changes influence them is key to disease diagnosis using optical techniques.

Light-Tissue Interaction: Absorption, Scattering, Reflection, and Fluorescence

When light interacts with biological tissue, four main phenomena occur, each providing valuable information about the tissue's properties:

Absorption

Absorption is the process where photon energy is converted into other energy forms (such as heat) by specific molecules within the tissue, known as chromophores. Each chromophore has a characteristic absorption spectrum, meaning it effectively absorbs only particular wavelengths of light (6). The degree of light absorption at different wavelengths can provide information about the concentration of chromophores in the tissue. For instance, hemoglobin (in blood) is a strong chromophore that absorbs light intensely in the visible and near-infrared regions. Changes in the oxygenation status of hemoglobin (from oxyhemoglobin to deoxyhemoglobin) alter its absorption spectrum, which can be used to monitor tissue metabolism (7).

Scattering

Scattering is the process where light is deflected from its original path upon collision with heterogeneous structures within the tissue. These heterogeneities include cell nuclei, mitochondria, collagen, and other organelles or tissue fibers (5). Light scattering depends on the size and shape of these heterogeneities, as well as the light's wavelength. Generally, scattering is higher for shorter wavelengths (blue light) and lower for longer wavelengths (red and near-infrared light). This is the same phenomenon responsible for the blue appearance of the sky and the deeper penetration of red light in biological tissues (8). The pattern and intensity of light scattering provide information about the microscopic structure and organization of the tissue. Changes in the size and density of cell nuclei (as occur in cancerous tissues) significantly impact scattering patterns (9).

Reflection

Light reflection from biological tissue arises from the combined effects of absorption and scattering. A fraction of the incident light is reflected directly from the tissue surface as specular reflection, whereas the remaining fraction penetrates the tissue, undergoes multiple scattering and absorption events, and subsequently re-emerges as what is termed diffuse reflectance (10). The diffuse reflectance spectrum can yield information about chromophore concentration and structural features of the tissue. For example, regions with increased vascularity (a potential sign of a tumor) show a stronger hemoglobin absorption signature in their reflectance spectrum.

Fluorescence

Fluorescence is a process where a molecule (fluorophore) absorbs light energy at a specific wavelength and then emits it at a longer, lower-energy wavelength (11). Due to its high sensitivity and ability to detect specific molecules, fluorescence is highly valuable in optical biopsy. Fluorophores can be endogenous (naturally present in the tissue) or exogenous (externally added to the tissue).

Effects of Endogenous and Exogenous Chromophores and Fluorophores

Endogenous Chromophores

These molecules are naturally found in body tissues and have various biological roles. Changes in their concentration or state can be indicative of disease:

- **Hemoglobin:** Found in red blood cells, responsible for oxygen transport. Changes in concentration or oxygenation status (oxyhemoglobin and deoxyhemoglobin) are indicators of metabolic activity and angiogenesis in tumors (7).
- **Melanin:** A pigment in the skin, hair, and eyes. Changes in its concentration can be useful in diagnosing melanoma (skin cancer) (12).
- **Water:** The major component of biological tissues, exhibiting strong absorption in specific infrared spectral regions.
- **Lipids:** Fats and lipids are present in various tissues with their own specific absorption and scattering spectra.

Endogenous Fluorophores

These molecules naturally emit fluorescence in biological tissues, and their fluorescence spectra are affected by the tissue's health status:

- **Collagen and Elastin:** Key structural proteins in connective tissue. Changes in their structure or quantity (e.g., in fibrosis or cancerous tissue remodeling) can alter their fluorescence (13).
- **NAD(P)H** (Nicotinamide Adenine Dinucleotide) and FAD (Flavin Adenine Dinucleotide): Coenzymes involved in cellular metabolism (cellular respiration). Their fluorescence ratio is an indicator of the cell's metabolic state. Metabolism is altered in cancer cells, which is observable in the fluorescence of these molecules (14).
- **Porphyrins:** Hemoglobin precursors that accumulate in certain bacteria or specific tumor types (after the administration of certain drugs) and exhibit significant fluorescence (15).

Exogenous Fluorophores

These agents are administered externally to label specific tissues or enhance contrast:

- **Fluorescent Dyes/Contrast Agents:** Molecules that selectively bind to cancer cells or specific structures. Examples include fluorophores that target DNA, specific proteins, or tissues with high permeability (16).
- **Photosensitizer Precursors:** Such as 5-Aminolevulinic Acid (5-ALA), which converts in vivo into photosensitizing porphyrins that accumulate in cancerous tissues. These porphyrins not only fluoresce (for diagnosis) but can also be used to destroy cancer cells when activated by light therapy (Photodynamic Therapy) (15).

Optical Biomarkers of Disease

Pathological changes in tissue lead to measurable alterations in its optical properties, which serve as optical biomarkers of disease:

- **Structural and Morphological Changes:**
- **Cell Nucleus Size and Density:** In cancer, cell nuclei are often larger and more irregular, and cellular density increases. These changes enhance light scattering, detectable by techniques such as Light Scattering Spectroscopy or OCT (9).
- **Collagen Fibril Structure:** In some cancers, the collagen surrounding the tumor is reorganized. These changes are observable in collagen autofluorescence and scattering patterns (13).
- **Angiogenesis:** The formation of new blood vessels to supply tumors leads to increased blood

volume and changes in oxygenation, identifiable by reflectance spectroscopy (monitoring hemoglobin) (7).

- **Chemical Composition Changes:**
  - **NAD(P)H/FAD Ratio:** Changes in the cellular redox state, indicating a shift from aerobic metabolism to anaerobic glycolysis (the Warburg effect) in cancer cells. This change is monitorable via the fluorescence of these molecules (14).
  - **Collagen and Elastin Content:** In diseases like cancer, the ratio and organization of collagen and elastin change, affecting light scattering and fluorescence (13).
  - **Tissue pH Changes:** Some diseases cause alterations in tissue pH, which can influence the fluorescence of certain molecules.
- **Metabolic Changes:**
  - Cancerous tissues exhibit abnormal metabolism, which can impact the concentration of endogenous fluorophores like NAD(P)H and FAD (14). These metabolic shifts manifest not only in fluorescence but sometimes in absorption and scattering as well.

By accurately measuring and analyzing these optical changes using advanced algorithms, optical biopsy can provide valuable diagnostic information non-invasively and in real-time, thus holding the potential to revolutionize disease diagnosis and management.

3.2. Principal Techniques of Optical Biopsy

Optical biopsy encompasses a wide array of sophisticated optical techniques, each operating on specific physical principles and providing unique information about the biochemical and structural properties of tissue. These techniques can be broadly categorized into two groups: Spectroscopy and Optical Imaging.

Spectroscopy

Optical spectroscopies focus on analyzing the manner in which light is absorbed, scattered, or emitted by tissue across various wavelengths. These methods yield valuable molecular and biochemical information but typically do not clearly image the structural or morphological features of the tissue.

Fluorescence Spectroscopy

Fluorescence is a phenomenon where specific molecules (fluorophores) absorb light energy (photons) at a shorter wavelength (excitation light) and subsequently re-emit this energy as light at a longer wavelength (fluorescence) (5). The difference between the absorption and emission wavelengths is termed the Stokes Shift.

- **Tissue Autofluorescence:** Many endogenous molecules in biological tissues, such as NAD(P)H (involved in cellular metabolism), FAD (Flavin Adenine Dinucleotide, a respiratory coenzyme), collagen, and elastin (structural proteins), possess natural fluorescence properties (11). Changes in the concentration, redox state, or microscopic environment of these endogenous fluorophores can alter the tissue's autofluorescence pattern, serving as an indicator of disease (such as cancer or inflammation). For instance, cancer cells often exhibit higher glycolytic metabolism, leading to a shift in the NAD(P)H/FAD ratio and a subsequent change in the fluorescence spectrum (14).
- **Exogenous Fluorescence:** To enhance contrast or target specific molecules, exogenous fluorophores can be used. These agents can be fluorescent dyes (like fluorescein or Indocyanine Green) or fluorescent nanoparticles (such as quantum dots) that are selectively taken up by diseased cells or bind to specific biomarkers (16).

Fluorescence spectroscopy has extensive medical applications. It is utilized for the early detection of precancerous and cancerous lesions in organs like the esophagus, lung, colon, and cervix. This technique is also employed to guide invasive biopsies to more suspicious areas and to precisely delineate tumor margins during surgery (17).

Raman Spectroscopy

Raman spectroscopy is based on the Raman Scattering effect. When monochromatic light (usually a laser) illuminates molecules, the majority of the light is scattered elastically (Rayleigh Scattering); however, a very small fraction (around 0.001%) is scattered inelastically (18). This inelastic scattering means the scattered photons have a different energy (and thus wavelength) than the incident photons. This energy change results from the photon's interaction with molecular vibrations. Every molecule possesses unique vibrations, acting as a "molecular fingerprint" (19). The advantages of this method include:

- **Molecular Fingerprint:** The Raman spectrum provides highly detailed chemical information regarding the molecular composition (e.g., proteins, lipids, nucleic acids) and structure. This feature allows for the differentiation between healthy and diseased tissues based on subtle changes in their biochemical composition.
- **Label-free:** Unlike fluorescence spectroscopy, Raman spectroscopy typically does not require exogenous fluorophores, as it operates based on the tissue's endogenous molecules.
- **Low Water Sensitivity:** The Raman signal from water is relatively weak, which is a significant advantage in water-based biological samples.

Raman spectroscopy is recognized as a potent tool for the detailed chemical analysis of tissues. Its clinical applications include cancer diagnosis in various sites (such as skin, breast, and gastrointestinal tract) as well as the accurate identification of infections (20).

Reflectance / Absorbance Spectroscopy

These techniques measure the amount of light reflected or absorbed by the tissue across different wavelengths. After penetrating the tissue, light interacts with chromophores (such as hemoglobin and melanin) and is scattered (6). The collected optical spectrum contains information about the concentration and status of these chromophores, as well as the tissue's scattering properties. Reflectance/Absorbance spectroscopy is used as a powerful diagnostic tool in medicine for:

- **Measuring Hemoglobin and Oxygenation Status:** Since oxyhemoglobin and deoxyhemoglobin have distinct absorption spectra, this method can monitor tissue oxygenation and blood supply (which changes in tumors) (21).
- **Tissue Microstructure:** Light scattering patterns provide information about the size and density of cell nuclei and collagen fibers. Changes in these structures, particularly in early stages of cancer, can be detected by reflectance spectroscopy (17).

The main advantage of this spectroscopy lies in its relative simplicity, high speed, and ease of implementation in compact and endoscopic instruments for clinical diagnostics.

3.3. Optical Imaging

In contrast to spectroscopy, which provides point or volume information, optical imaging techniques allow us to visualize the structure and morphology of the tissue with high spatial resolution.

Confocal Microscopy

Confocal microscopy was developed to overcome image blurring in thick samples. It employs a pinhole in front of the detector's focal plane, which collects only light emitted from the desired focal plane within the sample, blocking light from other planes (above and below the focal point) (22). By scanning point-by-point and layer-by-layer, high-resolution optical sections can be created deep within the tissue, allowing for subsequent 3D tissue reconstruction.

Confocal microscopy is utilized as an in situ optical biopsy tool for superficial tissues such as the skin (for diagnosing diseases like melanoma), and the oral and esophageal mucosa, enabling the real-time monitoring of cellular changes at a microscopic level without the need for physical tissue sampling (23).

Optical Coherence Tomography (OCT)

OCT is a high-resolution imaging technique analogous to ultrasound, but it utilizes light (typically near-infrared) instead of sound waves (24). The method relies on measuring the time delay (and thus depth) of light reflected from different structures within the tissue. By employing an interferometer (typically Michelson), the light reflected from the tissue is compared with a reference beam, and the resulting interference pattern provides depth information (A-scan). By scanning the beam, 2D cross-sectional images (B-scan) and even 3D reconstructions of the tissue structure are obtained with micrometre-scale resolution (25). Key advantages of this method include:

- **Depth Imaging:** Capability to penetrate several millimeters deep into the tissue (depending on light wavelength and tissue type).
- **High Resolution:** Spatial resolution typically around 10-15μm, allowing visualization of cellular and subcellular structures.
- **Non-invasive and Real-time:** Requires no direct physical contact and provides real-time images.

OCT is used in diagnosing ocular diseases (such as glaucoma and macular degeneration), skin cancer detection, examining mucosal structure in the gastrointestinal tract (Barrett's esophagus, colorectal cancer), and diagnosing cardiovascular diseases (imaging of atherosclerotic plaques) (26).

Multi/Hyperspectral Imaging

These techniques combine the benefits of spectroscopy and imaging, providing not only spatial information (image) but also spectral information (absorption, fluorescence, reflectance) for every pixel in the image (27).

- **Multispectral Imaging (MSI):** Images are acquired in several discrete, predefined spectral bands (e.g., at 3 to 10 specific wavelengths).
- **Hyperspectral Imaging (HSI):** Images are captured in hundreds or even thousands of continuous spectral bands, generating a "data cube" that contains the complete spectral signature for every pixel (28).

These techniques are utilized for differentiating healthy from diseased tissues based on their unique spectral signature; delineating tumor margins; monitoring tissue oxygenation and perfusion; and achieving more accurate cancer diagnosis in image-guided surgeries (29).

Advanced Optical Endoscopy

This approach entails the integration of optical spectroscopy and imaging modalities (including fluorescence, Raman, optical coherence tomography, and confocal techniques) with conventional endoscopic platforms. This combination allows clinicians to optically examine internal body tissues in vivo with high resolution, circumventing the need for surgical access (24).

This method is applied in the screening and early diagnosis of cancer in the gastrointestinal tract (esophagus, stomach, colon), respiratory tract (trachea and bronchi), and genitourinary system. These techniques assist physicians in identifying suspicious areas, performing more targeted biopsies, or even avoiding unnecessary biopsies (30).

The development and combination of these optical techniques have provided medical professionals with powerful tools to examine biological tissues at various levels (molecular, cellular, and structural), contributing to more accurate and rapid disease diagnosis.

3.4. Clinical Applications of Optical Biopsy

With its unique capabilities to provide real-time, non-invasive information on tissue status, optical biopsy has found extensive clinical applications in disease diagnosis, particularly cancer, as well as in monitoring non-cancerous conditions. This approach enables clinicians to make more accurate diagnoses, plan treatments, and guide surgical procedures.

Cancer Diagnosis

Early cancer detection is vital, and optical biopsy has demonstrated high potential in this area. These techniques can detect precancerous and cancerous lesions in their early stages by identifying pathological changes at the cellular and molecular levels:

- **Skin:**
  - **Melanoma:** Reflectance Confocal Microscopy (RCM) is a powerful tool for diagnosing melanoma and differentiating it from benign nevi. RCM is capable of visualizing cellular and subcellular skin structures at a resolution comparable to histopathology (31). Raman Spectroscopy also aids diagnosis by identifying biochemical changes related to melanin and other molecules (32).
  - **Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC):** These two common skin cancers are also detectable with RCM. Changes in cell nuclei, vascular patterns, and collagen structure serve as important optical biomarkers for the diagnosis of these malignancies (33).
- **Gastrointestinal Tract:**
  - **Barrett's Esophagus:** This precancerous condition of the esophagus can progress to esophageal adenocarcinoma. Optical Coherence Tomography (OCT) and Confocal Endomicroscopy can diagnose dysplasia and early-stage cancer in patients with Barrett's esophagus with high resolution, thereby guiding more targeted biopsies (34).
  - **Colorectal Cancer:** Optical biopsy, including fluorescence, Raman, and OCT, is applied during colonoscopy to identify polyps, adenomas, and colorectal cancer. These techniques can help distinguish benign from malignant lesions, potentially reducing the need for the removal of all small polyps (35).
- **Respiratory Tract:**
  - **Lung Cancer:** Fluorescence spectroscopy and OCT are useful in identifying precancerous and cancerous lesions in the bronchi (large airways). Changes in tissue autofluorescence (due to metabolic shifts) and alterations in the structural pattern of the bronchial mucosa can be indicative of malignancy (36).
- **Cervix:**
  - **Precancerous Lesion Detection:** Fluorescence, Raman spectroscopy, and OCT are valuable in diagnosing Cervical Intraepithelial Neoplasia (CIN) and invasive cancer. These methods can assist colposcopy in identifying suspicious areas and reducing unnecessary biopsies (37).
- **Other Sites:**
  - **Bladder:** Fluorescence spectroscopy utilizing 5-Aminolevulinic Acid (5-ALA) as an exogenous agent aids in the diagnosis of bladder cancer and tumor margin delineation during surgery (38).
  - **Oral Cavity:** Fluorescence and Raman spectroscopy are applied in the diagnosis of precancerous and cancerous lesions in the oral cavity, particularly in hard-to-reach areas (39).
  - **Breast:** OCT and optical spectroscopy are being investigated for breast cancer diagnosis, particularly for cystic and solid lesions, and for guiding needle biopsies (40).

Diagnosis of Non-Cancerous Diseases

Optical biopsy is not limited to cancer diagnosis but is also effective in identifying and monitoring a wide range of non-cancerous conditions:

- **Inflammatory Diseases:**
  - **Inflammatory Bowel Disease (IBD):** OCT can help assess the depth and severity of inflammation in Crohn's disease and ulcerative colitis. Changes in mucosal thickness, vascular patterns, and glandular structure are signs of inflammation observable by OCT (41). Fluorescence spectroscopy can also reveal metabolic changes associated with inflammation.
  - **Arthritis:** OCT imaging is utilized to examine joint changes caused by arthritis, especially in small joints (42).
- **Infection Detection:**
  - **Raman Spectroscopy** is capable of identifying the molecular fingerprint of microorganisms (such as bacteria and fungi) and can be used for the rapid diagnosis of infections without the need for prolonged laboratory culture (43). Fluorescence spectroscopy is also applied by identifying specific bacterial metabolites.
- **Wound Monitoring and Tissue Repair:**
  - OCT and optical spectroscopy can be used to monitor the wound healing process, assess burn depth, and detect signs of infection in wounds (44). This assists clinicians in selecting the

optimal treatment method and tracking the recovery progress. Changes in oxygenation, vascularity, and collagen structure during wound healing are monitorable with these techniques.

Intraoperative Applications

One of the most crucial and promising applications of optical biopsy is its use during surgery. These applications enable surgeons to make more informed, real-time decisions:

- **Differentiation Between Healthy and Diseased Tissue:** Optical biopsy techniques (such as fluorescence, Raman spectroscopy, or OCT) can help surgeons accurately delineate tumor margins. This is particularly vital in cancers lacking clear boundaries (e.g., glioblastoma in the brain) (45). By using these techniques, the surgeon can ensure the removal of all cancerous tissue while minimizing damage to healthy tissue, leading to reduced recurrence rates and improved surgical outcomes.
- **Real-time Monitoring of Vessels and Nerves:** In certain surgeries, such as brain surgery or complex abdominal procedures, OCT can be used for real-time monitoring of blood flow in vital vessels or assessing the health of peripheral nerves (46).
- **Diagnosis of Unprocessed Tissues:** Optical systems are being developed that can provide high-resolution microscopic images of freshly excised (unprocessed) tissue samples during surgery, offering near-immediate results for the pathologist and surgeon (47).

In summary, optical biopsy is transforming the clinical approach to the diagnosis and management of many diseases, especially in the fields of oncology, gastroenterology, and dermatology, by serving as a powerful diagnostic and guidance tool.

3.5. Advantages and Limitations of Optical Biopsy

Despite its transformative potential in medical diagnostics, optical biopsy, like any technology, possesses both advantages and limitations. Understanding these is essential for its effective application and future development.

Advantages

- **Non-invasive/Minimally Invasive:** One of the most prominent benefits of optical biopsy is its non-invasive or minimally invasive nature. This feature reduces or eliminates the need for tissue incision and bleeding, which benefits the patient (reduced pain, discomfort, and infection risks) and the healthcare system (reduced burden and costs associated with invasive procedures) (5, 48).
- **Real-time and Rapid Diagnosis:** Unlike conventional biopsies, which require laboratory processing and a long waiting time for results (several days to weeks), optical biopsy can provide diagnostic information in real-time during the clinical procedure (such as endoscopy or surgery). This capability allows for faster decision-making by the physician (37, 34).
- **Reduced Need for Repeat Sampling:** By providing precise, instantaneous information, optical biopsy can guide invasive sampling, ensuring that the specimen is taken from the truly suspicious area. This reduces the need for repeat biopsies, which are painful and costly for the patient (1).
- **Potential for Continuous Monitoring:** The non-invasive nature of these techniques allows for the continuous monitoring of tissue changes over time (e.g., in surveillance of precancerous lesions or monitoring treatment response), which is not feasible with invasive methods (4).
- **Reduced Side Effects:** Due to the minimal or non-invasive nature, the risks associated with traditional biopsies, such as bleeding, infection, damage to adjacent organs, and scarring, are significantly reduced (49).
- **Cost Reduction:** While the initial investment in optical biopsy equipment can be high, the long-term benefit of reducing the need for repeated biopsies, hospitalizations, and waiting times for results can lead to a overall reduction in healthcare costs (50).

Limitations

Despite its numerous benefits, optical biopsy faces challenges and limitations that affect its widespread adoption and development:

- **Limited Penetration Depth:** Light, especially visible and ultraviolet light, cannot penetrate biological tissues to a great depth. This limited penetration (typically a few millimeters for visible light and tens of micrometers for UV light) restricts the ability of optical biopsy to diagnose deep-seated lesions or tumors located in underlying tissue layers (6). To overcome this, Near-Infrared (NIR) light is used due to its higher penetration, but even then, it is unsuitable for very deep lesions.
- **Complexity of Data Interpretation and Need for Advanced Algorithms:** Optical spectroscopy and imaging data are often complex and multidimensional, requiring sophisticated statistical analysis and Machine Learning or Deep Learning algorithms to extract meaningful diagnostic information (27). The development and validation of these algorithms are time-consuming and challenging, requiring large, high-quality datasets.

- **Incomplete Replacement of the Gold Standard (Tissue Microscopy):** Despite advances, optical biopsy cannot yet fully replace traditional pathology (histopathological examination of tissue samples under a microscope). Histopathology remains the "gold standard" for the definitive diagnosis of many diseases, especially cancer (51). Optical biopsy functions primarily as a screening, guiding, or complementary tool that can reduce the need for invasive biopsy, but in many cases, the final definitive diagnosis still requires pathological examination.
- **Need for Calibration and Normalization of Signal Variance Across Individuals:** The optical properties of tissue can vary among individuals due to factors such as skin tone, vascular status, age, and medication use (52). These variances necessitate accurate calibration and data normalization methods to ensure the accuracy and reliability of diagnostic results. Lack of proper standardization can lead to diagnostic errors.
- **High Initial Equipment Cost and Need for Expertise:** Advanced optical equipment is often expensive and requires specialized personnel for operation and data analysis, which can be a barrier to widespread adoption in certain healthcare settings (53).

Despite these limitations, continuous research in optical biopsy aims to overcome these challenges and enhance the technology's capabilities and accessibility.

3.6. The Role of Artificial Intelligence (AI) in Overcoming Data Complexity

Addressing the key challenge of Data Interpretation Complexity (Section 5.2), Artificial Intelligence (AI) and its sub-field, Machine Learning (ML), have emerged as critical enablers in optical biopsy. The role of AI extends beyond simple analysis and includes the following capabilities:

- **Extraction of Hidden Diagnostic Features:** Deep Learning algorithms, particularly Deep Neural Networks, possess an unparalleled ability to extract hidden and non-linear diagnostic features from multi-dimensional spectra and images that are difficult to discern through conventional statistical analysis or by the human eye (54). These latent features may be subtle indicators of molecular and structural changes related to disease (such as cancer).
- **Automated Spectra and Data Classification:** AI dramatically improves the accuracy and speed of automated spectra classification. For instance, in Raman and Fluorescence spectroscopy, ML algorithms can automatically differentiate spectra associated with healthy tissues from those indicative of diseased (dysplastic or malignant) tissues, which reduces diagnosis time to a fraction of a second (20, 27).
- **Advanced Optical Image Processing:** In high-resolution imaging modalities such as Optical Coherence Tomography (OCT) or Confocal Microscopy, AI is essential for advanced image processing. These applications include automated Segmentation of tissue structures (such as nuclei or vasculature) and effective Denoising, which significantly enhances image quality for both human and machine diagnostic decision-making (55).
- **Mitigation of Inter-Individual Variability:** By training on large and diverse datasets, AI models can normalize and adjust for signal variations across different individuals (stemming from skin tone, age, or vascular status), thereby strengthening the diagnostic reproducibility and reliability (56).

3.7. Challenges and Future Outlook in Optical Biopsy: A Comparative Analysis of Recent Perspectives

Despite significant progress, the translation of optical biopsy into routine clinical applications still faces technical, clinical, and regulatory challenges. This section discusses and compares these challenges by referencing recent scientific literature and examines the core recommendations driving the future roadmap.

Fundamental Challenges: Limitations and Their Impact (Discussion and Comparison)

The key challenges for optical biopsy are primarily centered in the areas of light-tissue physics, data analysis, and clinical adoption:

Challenge	Summary of Recent Literature Perspectives	Impact and Comparison with Traditional Methods (e.g., Histological Biopsy)
Limited Penetration Depth	This physical limitation is recognized as one of the main obstacles for application in internal tumors or deep organs [6]. Recent articles emphasize developing methods like Near-Infrared (NIR) optical imaging or hybrid techniques	Compared to deep imaging modalities such as MRI or CT, optical biopsy is still limited in diagnosing subsurface lesions. This primarily restricts its use to

	(such as endoscopy with longer probes) to overcome this limitation.	superficial lesions or those accessible via endoscopy.
Data Interpretation Complexity	Extracting precise diagnostic information from complex optical signals resulting from light scattering and absorption in heterogeneous biological media requires advanced physical-biological models and sophisticated analysis algorithms (5). Many recent articles emphasize the importance of Artificial Intelligence (AI) and Deep Learning to overcome this complexity, as traditional statistical models are often incapable of managing data variability (27, 20)	This challenge necessitates more expertise compared to traditional pathology assessments. However, the use of AI promises to significantly reduce interpretation time, which is a vital advantage compared to the long waiting times for traditional biopsy results.
Lack of Standardization and Regulatory Approvals	The dominant view in the literature agrees that the absence of standard protocols for data acquisition, analysis, and instrument calibration is a serious barrier (54) This deficiency makes comparing results across different studies and centers difficult, directly impacting the Reproducibility and Reliability of outcomes. The lengthy and costly regulatory approval process (FDA, CE Marking) for new medical devices slows down the rapid translation of innovations to the market.	Poor standardization reduces physician confidence and slows the transfer of technology from the lab to the clinic, whereas traditional biopsy methods have established protocols and widespread clinical acceptance.
Need for Operational Expertise	Operators and data analysts for optical biopsy require specialized knowledge in optics, biology, and data analysis, which can limit the availability of this technology in healthcare centers.	This highlights the need for specialized training and infrastructure development. In contrast, traditional histopathology is readily available but requires long-term trained specialists.
Reproducibility and Reliability	Ensuring that optical biopsy results are reproducible and reliable under various conditions and by different operators is a critical challenge (57, 58). Variations in instrument settings, environment, or even patient status can influence the outcomes.	To overcome this challenge, recent articles emphasize the importance of internal calibration tools and robust analysis algorithms, which are essential for ensuring the reliability of optical systems.

Future Outlook: Comparing Recommendations and Innovation Pathways

Despite the challenges, the recommendations put forth in the scientific literature outline a clear path for the future development of optical biopsy. Innovations in the following areas are strategically aimed at overcoming the core challenges:

Area of Innovation (Literature Recommendation)	Primary Focus in Recent Articles	Main Objective (Overcoming Which Challenge)
1. Advanced Instrumentation Development	Focus on developing smaller, lighter, and more affordable devices (55) Improving spatial and spectral resolution, increasing penetration depth, and accelerating data acquisition times through advancements in laser sources and detectors.	Limited Accessibility, Limited Penetration Depth, and High Cost.
2. AI and Data Analysis Algorithms	The most frequent recommendation is the application of Machine Learning and Deep Learning (e.g., CNNs in OCT) to analyze vast spectroscopic and imaging data volumes (27, 20).	Data Interpretation Complexity and improving Reproducibility.

	These algorithms can identify complex patterns imperceptible to the human eye, aiding in automated interpretation and decision support.	
3. Multimodal Approaches	Targeted combination of multiple optical techniques (e.g., integrating Raman spectroscopy with OCT for chemical and structural data) (13)Also, integration with other clinical modalities like ultrasound or MRI [56], which allows for accurate optical guidance to deep lesions.	Limited Penetration Depth (via guidance) and increasing Diagnostic Comprehensiveness and Accuracy.
4. Standardization and Regulation	The critical recommendation is the establishment of standardized protocols for device design, calibration, and analysis methods (30). Collaboration with regulatory bodies to create clear and efficient pathways for new device approval.	Lack of Standardization and increasing Clinical Trust (59).

The future of optical biopsy hinges on the convergence of innovations in optics and data science. Successful implementation of multimodal solutions and AI algorithms to overcome the challenges of penetration depth and data complexity will be key to the widespread adoption of this technology. Ultimately, by conducting large, multi-center, and well-controlled clinical trials [57] and implementing standardized protocols, optical biopsy will evolve into a vital, non-invasive diagnostic tool with the potential to significantly improve patient outcomes.

4. Conclusion

Optical biopsy, as a dynamic and interdisciplinary field at the intersection of optics, biology, and medicine, offers significant potential for transforming diagnostic processes. This review article has explored the fundamental physical and biological principles of this approach, the principal techniques of optical spectroscopy and imaging, and its diverse clinical applications in the diagnosis of cancer and non-cancerous diseases.

The main findings indicate that optical biopsy is capable of identifying optical biomarkers of disease through the complex interaction of light with tissues (including absorption, scattering, reflection, and fluorescence) and by leveraging changes in endogenous and exogenous chromophores and fluorophores. Spectroscopic techniques like fluorescence, Raman, and reflectance provide valuable biochemical information, while optical imaging methods such as confocal microscopy and OCT enable high-resolution visualization of tissue structure. The clinical utility of these methods in the early and accurate diagnosis of cancers of the skin, gastrointestinal, respiratory, and cervical tracts, as well as in identifying inflammatory and infectious diseases and monitoring wounds, has been highlighted.

The key advantages of optical biopsy—including its non-invasive/minimally invasive nature, real-time and rapid diagnosis capability, reduced need for repeat sampling, potential for continuous monitoring, and reduction of side effects and costs—make it a highly appealing option in modern medicine. However, limitations such as limited light penetration depth, complexity of data analysis and the need for advanced algorithms, and the incomplete replacement of histopathology as the "gold standard" for definitive diagnosis remain significant challenges.

Despite these limitations, the future prospects of optical biopsy remain highly promising. The continuous development of new instruments with higher portability, lower cost, and greater accuracy, alongside remarkable advancements in AI and deep learning-based data analysis algorithms, has the potential to significantly improve the efficacy and accessibility of this technology. Furthermore, multimodal approaches that integrate multiple optical techniques with each other or with other clinical imaging modalities promise to deliver more comprehensive and precise information. Conducting broader clinical studies to prove efficacy and utility, and standardizing protocols and regulations, are the next vital steps for the widespread adoption and entry of this technology into mainstream healthcare.

Ultimately, optical biopsy should not be viewed as a replacement for existing diagnostic methods, particularly invasive biopsy and pathology. Instead, its primary role is as a complementary and powerful tool that can optimize, accelerate, and minimize the invasiveness of the diagnostic process. By providing real-time and accurate information, this technology significantly enhances the ability of physicians to perform early diagnosis, guide therapeutic interventions more precisely, and monitor diseases more effectively, thereby greatly contributing to improved clinical outcomes and patient health.

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Conflict of interest

The authors declare no conflict of interest, financial, or otherwise.

AI Using Declaration

During the preparation of this work, the authors used ChatGPT in order to check the grammar and improve readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Author's contributions

The author (single author) was responsible for the conceptualization, literature search, data collection, analysis, and writing and editing of the manuscript.

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