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Methods and Systems of Optical Mapping in Biomedical Research

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1.1 Overview of Modern Optical Mapping Techniques

1.1.1 Diffuse, Fluorescence Molecular, Bioluminescence, Photoacoustic, Laser Coherent, and Laser Polarization-sensitive Systems

1.1.1.1 Diffuse Optical Tomography Methods and Systems

Diffuse optical tomography (DOT) represents a noninvasive biomedical imaging modality that generates tomographic reconstructions of the spatial distribution of optical properties, notably absorption and scattering coefficients, within biological tissues. The methodology involves surface illumination of the tissue and the subsequent detection of the emergent scattered radiation field [1–7]. Utilizing near-infrared (NIR) wavelengths ensures significant depth of penetration, where the probe beam is primarily attenuated by oxy- and deoxyhemoglobin. This allows for the precise quantification of hemoglobin concentrations and oxygen saturation levels, facilitating the diagnosis of breast malignancies and pathologies characterized by angiogenesis [8–12]. Furthermore, DOT is used in neuroimaging to monitor cortical activity [13] and to assess cerebral oxygenation in neonatal intensive care settings [14]. These clinical applications leverage the tracking of regional hemodynamic variations and scattering coefficients as critical biomarkers of physiological tissue status.

While DOT is a promising biomedical imaging modality, it faces significant technical limitations primarily due to the intense multiple scattering and absorption of light within biological media. Unlike X-ray computed tomography (CT), which assumes linear, ballistic photon travel, DOT must account for highly diffusive light propagation, which makes standard back-projection algorithms ineffective for image reconstruction. Consequently, DOT requires complex, nonlinear inverse modeling to accurately map optical properties against a background of scattered radiation. Image reconstruction in DOT is fundamentally comprised of two interconnected procedures. Initially, forward analytical modeling is used to characterize light propagation and generate predicted measurement data derived from defined absorption and scattering coefficients. Subsequently, an optimization framework is utilized to minimize the variance between empirical observations and these theoretical predictions. The mathematical foundation of the forward problem is established via the radiative transfer equation (RTE) or its approximations, notably the photon diffusion equation (PDE), the Spherical harmonics (PN) approximation, and Monte Carlo simulations, which facilitate the analytical derivation of surface measurements from biological specimens. The optimization phase involves the iterative refinement of initial scattering and absorption coefficients to attenuate the residuals between predicted and experimental datasets. This objective is achieved through diverse numerical optimization strategies, most notably the Newton–Raphson method, which minimizes the squared error functional, complemented by regularization techniques and the integration of a priori constraints. Such methodologies demonstrate robust efficacy across a spectrum of practical optimization challenges. Consequently, addressing the inverse problem in NIR reconstruction necessitates a synergistic application of forward modeling and optimization algorithms.

In clinical practice, this integrated framework is indispensable for neutralizing the confounding effects of diffuse background signals inherent in biological media. *Current research aimed at optimizing analytical reconstruction in NIR imaging focuses on addressing critical technical constraints, specifically the suboptimal spatial resolution and limited quantitative accuracy inherent in DOT of heterogeneous biological media*. To enhance spatial resolution, advanced methodologies such as analytical sparsity regularization – frequently derived from compressed sensing paradigms – are being implemented. Furthermore, the integration of a priori morphological data regarding internal tissue structures serves as a robust strategy for refining the precision of DOT image reconstruction. Structural constraints are typically derived from multimodal imaging frameworks, such as CT or magnetic resonance imaging (MRI). Additionally, spectral datasets offer a distinct category of a priori knowledge, facilitating enhanced quantitative precision in characterizing chromophore concentrations within multispectral DOT paradigms. A pivotal shift in the field is the emergence of reconstruction algorithms leveraged by artificial intelligence (AI) and high-performance computing. Recent progress in deep learning has fundamentally transformed both diffuse optical reconstruction [15–19] and the computational modeling of radiative transport [20]. These cutting-edge developments in biomedical optics now permit the high-fidelity visualization of deeply embedded vascular structures [21, 22]. Significant advancements in DOT are expected to soon enable a qualitative breakthrough in characterizing sub-surface chromophore distributions. Such progress underscores the potential of DOT as a cornerstone modality for early cancer detection and the assessment of cortical hemodynamics. *Despite these prospects, the inverse problem in infrared DOT remains complicated by multiple scattering events. These phenomena continue to pose significant hurdles, often limiting achievable spatial resolution and necessitating more robust algorithms to ensure quantitative accuracy*.

1.1.1.2 Fluorescence Molecular Tomography Methods and Systems

Optical molecular imaging methodologies are extensively utilized for disease diagnostics, the assessment of therapeutic efficacy, and pharmaceutical development. These techniques analyze the interaction of optical radiation with biological media to quantify the concentration of fluorescent or bioluminescent probes within target specimens. Among these, fluorescence molecular tomography (FMT) stands as a highly informative diagnostic tool, enabling the three-dimensional reconstruction of fluorescence distributions derived from surface photon flux density measurements [1, 2]. Quantitative analysis through FMT facilitates the identification of oncological biomarkers, typically employing short laser pulses for fluorescence excitation. Furthermore, time-resolved recording of fluorescence fields ensures high temporal resolution, permitting the extraction of critical preclinical data regarding the morphological and biochemical optical properties of the examined subjects. Time-resolved imaging facilitates the direct characterization of the intrinsic fluorescence lifetimes of molecular probes under pulsed laser excitation. While the fluorescence quantum yield – defined as the efficiency ratio of emitted to absorbed photons – enables the visualization of fluorophore topography and the spatiotemporal dynamics of molecular interactions, the fluorescence lifetime offers a more nuanced diagnostic parameter. Modulated by microenvironmental quenching factors such as local pH, ionic concentrations, and oxygen saturation, this lifetime is uniquely sensitive to pathological transitions. Furthermore, it demonstrates remarkable robustness against experimental fluctuations, remaining largely invariant to changes in fluorophore concentration and excitation power [3, 4]. Consequently, fluorescence lifetime imaging (FLIM) offers distinct analytical advantages over intensity-based approaches. Surface fluorescence intensity is inherently confounded by a complex interplay of fluorophore concentration, intrinsic optical properties, and underlying tissue anatomy. While conventional micro-CT provides essential anatomical frameworks for small-animal models, the recent maturation of photon-counting micro-CT (PCMCT) has revolutionized this integration. PCMCT facilitates sophisticated material decomposition and contrast enhancement, thereby refining the accuracy of structural *a priori* assessments. In preclinical oncology, PCMCT enables the precise delineation of diverse tumor morphologies in vivo, providing the necessary spatial constraints to stabilize FMT reconstructions [5]. The modeling of photon migration within biological media typically relies on the radiative transfer equation (RTE) [6] or Monte Carlo simulations [7]. The fidelity of tomographic reconstruction is fundamentally dependent on the precision of the underlying optical parameters; inaccuracies in these values can severely distort image data, compromising both the localization and quantitative characterization of molecular probes [8]. Addressing this challenge, a novel methodology leveraging PCMCT has been developed to derive high-fidelity optical parameters for heterogeneous biological tissues [9]. A salient feature of this methodology is its capacity for multienergy image acquisition, which facilitates the segmentation of various organs and histological components. By integrating the specific optical characteristics of these segmented regions, it becomes possible to analytically derive a comprehensive set of absorption and scattering coefficients [9]. Nevertheless, the high degree of infrared scattering within voluminous biological media significantly complicates the

reconstruction of fluorescence yield and lifetime via FMT [10, 11]. To address these challenges, gradient-based optimization frameworks incorporating Tikhonov regularization are frequently employed [12, 13]. However, the efficacy of such analytical approaches is often constrained by the existence of multiple local minima, which are sensitive to initial conditions, model approximations, measurement noise, and specific algorithmic architectures. To identify the global optimum within this ill-posed inverse problem, a range of sophisticated analytical regularization techniques has been proposed [11, 14]. A prominent methodology in this field is Tikhonov regularization, which leverages structural *a priori* constraints on the initial fluorescence distribution; specifically, the L1 norm and its derivatives are utilized to enforce sparsity [15, 16]. While subsequent minimization of total variation (TV) promotes piecewise smoothness [17], nonconvex Lq $(0 < q < 1)$ and logarithmic regularization schemes frequently yield superior tomographic reconstruction quality compared to standard L2-norm approaches [18–20]. Despite these advancements, the framework is fundamentally challenged by the severe ill-posedness inherent in fluorescence inverse problems and by physical measurement constraints. Furthermore, the fidelity of these reconstructions remains highly contingent upon the empirical selection of regularization parameters and iteration counts, often introducing significant uncertainty and interpretive ambiguity into the resulting datasets. The preclinical PCMCT workflow comprises a sequence of rigorous analytical stages. Initially, *in vivo* scanning of the specimen (e.g., a murine model) is conducted to acquire a series of projection datasets across multiple energy thresholds. Subsequently, these projections are utilized to reconstruct multienergy PCMCT images, followed by sophisticated image segmentation to delineate internal anatomical features [5]. This process yields an extensive repository of data regarding both anatomical topography and the biochemical composition of tissues. Such granular information facilitates the derivation of optical absorption and scattering coefficients with significantly higher precision than that achievable via conventional current-integrated micro-CT [9]. Furthermore, the application of contrast-enhanced PCMCT markedly increases diagnostic sensitivity for tumor detection [21]. The administration of exogenous contrast agents enhances the visibility of organs and neoplastic tissues [22]. Within this framework, fluorescent probes are delivered to the subject to establish optical contrast; following a specific incubation period, these biomarkers accumulate within target tissues at concentrations sufficient for oncological assessment. Consequently, the signal-to-noise ratio is optimized as the concentration of the fluorescent agent in the localized pathology significantly exceeds that of the surrounding background parenchyma. These accumulated biomarkers are subsequently excited using pulsed laser radiation for time-resolved analysis [23]. Incident laser pulses propagate through the biological volume to reach the target site, where they induce the excitation of embedded fluorophores. This ensemble of excited molecules acts as a secondary emission source, subsequently emitting fluorescence photons at a redshifted wavelength. These emitted photons undergo further scattering and absorption as they migrate through the heterogeneous tissue media. A fraction of this diffuse radiation exits the boundary and is captured by time-resolved optical detectors equipped with appropriate spectral filters. While these datasets provide the necessary input for fluorescence image reconstruction, resolving the inverse source problem remains a formidable challenge. The severe ill-posedness, driven by the high degree of multiple scattering from morphological inhomogeneities, often leads to nonunique solutions. Consequently, the presence of diffuse scattering noise and signal ambiguity frequently results in uncertain reconstructions, hindering the clinical and preclinical translation of this imaging modality.

1.1.1.3 Bioluminescence Imaging Methods and Systems

Bioluminescence imaging (BLI) methodologies utilize a diverse range of light-emitting biological systems [1]. The phenomenon of bioluminescence involves the enzymatic oxidation of a low-molecular-weight substrate, luciferin, catalyzed by the enzyme luciferase within living organisms [1].

Currently, technological advancements in BLI are progressing across three interconnected domains [2–19]:

1. The identification of novel luciferases and the chemical synthesis of innovative luciferin analogs;
2. The application of protein engineering techniques to develop genetically encoded probes incorporating luciferases;
3. The integration of these components and detection systems into comprehensive imaging platforms for monitoring molecular events *in vivo*.

The phenomenon of bioluminescence is extensively utilized across diverse bioassays and molecular imaging modalities. As detailed in the comprehensive review [20], the primary advantages of this optical imaging technique stem from a unique combination of the following factors [21–51]:

- Exceptional Sensitivity: BLI facilitates the detection of molecular events even at trace concentrations of luciferase and its cognate substrate, luciferin.

- Minimal Background Interference and Superior Signal-to-noise Ratio (SNR): Unlike fluorescence-based methods, BLI systems exhibit negligible intrinsic background luminescence in mammalian tissues. This absence of autoluminescence ensures a high SNR by effectively eliminating presignal noise.
- High Quantum Yield (QY): The luciferase–luciferin reaction typically demonstrates a superior QY compared to alternative chemiluminescent processes, resulting in high photon emission per chemical reaction.
- Extended Dynamic Range: BLI platforms generally offer a broader dynamic range of signal detection than other optical methods, such as fluorescence imaging (FLI).
- Independence from External Illumination: By eliminating the need for an external excitation source, BLI avoids phototoxic effects on biological specimens and simplifies the instrumentation by removing the requirement for complex optical filtering systems.
- Analytical Versatility: BLI serves as a highly adaptable optical readout across various bioassay formats and model organisms, ranging from prokaryotes to complex vertebrates.
- Rapid Temporal Response: BLI allows for near-instantaneous signal acquisition without extensive sample pretreatment. While fluorescent proteins require a maturation period for fluorophore oxidation, luciferase reporters become functional immediately upon expression.
- Cost-effectiveness: BLI represents a significantly more economical imaging modality compared to high-end diagnostic techniques such as PET, SPECT, or MRI.
- Deep Tissue Penetration: The technique is suitable for noninvasive imaging of internal structures at depths ranging from several millimeters to centimeters.
- Biocompatibility and Safety: Derived from natural biological systems, BLI components are inherently biocompatible and nontoxic, offering a nonradioactive alternative for longitudinal studies *in vivo*.

The limitations and technical constraints of BLI can be categorized as follows:

- Inferior Optical Intensity: Compared to FLI, BLI typically exhibits lower photon emission flux, which can limit the absolute signal strength.
- Obligatory Substrate and Cofactor Requirements: The generation of a bioluminescent signal is strictly dependent on the availability of a specific substrate (luciferin) and, in many cases, essential cofactors such as Mg^{2+} and ATP.
- Substrate-dependent Optical Properties: The spectral profile and emission intensity are inherently dictated by the chemical structure and properties of the substrate, which serves as the primary luminophore.
- Sensitivity to Microenvironmental Variables: As an enzyme-mediated process, the kinetics of the bioluminescent reaction are susceptible to fluctuations in pH, ionic strength, temperature, and buffer composition. Such dependencies can potentially compromise the precision of quantitative longitudinal measurements.
- Limited Spatial Resolution: BLI generally offers lower spatial resolution relative to FLI. Whereas fluorescence techniques provide high-contrast, sharp cellular images, BLI signals often appear more diffuse and lack fine structural detail.

A comprehensive analysis of the mechanisms, methodologies, and practical applications of bioluminescence is available in the literature cited in the following sections.

1.1.1.4 Methods and Systems of Diffuse Photoacoustic Tomography

The investigation of inhomogeneities within continuous media holds significant implications across scientific, industrial, ecological, and medical sectors, necessitating the systematic categorization of empirical data [1–3]. Visualization systems operating in nonvisible spectral regions enable the integration of human cognitive visual processing into the interpretation of complex measurement datasets. By utilizing integral information channels, researchers can evaluate radiative power, spatial dimensions, the relative positioning of objects, and temporal dynamics. Historically, the detection and identification of such subjects have been facilitated by integral and differential infrared (IR) technologies [4].

This study aims to delineate contemporary advancements in photoacoustic imaging (PAI) toward clinical translation, identify persisting technical and regulatory barriers, and explore prospective trajectories for establishing PAI as a standard clinically relevant modality.

Photoacoustics represents an emergent biomedical imaging paradigm that characterizes optical absorbers within biological tissues via acoustic detection – essentially converting optical excitation into acoustic output. This modality offers substantial clinical potential due to its high spatial resolution, significant penetration depth, and compatibility with a diverse array of endogenous and exogenous contrast agents, all while remaining inherently nonionizing.

In recent years, substantial advancements in instrumentation and methodological frameworks have expanded the clinical utility of imaging technologies. These developments have facilitated innovative applications in functional neuroimaging, oncology – specifically for breast cancer screening, sentinel lymph node metastasis detection, and urological malignancies – as well as in dermatological diagnostics, surgical navigation, and reproductive therapy [1].

Concurrently, the rapid evolution of molecular imaging has led to its integration into standardized clinical practice, enhancing the characterization and longitudinal monitoring of various pathologies. While magnetic resonance imaging (MRI) and radionuclide-based modalities have driven the discovery of novel contrast agents, their broader adoption is often constrained by high operational costs and logistical requirements, such as magnetic shielding and radiation safety protocols [4–6]. Conversely, the increasing portability of ultrasound systems, coupled with the emergence of molecular techniques like elastography and microbubble-enhanced imaging, has established ultrasonography as an essential tool for both anatomical and molecular assessment [7].

PAI, also referred to as optoacoustic imaging, represents a burgeoning technology capable of augmenting ultrasound with high-contrast optical data. This modality offers a portable, cost-effective solution for the localized imaging of vasculature and other optically active agents, functioning either as a supplementary tool or a standalone diagnostic platform [8–16].

The principal advantages of PAI encompass high potential for excellent spatial and temporal resolution, clinically relevant imaging depths, the capacity to visualize both endogenous and exogenous chromophores, and the inherent safety of non-ionizing radiation. Key endogenous chromophores include water (in both free and bound states), oxyhemoglobin, deoxyhemoglobin, melanin, and various lipids. Exogenous agents commonly comprise low-molecular-weight dyes like indocyanine green and methylene blue, nanoparticles, and reporter gene agents. Unlike conventional microbubble-based ultrasound contrast agents, PAI facilitates the imaging of small molecules that readily extravasate, target cell membrane components, or even penetrate intracellular compartments, thereby providing valuable molecular data for clinical assessment.

The underlying photoacoustic effect, characterized by the conversion of absorbed optical energy into acoustic pressure waves, was initially described by Alexander Graham Bell over a century ago. However, significant technological advancement remained stagnant until the advent of the laser, which dramatically enhanced signal generation efficiency. Subsequently, photoacoustics gained traction, initially in gas spectroscopy and later within the realm of biomedical applications.

Over the past two decades, PAI has rapidly transitioned from a theoretical concept to a sophisticated tomographic modality utilized in both small animal research and clinical diagnostic devices. The effect relies on the generation of transient pressure waves following the absorption of pulsed or modulated light. These induced acoustic waves experience significantly less scattering and absorption in biological tissue compared to visible or NIR light. The detection of these pressure transients at the tissue surface via acoustic sensors enables the precise reconstruction of the original optical absorption sites.

Owing to the inherent scalability of the PAI principle, the technology can be optimized across a broad range of depths and resolutions. Acoustic-resolution photoacoustic tomography can achieve submillimeter resolution at depths extending up to several centimeters, while optical-resolution photoacoustic microscopy offers submicron resolution, albeit limited to imaging depths of a few hundred microns. The typical depth-to-resolution ratio remains consistently around 200, and the field of view generally scales proportionally with the imaging depth and acquisition duration.

In recent years, advancements in light source technologies have facilitated the development of more portable, tunable, and cost-effective compact systems. Furthermore, innovative reconstruction algorithms now enable real-time mapping of initial pressure distributions with high fidelity and precision, even under suboptimal conditions, such as restricted fields of view, sparse detector coverage, or limited sensor bandwidth. By employing multiwavelength excitation, it is theoretically possible to quantify the concentrations of diverse chromophores simultaneously, thereby providing molecularly specific contrast [17].

Despite these advancements, quantitative accuracy and spectral resolution remain persistent challenges in PAI, primarily due to the phenomenon of spectral coloring [18]. A critical factor governing the clinical utility of PAI is its penetration depth. PAI is capable of resolving structures at depths of several centimeters, significantly outperforming purely optical modalities, such as confocal microscopy, which rely on the rejection of out-of-focus light [15].

Moreover, at comparable imaging depths, PAI offers superior spatial resolution relative to techniques based on diffuse photon migration, such as DOT. However, achieving reliable and clinically actionable *in vivo* imaging at depths exceeding 2–3 cm remains a formidable challenge [19]. This limitation is exacerbated by the fact that, unlike B-mode ultrasonography, PAI is fundamentally designed to be a quantitative or semi-quantitative modality. Consequently, while image intensity is expected to correlate positively with local optical absorption, this linear relationship becomes increasingly tenuous at greater depths due to the nonlinear attenuation of light.

Firstly, the optical fluence undergoes significant attenuation and its spatial distribution increases in complexity by at least an order of magnitude for every centimeter of tissue depth. In the absence of a robust fluence compensation scheme, a reconstructed photoacoustic image reflects the product of the local optical flux and the absorption coefficient, rather than the intrinsic optical absorption alone. Consequently, regions may appear hypo-intense due to insufficient light penetration rather than low absorption, and vice versa [20]. While optical modeling can partially mitigate these effects by predicting light attenuation [21], the accuracy of such compensation diminishes substantially as imaging depth increases. This challenge is further compounded in multispectral applications, where light fluence is nonuniformly perturbed across different wavelengths.

Secondly, many clinical implementations of PAI are constrained by a limited detection aperture, as it is often impossible to surround the volume of interest with sensors. Reconstructing the initial pressure distribution from such incomplete datasets leads to artifacts and errors that increase exponentially as the viewing angle narrows – a situation that worsens with increasing depth relative to the aperture size [22]. Furthermore, even under full-view conditions, the SNR deteriorates significantly at greater depths due to both optical and acoustic attenuation and dispersion. Consequently, the fidelity of the reconstruction regarding local fluence and absorption is compromised as imaging depth increases [23].

Thirdly, the acoustic properties of biological media – specifically the speed of sound and the Grüneisen parameter, which dictates the efficiency of thermoelastic conversion – are heterogeneous across various tissue types, exhibiting variations of several tens of percent. Such variability introduces further complexities in ensuring the quantitative accuracy of the imaging modality.

Specifically, the acoustic properties of tissue are temperature-dependent, which introduces variability as thermal profiles shift with increasing depth [24]. This temperature sensitivity may account for the notable discrepancy between the penetration depths achieved in phantoms (exceeding 6 cm) and those observed in *in vivo* clinical settings (typically restricted to 2–3 cm).

Another substantial barrier to the clinical adoption of PAI pertains to image contrast. Biological tissues comprise a complex milieu of endogenous chromophores, such as water, various hemoglobin states, and pigments, possessing overlapping absorption spectra [25]. While multispectral PAI can theoretically differentiate these components to monitor pathological shifts (e.g., lipid deposition in atherosclerosis or neoangiogenesis in malignancies), the diagnostic utility of visualizing vascular architecture and oxygenation alone remains limited for many conditions [26]. Such vascular alterations are often insufficiently pronounced for early-stage detection or lack the specificity required to differentiate between benign and malignant lesions. Furthermore, the inherent difficulties in achieving precise quantification at depth mean that the diagnostic accuracy of PAI-derived parameters remains a subject of ongoing investigation [27].

Technical constraints also include the requirement for "wet" acoustic coupling between the transducer and the tissue. Although alternative "dry" or noncontact coupling methods have been proposed [28], they typically suffer from reduced sensitivity and increased system complexity, hindering their practical implementation.

Many of these limitations could be mitigated through the use of targeted exogenous molecular agents [29]. These contrast agents offer superior absorption and distinct spectral signatures, facilitating their separation from background signals and providing critical data on biomarker expression or enzymatic activity [30]. However, despite extensive preclinical validation, such agents have yet to receive FDA approval. Currently, the clinical application of PAI is further hampered by depth constraints, quantification uncertainties, and a lack of standardized clinical systems. The future integration of PAI into routine medical practice likely depends on the development of robust hardware and the demonstration of clinical benefit that outweighs the current operational complexities [32–41].

1.1.1.5 Methods and Systems for Polarization Mapping and Biomedical Imaging

This section provides a concise overview of methodologies and systems for laser polarization mapping and biomedical imaging. A more exhaustive treatment of this subject is available in the authoritative review [18], which offers the most comprehensive synthesis of the field and its extensive literature to date [1–286].

The integration of polarimetric modalities with established optical techniques facilitates the acquisition of unique vector-based data, enhancing existing technologies such as microscopy and endoscopy [16, 24, 32, 33, 92, 180]. A significant methodological advantage of biomedical polarimetry is its capacity for label-free imaging, precluding the need for exogenous dyes [16, 22, 24]. Furthermore, polarimetry serves as a powerful tool for characterizing the vector properties of fluorescent markers, as the dipole orientation of a fluorophore is intrinsically encoded within the polarization state of the emitted radiation [154, 155]. Research has demonstrated that the polarization azimuth (PA) and the degree of linear polarization (SOP) are critical parameters for advancing biomedical super-resolution microscopy [153, 181, 182].

To date, a diverse array of biomedical polarimetry methods has been developed and successfully implemented, including:

- Polarization wide-field and confocal microscopy [16, 24, 183, 197];
- Spatial-frequency domain imaging in polarized light [184];
- Polarization-based endoscopy [185–190];
- Spectral polarimetry of scattered light and polarization fluorescence spectroscopy [18, 82, 191–196];
- Polarization Raman spectroscopy [198, 199];
- Polarization-sensitive optical coherence tomography (PS-OCT) [200–218];
- Polarization-resolved nonlinear microscopy and polarization speckle imaging [213, 227].

While certain polarimetric modalities have been extended to three-dimensional (3D) imaging through signal integration or volumetric segmentation, the majority of contemporary methods are primarily focused on two-dimensional (2D) analysis. The theoretical framework for modeling the interaction between polarized photons and biological media is predominantly based on Monte Carlo (MC) simulations. Within these computational models, the microstructural architecture of biological tissues is typically represented by fundamental geometric elements, such as spherical or cylindrical scatterers, birefringent domains, and multilayered geometries. MC simulations have proven highly effective in reproducing the essential polarimetric characteristics observed in biomedical specimens.

As previously established, biomedical polarimetry and imaging are categorized into label-free and label-based methodologies:

- Label-based Polarimetry: This approach is widely utilized in biomedical microscopy to extract vector-based information from dipole emitters, which is encoded in the polarization state of the detected light. Polarimetric detection of dipole orientation and fluorescence intensity is a cornerstone of fluorescence polarization microscopy (FPM). This technique has been instrumental in characterizing cytoskeletal components, such as actin, myosin, kinesin, microtubules, and septin, and in investigating dynamic molecular processes, including ATP/ADP binding kinetics and the heterogeneous behavior of subcellular lipid membranes.

The second avenue of research, label-free biomedical polarimetry, has demonstrated significant clinical utility in the detection and characterization of various malignancies [22–24]. Extensive studies have confirmed its efficacy in identifying pathologies of the skin [242], cervix [243–246], colon [166, 247–250], liver [163, 251], breast, and gastrointestinal tract [93–95, 252]. Bioinformatic processing of polarimetric datasets permits the quantitative evaluation of fibrosis across different oncological stages [94, 163]. Beyond assessing the degree of fibrotic progression, polarization data facilitates the pathological diagnosis of morphological distributions within fibrotic regions [164, 165, 171]. Furthermore, recent applications have extended polarimetric analysis to the detection of nonmalignant conditions, including Alzheimer's disease and bladder outlet obstruction [24, 253, 254].

Polarization-based techniques enhance the contrast of superficial tissue imaging by effectively filtering out multiply scattered photons originating from deeper structural layers [20–24]. Given that more than 85% of cancers develop within the superficial epithelium, these methods hold substantial promise for early cancer screening and diagnostic identification, particularly within the context of minimally invasive surgery (MIS) [24, 255].

A salient feature of polarimetric measurement is the capacity to characterize the optical anisotropy inherent in biological tissues of varying physiological and morphological states. For instance, structural anisotropy and linear birefringence are prominent in collagen-rich tissues, such as tendons, skin, and the bladder [156, 256–259], as well as in skeletal and myocardial muscle fibers and elastin [20–23, 164, 260–262]. The magnitude of this optical anisotropy is intrinsically linked to the orientation of the fast axes of linear birefringence [16, 165, 171]. Consequently, the extraction of such anisotropic vector data via polarimetry provides a robust framework for the differentiation and identification of diverse fibrous architectures within complex biological media [164, 165, 167]. To date, however, the majority of *ex vivo* investigations have focused primarily on fundamental research applications [24, 152, 171].

Typical polarimetric configurations in backscattering mode encompass a variety of modalities designed for *in vivo* clinical diagnostics, including polarization endoscopy [24], reflectance Mueller matrix (MM) microscopy [16, 266], MM colposcopy [180, 267], wide-field handheld polarimetry [16], and PS-OCT [268]. As a promising label-free diagnostic instrument, polarization endoscopic imaging has been utilized in murine abdominal studies, effectively distinguishing the small intestine, stomach, liver, and adipose tissues based on their unique polarimetric signatures [92].

Recent advancements have further introduced several specialized MM endoscopic systems [188–190, 256], some of which have been expanded into the multispectral domain using discrete fixed wavelengths [92]. Furthermore, volumetric analysis

of complex biological structures, such as human pulmonary malignancies [159] and cutaneous tissues [269], underscores the significant potential of these techniques for future clinical diagnostic integration [16, 22–24].

Notwithstanding the substantial progress achieved in polarization mapping and biomedical imaging, several persistent limitations and technical challenges must be addressed to facilitate further maturation and clinical translation of the field.

The following are key limitations hindering the advancement and clinical implementation of laser polarimetry [287]:

1. Lack of a Unified Phenomenological Model: There is currently no standardized analytical model capable of comprehensively describing the transformation of laser radiation polarization parameters as light propagates through biological tissues characterized by diverse morphological structures and physiological states.
2. Absence of Standardized Analytical Frameworks: A unified system of analytical relationships is needed to link the measured distributions of scattered radiation polarization parameters directly to the specific optical characteristics, such as linear and circular birefringence, and dichroism, of the polycrystalline architecture found in biological tissues.
3. Paucity of Data on Biological Fluid Films: There is a notable absence of systematic data regarding the applicability and capabilities of laser polarimetry for analyzing the optical parameters and structures of human biological fluid films, an extremely critical class of biological objects.
4. Influence of Multiple Scattering Artifacts: Experimental results are frequently compromised by the significant informational distortions introduced by multiply scattered, depolarized background radiation, which obscures the primary signal.
5. Deficiency in Quantitative Evaluation Systems: A unified system for the multifunctional, quantitative evaluation of the statistical, correlational, scale-selective, and fractal properties within coordinate distributions or maps of polarization parameters is currently lacking.

1.1.1.6 Optical Coherence Tomography Methods and Systems

Optical coherence tomography (OCT) has established itself as a sophisticated modality for noninvasive optical imaging, offering substantial potential for biological investigation and *in situ* medical biopsy, particularly within 3D *in vivo* contexts. Typically utilizing broadband NIR laser sources, OCT achieves significantly greater penetration depths, higher resolution, and superior SNR compared to conventional microscopy. This spectral advantage minimizes blurring associated with multiple scattering, thereby facilitating the high-fidelity visualization of internal morphological architectures beyond superficial layers. The integration of advanced probing sources, such as multiplexed superluminescent diodes and ultrabroadband supercontinuum lasers, has further expanded clinical utility by enabling submicron resolution and high-speed data acquisition.

Initially conceptualized by David Huang in 1991, OCT represents a cost-effective, high-resolution imaging paradigm that reconstructs transverse cross-sections of biological tissues *in situ* by measuring backscattered light signals [1]. Over the subsequent three decades, the transition to ultra-high-resolution OCT, powered by broadband femtosecond laser sources, has realized axial and transverse resolutions of 1 and 3 μm, respectively, facilitating subcellular imaging [2]. Furthermore, the implementation of Fourier-domain OCT (FD-OCT) has catalyzed a qualitative advancement in acquisition speeds, solidifying the role of this 3D biomedical imaging technology in contemporary clinical and research settings.

Owing to advancements in acquisition speed and image fidelity, OCT has been extensively adopted across engineering and biomedical disciplines. In forensic science, for instance, OCT facilitates the reconstruction and analysis of latent evidence, including subsurface fingerprints [3], layered handwriting [4], and multilayered automotive coatings [5–7]. Furthermore, the increasing accessibility of high-power mid-infrared sources has expanded the utility of mid-infrared OCT in nondestructive testing, ceramic manufacturing, and the diagnostic conservation of historical artwork [8, 9]. Despite these diverse industrial applications, the primary impact of OCT remains in the biomedical sector, where it has become an indispensable standard for ophthalmic examinations.

The evolution of these imaging technologies has significantly enhanced the visualization and diagnosis of various pathologies [10–13]. The capacity to resolve finer morphological details within lesions supports earlier clinical intervention and improved therapeutic outcomes. Notably, OCT offers distinct advantages in spatial resolution and cost-effectiveness compared to established modalities such as MRI and CT.

Beyond structural visualization, various functional extensions of OCT have been developed to assess physiological parameters [14–21]. Doppler OCT, for example, enables the visualization of microvasculature and the estimation of hemodynamic velocity by differentiating between signals originating from flowing erythrocytes and stationary anatomical structures [22–25]. Additionally, specialized techniques such as polarization-sensitive OCT (PS-OCT) and phase-sensitive OCT provide enhanced structural contrast. PS-OCT, in particular, leverages the polarization state and tissue birefringence to resolve

intricate structural data with high spatial resolution [26–30]. Consequently, modern commercial OCT systems, characterized by micron-scale resolution and millimeter-scale penetration depth, have become integral to fields such as ophthalmology, endoscopy, cardiology, and otology [14, 31–33].

A primary challenge associated with the aforementioned OCT methodologies is the prevalence of significant speckle noise within the laser-scanned cross-sectional images of the internal architecture of diffusely scattering biological tissues.

Current OCT techniques predominantly rely on diverse modeling approaches to characterize the structural and optical properties of reconstructed tomograms, including intensity distributions, polarization maps, and Mueller or Jones matrix representations. However, the quantitative analysis of these datasets remains neither systematically organized nor standardized across the field [34–58].

Furthermore, there is a notable deficiency in OCT-based data regarding the polycrystalline structure of dehydrated biological fluid films. These specimens represent a critical diagnostic resource that is arguably as informative as tissue biopsies while being significantly more accessible for clinical evaluation.

1.1.2 Unsolved Problems and Proposed Solutions via 3D Jones–Mueller Digital Holographic Mapping

This section provides a systematic overview of the critical unresolved challenges (italicized in Section 1.1) inherent to the current landscape of optical biomedical imaging modalities:

1. Diffuse Optical Tomography
   - Propagation Constraints: Significant technical barriers persist regarding multiple scattering and photon absorption during light propagation through biological media.
   - Reconstruction Inefficiency: Unlike X-ray CT, the application of back-projection for infrared image reconstruction is ineffective due to the high background of scattered radiation.
   - Analytical Limitations: DOT exhibits suboptimal spatial resolution and a lack of precision in the quantitative evaluation of optically inhomogeneous diffuse tissues.
   - Parametric Deficiencies: These systems are currently unable to extract data regarding the optical anisotropy of biological structures.
2. Fluorescence Molecular Tomography
   - Computational Complexity: Extensive multiple scattering of infrared radiation within biological volumes complicates the accurate reconstruction of fluorescence yield.
   - Algorithmic Instability: Analytical optimization is constrained by the existence of multiple local solutions, which are highly sensitive to initial conditions, model approximations, stochastic noise, and algorithmic architecture.
   - Methodological Ambiguity: The modality is characterized by ill-defined measurement limitations and a high degree of uncertainty in the derived informational content.
   - Regularization Sensitivity: Reconstruction fidelity is heavily dependent on the subjective selection of regularization parameters and iteration counts.
   - Structural Information Gap: FMT lacks the capability to characterize the structural anisotropy and optical activity of biological specimens.
3. Bioluminescence Imaging
   - Signal Flux Constraints: BLI typically exhibits lower optical intensity relative to fluorescence-based modalities.
   - Biochemical Dependencies: The technique requires specific exogenous substrates, with the resulting optical signatures (spectral profile and intensity) being strictly dictated by the chemical properties of these substrates and the localized reaction conditions.
   - Resolution and Fidelity Issues: BLI is characterized by low spatial resolution, often yielding diffuse and low-contrast cellular images.
   - Vectorial Limitations: Similar to the aforementioned modalities, BLI cannot currently retrieve information pertaining to structural anisotropy or the optical activity of the target tissue.
4. Diffuse Photoacoustic Tomography/PAI
   - Spectral Interference: Quantitative accuracy and spectral unmixing remain compromised by the persistent challenge of "spectral coloring," which distorts the spectral signatures of deep-seated chromophores.
   - Clinical Depth Limitations: There is significant ambiguity regarding the clinical efficacy of this modality at imaging depths exceeding 2–3 cm *in vivo*.

- Fluence Dependency: Photoacoustic signals do not directly represent intrinsic optical absorption; rather, they reflect the product of local optical fluence and the absorption coefficient, leading to potential misinterpretation of hypointense regions.
- Detection Aperture Constraints: Clinical implementation is frequently hindered by "limited-view" architectures, where the inability to surround the target volume with sensors results in reconstruction artifacts.
- Heterogeneity of Acoustic Parameters: Essential parameters, such as the speed of sound and the Grüneisen coefficient, are nonuniform across different tissue types and exhibit significant fluctuations (up to several tens of percent).
- Polarimetric Information Gap: The modality is currently unable to preserve or retrieve structural polarization data.

5. Laser Polarization Mapping and Biomedical Imaging
   - Theoretical Deficiencies: There is an absence of a unified phenomenological model to analytically describe the transformation of polarization parameters as laser radiation propagates through diverse morphological and physiological tissue states.
   - Analytical Disconnection: A standardized system of relationships between measured polarization distributions and the specific optical properties (linear/circular birefringence and dichroism) of polycrystalline biological architectures has yet to be established.
   - Paucity of Biofluid Data: Systematic empirical data regarding the polarimetric characterization of human biological fluid films-a critically informative class of specimens-is virtually nonexistent.
6. Methods and Systems for Polarization Mapping and Biomedical Imaging
   - Multiple Scattering Interference: The integrity of experimental results is frequently undermined by the distorting effects of a multiply scattered, depolarized background.
   - Quantitative Standardization: There is a lack of a unified framework for the multifunctional quantitative assessment of the statistical, correlational, scale-selective, and fractal dimensions of polarization coordinate maps.
7. OCT Methods and Systems
   - Speckle Noise Interference: A primary technical challenge is the high level of speckle background noise present in layered laser images of the internal architecture of diffusely scattering biological tissues.
   - Modeling Variability: Current approaches rely on diverse and often disparate model considerations for the formation of optical characteristics in reconstructed images, including intensity maps, polarization maps, and Mueller/Jones matrix representations.
   - Lack of Standardization: The quantitative analysis of OCT data is currently neither systematized nor standardized across research and clinical practice.
   - Data Deficit in Biofluids: There is a notable absence of systematic OCT data pertaining to the polycrystalline structure of dehydrated biological fluid films.

Comparative Analysis and Future Directions

A comparative analysis of existing unresolved issues across the spectrum of biomedical optical imaging modalities highlights promising avenues for qualitative enhancement and future development:

- Development of Integrated Systems: The creation of a new generation of "interdisciplinary" hardware and software information and diagnostic systems is proposed, synthesizing principles from polarimetry, interferometry, and tomography.
- Background Elimination and Sensitivity Enhancement: New diagnostic systems aim to mitigate the influence of depolarized background noise through polarization-interference recording of biospeckles. This is followed by digital holographic reconstruction and phase scanning to isolate the single-scattered component within the object field.
- Standardized Layer-by-layer Reconstruction: The objective is to develop unified principles for the layer-by-layer phase reconstruction of maps detailing the linear/circular birefringence and dichroism of biological tissues and fluid samples, utilizing the rigorous Mueller–Jones matrix formalism.
- Objective Multiparametric Analysis: Future work will focus on the objective, multifunctional quantitative evaluation of medical Mueller–Jones matrix images and optical anisotropy maps, integrating statistical, correlational, fractal, singular, and other advanced analytical approaches.
- Biomarker Development: The final direction involves the development and clinical validation of a complex suite of digital markers for the early diagnosis and differentiation of pathological and necrotic changes in the polycrystalline architecture of biological specimens.

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Section 1.1.1.6

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