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UNIVERSITY OF CALIFORNIA,
IRVINE

Quantitative Optical Coherence Elastography for Multiscale Biomechanical Characterization of
Ocular and Neural Tissues

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biomedical Engineering

by

Fengyi Zhang

Dissertation Committee:
Professor Zhongping Chen, Chair
Professor Andrew Browne
Professor Liangzhong Xiang

2025

DEDICATION

To

my parents and friends

in recognition of their worth

an apology

“A feeling bears on itself the scars of its birth; it recollects as a
subjective
emotion its struggle for existence;
it retains the impress of what might have been, but is not.”

(Alfred North Whitehead
Process and Reality)

and hope

“If the fool would persist in his folly he would become wise.”

William Blake
Proverbs of Hell

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ABSTRACT OF THE DISSERTATION

**Quantitative Optical Coherence Elastography for Multiscale Biomechanical Characterization of
Ocular and Neural Tissues**

by

Fengyi Zhang

Doctor of Philosophy in Biomedical Engineering

University of California, Irvine, 2025

Professor Zhongping Chen, Chair

Understanding the mechanical properties of soft biological tissues is fundamental to interpreting their physiological function and pathological remodeling. In the eye and brain—two of the most mechanically sensitive organs in the human body—local stiffness governs essential processes including optical focusing, intraocular pressure (IOP) homeostasis, axonal transport, extracellular matrix (ECM) regulation, and neurovascular coupling. Yet, despite the centrality of biomechanics, quantitative measurement of soft-tissue elasticity at micrometer scale remains an unresolved challenge. Conventional elastography techniques such as magnetic resonance or ultrasound elastography lack the spatial resolution required to resolve thin ocular structures, while mechanical testing is restricted to excised samples and cannot characterize in situ behavior. Optical coherence elastography (OCE), an extension of optical coherence tomography (OCT), has emerged as a promising solution by enabling nanometer-scale displacement detection and quantitative elasticity mapping in living tissues. However, full realization of OCE’s potential requires advances in system engineering, signal processing, excitation control, and biomechanical modeling.

This dissertation develops a unified OCE framework that integrates phase-sensitive detection, multimodal excitation strategies, and quantitative inversion algorithms to enable high-precision mechanical imaging across ocular and neural systems. At the systems level, three

complementary OCE platforms were established. First, a high-speed phase-resolved spectral-domain OCT system was engineered with a broadband 890-nm light source, optimized interferometer design, and nanometer-level phase stability. This system was coupled with a mechanical shaker that generated broadband elastic waves capable of propagating through the entire globe, enabling quantitative in vivo assessment of optic nerve head (ONH) stiffness under controlled IOP modulation. Second, a confocal acoustic-radiation-force OCE system was constructed to image equatorial scleral anisotropy. The system used a ring-shaped 6.8-MHz ultrasound transducer integrated with the OCT detection path, allowing spatially localized generation of guided waves and direction-dependent elasticity mapping. Third, a wide-field compressional OCE platform was developed for ex vivo mouse-brain imaging. This system incorporated a calibrated silicone reference layer for stress estimation, synchronized preload/actuation control, and optional optical clearing for improved depth penetration.

The first biological application focused on the ONH, a biomechanically vulnerable site central to glaucoma. Using the shaker-based platform, both ex vivo and in vivo experiments demonstrated that shear-wave velocity and reconstructed Young's modulus increased monotonically with IOP, confirming nonlinear stiffening under load. This work provided the first demonstration of in vivo ONH OCE in rabbits and established direct measurement of ONH biomechanics under physiological pressure regulation.

The second application investigated multiscale anisotropy in the equatorial sclera, a region essential to ocular shape regulation and load transmission. Direction-dependent wave propagation was quantified across nasal, temporal, superior, and inferior quadrants at multiple IOP levels. Both OCE and complementary ultrasonic elastography revealed consistently higher wave speeds in the circumferential (equatorial) direction than in the meridional direction.

Structural correlation using polarized light microscopy, scanning electron microscopy, and transmission electron microscopy revealed that mechanical anisotropy diverged from planar fiber orientation alone and instead reflected a multiscale organization of interwoven collagen lamellae.

The third application extended OCE to neural tissue, introducing a wide-field compressional system capable of mapping elasticity across entire mouse-brain slices. Strain and elasticity maps revealed clear contrast between gray and white matter, laminar structure in the hippocampus and cerebellum, and strong correspondence with histological microarchitecture. Optical clearing improved depth visibility without altering intrinsic stiffness distribution, demonstrating compatibility between OCE and tissue-transparency methods.

Collectively, this dissertation advances OCE as a quantitative, multi-regime elastography platform capable of probing biomechanics across ocular and neural tissues. The engineering innovations and biological findings presented here establish the foundation for future in vivo clinical translation, MHz-rate OCE, and integration of elastography with machine-learning-enabled analysis for real-time mechanical assessment.

INTRODUCTION

Mechanical forces play a central role in shaping the structure, function, and pathological remodeling of biological tissues[1-3]. Across molecular, cellular, and organ-level scales, tissue stiffness governs how structures deform under load, how fluids flow within confined compartments, and how biochemical signaling pathways respond to physical cues[4-6]. These principles are especially prominent in the eye and central nervous system, where delicate microarchitecture and extreme mechanical sensitivity render even subtle changes in elasticity clinically significant[7-9]. In the eye, the cornea and sclera maintain the globe's shape and transmit intraocular pressure (IOP)[10, 11], the crystalline lens modulates optical power through controlled deformation[12, 13], and the optic nerve head (ONH) acts as a biomechanical gateway where pressure-related stresses influence retinal ganglion cell injury in glaucoma[14, 15]. In the brain, the mechanical environment regulates neuronal organization, axonal integrity, and extracellular matrix composition[16, 17], while changes in stiffness accompany demyelination, inflammation, trauma, and neurodegeneration[18-20]. Across these tissues, mechanical alterations may precede detectable structural changes, making biomechanics an important but underexplored dimension of disease characterization[21, 22].

Despite its biological relevance, quantitative measurement of soft-tissue elasticity remains challenging. Traditional mechanical testing tools provide direct force–displacement measurements but require excised tissue and cannot capture in situ geometry or physiological loading[6, 23]. Clinical elastography techniques, such as ultrasound elastography and magnetic resonance elastography (MRE), enable noninvasive assessment but offer millimeter-scale spatial resolution and are limited in optical tissues with thin layered structures[24-26]. The spatial precision required to resolve laminar microarchitecture, collagen fiber organization, and local tissue heterogeneity far exceeds the capabilities of these modalities. In particular, the ocular system—with its transparent anterior tissues, highly scattering posterior shell, and submillimeter structures—demands an imaging modality capable of simultaneously achieving micrometer-scale resolution, nanometer-scale displacement sensitivity, and compatibility with both transparent and scattering environments.

Optical coherence tomography (OCT) meets these requirements through depth-resolved interferometric imaging that offers micrometer-scale axial resolution and exceptional phase sensitivity[27-29]. By detecting minute phase shifts between sequential A-scans, OCT can

measure nanometer-scale changes in optical path length[30-33]. When these phase changes arise from externally induced mechanical deformation, the technique becomes optical coherence elastography (OCE). OCE enables quantitative elasticity mapping by reconstructing displacement fields or tracking elastic-wave propagation within tissue[34]. Unlike conventional elastography, OCE requires no physical coupling medium, imposes negligible perturbation on the sample, and is naturally compatible with transparent and weakly scattering structures[35, 36]. These advantages position OCE as an ideal platform for high-resolution biomechanical imaging in ocular and neural tissues.

Realizing truly quantitative OCE, however, requires advancements in system engineering, mechanical excitation, signal processing, and biomechanical modeling. High-fidelity elastography depends critically on phase stability, optical bandwidth, and synchronization between mechanical loading and OCT acquisition[37]. Mechanical excitation must be well controlled and capable of generating either quasi-static compression or coherent elastic waves suited to the specific tissue architecture. Signal processing must isolate elastographically relevant motion from physiological noise, blood flow, and system jitter[33, 38]. Finally, conversion of displacement fields into elastic moduli demands appropriate constitutive modeling, whether through stress–strain relationships in compressional OCE or wave-speed–based inversion in dynamic OCE[39, 40]. Addressing these challenges is essential to translating OCE from a qualitative imaging tool into a quantitative biomechanical metrology platform.

The eye provides a compelling clinical motivation for these developments. Ocular tissues exhibit strong mechanical specialization: the cornea demonstrates nonlinear anisotropy governed by lamellar collagen organization[41, 42]; the sclera combines circumferential and meridional fiber families to resist IOP-induced stress[43, 44]; the crystalline lens stiffens markedly with age, contributing to presbyopia[13, 45]; and the ONH, as the site where retinal ganglion cell axons exit the eye, is uniquely susceptible to mechanical insult from elevated IOP[14, 15, 46, 47]. Changes in stiffness within these structures often precede gross morphological deformation, suggesting that biomechanical contrast could serve as an early indicator of disease[48, 49]. However, the small dimensions, curved geometry, and heterogeneous optical properties of ocular tissues have limited the reliability of conventional elastography methods. OCE overcomes these limitations by enabling noncontact, micrometer-scale biomechanical assessment across both anterior and posterior segments, as demonstrated throughout this dissertation.

Neural tissue presents similarly compelling challenges and opportunities. Brain tissue is among the softest in the body, with shear moduli typically below 2 kPa[50, 51], and displays pronounced viscoelastic and regionally heterogeneous behavior[52, 53]. Stiffness variations correlate with axon density, myelination, extracellular matrix composition, and vascular organization[18, 54]. Importantly, mechanical alterations accompany major neuropathologies including Alzheimer’s disease, multiple sclerosis, and traumatic brain injury[19, 20, 55]. Traditional histology provides exquisite structural contrast but offers no direct information about intrinsic biomechanics, while MRE provides bulk mechanical information but lacks the spatial resolution to distinguish fine anatomical layers[56, 57]. OCE bridges this gap by offering high-resolution, label-free mechanical mapping that can reveal microstructural differences between cortical layers, hippocampal regions, white-matter tracts, and cerebellar laminae. These capabilities open new avenues for studying neural microstructure and the mechanical underpinnings of disease[58].

This dissertation advances OCE as a unified, quantitative, and multiscale imaging framework capable of assessing biomechanics across ocular and neural tissues. At the core of this work is the development of three complementary OCE platforms: a high-speed phase-resolved spectral-domain OCT system integrated with a mechanical shaker for in vivo ONH elastography; a confocal acoustic radiation force excitation system paired with OCE to resolve anisotropy in the equatorial sclera; and a wide-field compressional OCE system with reference-layer calibration and optional optical clearing for ex vivo mouse-brain imaging. These platforms enable interrogation of biomechanics across a broad range of spatial scales, mechanical regimes, and tissue types. In the ONH, OCE quantifies nonlinear, pressure-dependent stiffening both ex vivo and in vivo; in the sclera, OCE reveals circumferential stiffness reinforcement and multiscale anisotropy not predictable from planar fiber orientation alone; in the brain, OCE delineates gray- and white-matter regions, hippocampal layering, and cerebellar microstructure with strong correspondence to histology.

The broader significance of this work lies in establishing OCE as a precise, high-resolution, and biologically meaningful tool for biomechanical characterization. The methods and findings presented here demonstrate the feasibility of in vivo ocular elastography, the ability to detect multiscale structural–mechanical relationships, and the value of combining OCE with tissue clearing and structural imaging. By advancing both the engineering foundation and

biological application of OCE, this dissertation lays the groundwork for future developments including MHz-rate OCE and real-time ophthalmic elastography.

The chapters that follow are organized as follows.

Chapter 1 provides background on tissue biomechanics, OCT principles, and the motivation for high-resolution biomechanical imaging in ocular and neural systems.

Chapter 2 develops the theoretical and system-level framework of OCE, including OCT physics, phase-resolved displacement extraction, dynamic and compressional elastography principles, and noise limitations.

Chapter 3 presents the development and in vivo application of a shaker-based OCE system for quantifying ONH elasticity under controlled IOP, representing the first such demonstration in a live animal model.

Chapter 4 introduces a multiscale investigation of scleral anisotropy using OCE, ultrasonic elastography, and structural imaging, revealing complex relationships between collagen organization and functional stiffness.

Chapter 5 describes wide-field compressional OCE of ex vivo mouse brain, including phantom validation, strain and elasticity mapping, and correlation with histological anatomy.

Chapter 6 consolidates findings across all tissues, discusses system-level innovations and future directions—such as MHz OCE and real-time ophthalmic translation.

CHAPTER 1: Background and Motivation

1.1 Overview of tissue biomechanics and clinical relevance

The mechanical behavior of biological tissues underlies many essential physiological functions and serves as a key determinant of disease progression. Mechanical properties such as stiffness, elasticity, and viscoelasticity govern how tissues deform, bear load, and recover from strain. Across scales—from the molecular organization of the extracellular matrix (ECM) to the coordinated response of whole organs—biomechanics integrates with biochemical and genetic regulation to maintain tissue homeostasis. Variations in the local mechanical microenvironment influence a broad range of cellular behaviors, including proliferation, migration, and differentiation, thereby linking physical forces with morphogenesis and pathology [1, 2]. Consequently, quantitative understanding of tissue mechanics is indispensable for elucidating normal physiology and for developing diagnostic and therapeutic approaches in biomedicine.

Soft tissues display marked mechanical diversity, spanning several orders of magnitude in modulus and exhibiting pronounced anisotropy and viscoelasticity. In ocular tissues, the cornea and sclera are composed primarily of organized collagen lamellae that confer tensile stiffness ranging from tens to hundreds of kilopascals [41, 43]. The crystalline lens exhibits a gradient in stiffness from the compliant cortex ($\sim 1\text{--}3$ kPa) to the stiffer nucleus (>10 kPa), contributing to accommodation and its age-dependent decline [12]. Neural tissues, by contrast, are among the softest in the body, with shear moduli typically below 2 kPa, reflecting their high water content and delicate cellular architecture [50, 51]. Such mechanical specialization enables precise optical and neural function but also renders these tissues highly susceptible to biomechanical disruption. For instance, corneal ectasia in keratoconus arises from localized weakening and collagen disorganization, lens stiffening contributes to presbyopia and cataract formation, and abnormal strain at the optic nerve head under elevated intraocular pressure is implicated in glaucomatous damage [10, 14, 15]. In the brain, altered stiffness has been associated with edema, tumor growth, demyelination, and traumatic injury [18-20]. These examples underscore that biomechanical homeostasis is both a marker and mediator of health and disease.

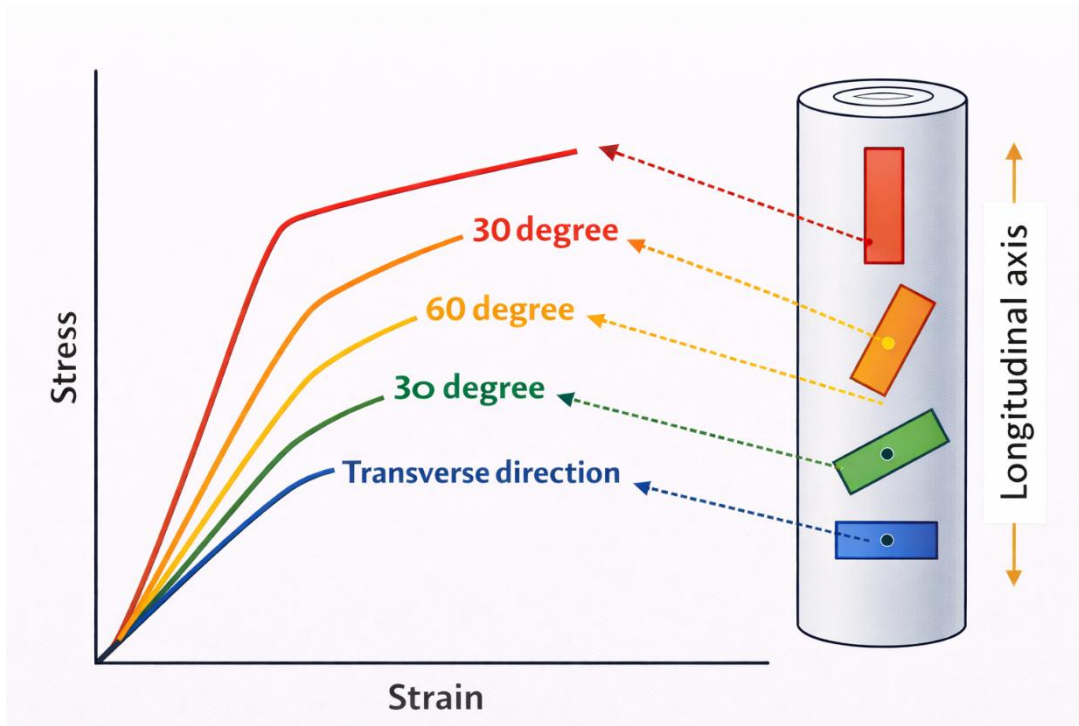


Figure 1.1. Anisotropic biomechanical properties of soft tissue. Specimens extracted along different orientations within the same tissue exhibit distinct stress–strain relationships, demonstrating direction-dependent mechanical behavior. (Redrawn from Frankel and Nordin.)

Reliable quantification of tissue elasticity is thus essential for advancing both mechanistic understanding and clinical assessment. Conventional techniques—including uniaxial or biaxial mechanical testing, indentation, rheometry, and atomic-force microscopy—provide direct measurements of material properties but are generally restricted to excised samples or surfaces [6]. Magnetic resonance elastography (MRE) and ultrasound elastography extend these assessments to in vivo settings but are limited by spatial resolution, sensitivity, or acoustic coupling constraints [26]. In ophthalmology, clinical elasticity measurements are typically inferred indirectly through intraocular pressure or corneal deformation metrics, which cannot resolve spatially heterogeneous stiffness distributions. In neuroimaging, the extreme softness and structural complexity of brain tissue hinder accurate, noninvasive characterization. These limitations highlight the need for imaging modalities that can map tissue mechanics with micrometer-scale resolution, high displacement sensitivity, and minimal perturbation.

Optical coherence tomography (OCT) has emerged as an ideal platform to address these challenges. OCT provides cross-sectional imaging of scattering tissues with micrometer-scale resolution and millimeter-scale depth using interferometric detection of backscattered light [27]. When combined with controlled mechanical stimulation and phase-sensitive signal processing, OCT can be extended to optical coherence elastography (OCE), enabling spatially resolved mapping of local mechanical properties through measurement of induced displacements or propagating elastic waves [31, 32]. This integration of optical imaging and mechanical contrast offers unique advantages for soft-tissue systems: OCE is inherently noncontact, compatible with transparent and weakly scattering media, and sensitive to nanometer-scale motion—features ideally suited to ocular and neural applications where delicacy and precision are paramount.

In summary, the study of tissue biomechanics provides critical insight into how mechanical integrity underpins biological function and disease. Quantitative, high-resolution mechanical mapping—particularly in the eye and brain—has the potential to reveal microstructural alterations before morphological changes become apparent. The work presented in this dissertation builds upon this motivation by developing and applying optical coherence elastography to quantify the elasticity of ocular and neural tissues with unprecedented sensitivity and spatial specificity. Through this approach, new biomechanical biomarkers may be established, advancing both fundamental understanding and translational diagnosis of vision and neurological disorders.

1.2 Introduction of OCT and extension to OCE

Optical coherence tomography (OCT) is an interferometric imaging modality that provides cross-sectional visualization of biological tissues with micrometer-scale resolution and millimeter-scale penetration in scattering media. Originally developed as an optical analog of ultrasound imaging, OCT measures the echo time delay and magnitude of backscattered light to reconstruct depth-resolved reflectivity profiles, or A-scans. By laterally scanning the probe beam, a two-dimensional cross-section (B-scan) or three-dimensional volume can be generated. Because light travels too rapidly for direct time-of-flight detection, OCT employs low-coherence interferometry to encode optical path length differences as interference fringes within the temporal or spectral domain [27]. The axial resolution is determined by the coherence length of

the light source, typically on the order of 2–10 μm , while the lateral resolution depends on the focusing optics and numerical aperture.

Two primary detection schemes are used in OCT: time-domain (TD-OCT) and Fourier-domain (FD-OCT). In TD-OCT, the reference arm path length is mechanically scanned to retrieve depth information sequentially, whereas in FD-OCT—comprising both spectral-domain and swept-source implementations—depth-resolved information is encoded in the frequency spectrum of the interferogram and reconstructed via Fourier transformation). FD-OCT offers orders-of-magnitude improvement in acquisition speed and sensitivity, enabling real-time three-dimensional imaging and functional extensions such as angiography and polarization-sensitive OCT. These capabilities have led to widespread clinical adoption, particularly in ophthalmology, where OCT has become indispensable for diagnosing and monitoring retinal and anterior-segment diseases.

Beyond morphological imaging, the interferometric phase of OCT signals carries exquisite sensitivity to nanometer-scale displacements, providing access to dynamic mechanical information. Optical coherence elastography (OCE) builds upon this phase-sensitive capability to quantify tissue biomechanics by monitoring how tissue responds to a controlled mechanical excitation [31, 58]. In OCE, a mechanical load—delivered by acoustic radiation force, air-coupled or contact transducers, piezoelectric actuators, or photothermal sources—is applied to induce minute deformations or elastic waves within the tissue. The resulting motion is captured by phase-resolved OCT detection, where sub-nanometer path length changes produce measurable phase shifts between sequential A-scans. The local displacement $u(z, t)$ can be estimated from the phase difference $\Delta\phi(z, t)$ through

$$u(z, t) = \frac{\lambda_0 \Delta\phi(z, t)}{4\pi n}$$

where λ_0 is the center wavelength of the light source and n is the refractive index of the medium [38]. By analyzing the spatial and temporal evolution of these displacements, OCE reconstructs strain, elastic modulus, or wave velocity maps that reveal local mechanical properties.

Depending on excitation and analysis methods, OCE techniques can be broadly classified as quasi-static or dynamic. Quasi-static OCE applies low-frequency compression or indentation and infers stiffness from the local strain response under a known load [58]. Dynamic OCE, in contrast, launches elastic waves whose propagation velocity or wavelength is related to the

tissue's shear modulus, enabling quantitative elasticity mapping analogous to ultrasound or magnetic resonance elastography but with much higher spatial resolution [32, 33]. The use of optical detection also allows interrogation of tissues that are optically transparent or weakly scattering—such as the cornea, lens, and retina—without requiring physical contact or immersion coupling, which is particularly advantageous for delicate ocular and neural structures.

Several signal-processing and system-engineering advances have further enhanced OCE performance. Phase-stabilized acquisition schemes and high-speed swept-source OCT systems minimize phase noise, enabling displacement sensitivity below a few nanometers. Computational inversion algorithms, including wave-based elasticity reconstruction and finite-element modeling, facilitate conversion from measured displacements to quantitative mechanical moduli. The integration of broadband optical sources, parallel detection, and optimized excitation geometries continues to expand OCE's spatiotemporal range, allowing both localized and wide-field elasticity assessment. Collectively, these developments position OCE as a versatile and quantitative imaging platform that bridges optical microstructural visualization with mechanical property mapping.

In the context of ocular and neural biomechanics, OCE offers unique advantages. Its noncontact nature preserves delicate surfaces such as the cornea or brain cortex, while its micrometer-scale resolution enables differentiation of thin layers and heterogeneous subregions that are inaccessible to conventional elastography. The capability to measure minute mechanical changes in situ, combined with the existing clinical infrastructure of OCT, paves the way for translation into diagnostic and research applications. By capturing both structure and mechanics within a unified optical framework, OCE extends the role of OCT from purely morphological imaging to functional biomechanical characterization—an advancement central to the objectives of this dissertation.

1.3 Motivation for quantitative OCE in ocular and neural systems

The eye and brain are among the most mechanically specialized and physiologically sensitive organs in the human body. Their function depends on finely tuned elasticity that maintains optical geometry, supports neural signaling, and preserves tissue integrity under dynamic physiological loads. Even subtle alterations in local stiffness can disrupt these processes and lead to disease. Yet, most conventional imaging modalities—including optical coherence tomography (OCT), ultrasound, and magnetic resonance imaging—provide primarily

morphological or biochemical information, leaving mechanical properties largely inaccessible in vivo. Optical coherence elastography (OCE) uniquely bridges this gap, offering noncontact, quantitative mapping of local elasticity with micrometer-scale resolution[59]. By combining the structural sensitivity of OCT with mechanical contrast, OCE provides a means to study how localized elasticity variations correlate with physiological function and pathological change in both ocular and neural systems.

1.3.1 Ocular Biomechanics

The eye is a biomechanical system that relies on the coordinated elasticity of the cornea, sclera, lens, and retina to maintain shape, optical focus, and intraocular pressure (IOP) homeostasis. The cornea accounts for roughly two-thirds of the eye's refractive power and must balance optical transparency with mechanical strength. Its lamellar collagen structure confers nonlinear, anisotropic stiffness that ranges from 100 kPa to over 1 MPa, depending on hydration, orientation, and age [60, 61]. Local softening and thinning in keratoconus cause conical protrusion and severe optical aberration, while surgical reshaping and ultraviolet crosslinking alter corneal rigidity to modify refractive power [62]. Accurate, depth-resolved stiffness assessment is therefore critical for evaluating disease progression and treatment efficacy.

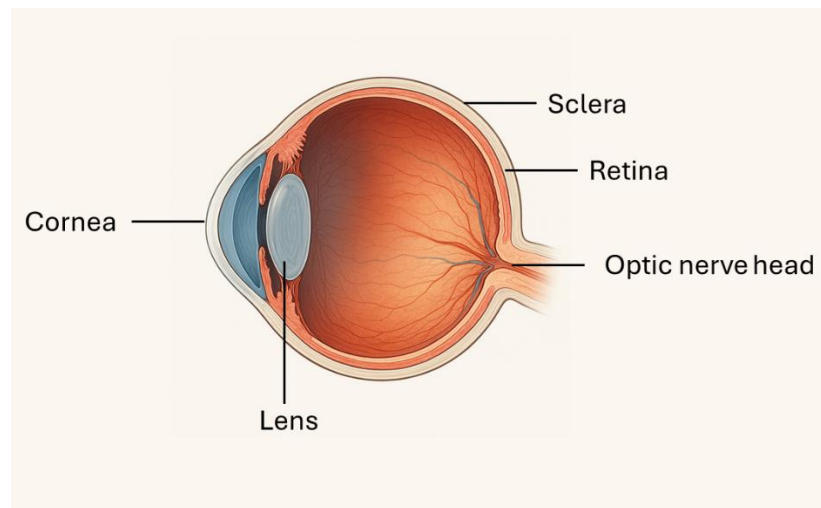


Figure 1.2 Eye anatomy

The sclera provides the primary load-bearing support of the eye, transmitting IOP-induced stress to the optic nerve head (ONH). Studies have shown that scleral stiffening or remodeling alters the strain distribution within the ONH, influencing the susceptibility of retinal

ganglion cell axons to damage in glaucoma [15]. The lens, a transparent, gradient-stiffness structure with cortical shear moduli near 1–3 kPa and nuclear moduli exceeding 10 kPa, progressively stiffens with age, leading to presbyopia and reduced accommodation [13]. Traditional elastography and indentation techniques provide only bulk mechanical data, insufficient to resolve layer-specific variations or the anisotropic lamellar structure of ocular tissues.

Early demonstrations of corneal OCE showed that phase-resolved imaging could visualize nanometer-scale deformation under acoustic or air-pulse excitation [63]. Subsequent developments, including wave-based OCE, enabled measurement of elastic wave propagation across the corneal surface to estimate Young’s modulus with depth specificity [64]. For the crystalline lens, compressional OCE and acoustic radiation force methods have revealed radial gradients in elasticity and age-dependent stiffening in both *ex vivo* and *in vivo* settings [64]. Quantitative OCE thus provides a unique capability to assess mechanical heterogeneity within transparent tissues, offering potential applications in keratoconus screening, crosslinking optimization, and lens biomechanics relevant to cataract surgery and presbyopia management.

1.3.2 Neural Biomechanics

The central nervous system (CNS) exhibits mechanical properties that are orders of magnitude softer than those of most other organs. Neural tissues have shear moduli below 2 kPa and display pronounced viscoelasticity, reflecting their high water content and delicate architecture of neurons, glia, and extracellular matrix [65]. Mechanical cues play vital roles in neurodevelopment, axon guidance, and neurovascular regulation [16], while aberrant stiffness changes accompany a variety of neurological disorders. Increased stiffness in glial scars and tumors, softening in demyelinated or edematous tissue, and heterogeneous stiffening in neurodegenerative diseases such as Alzheimer’s, Parkinson’s, and multiple sclerosis have all been reported [66, 67]. These mechanical alterations often precede observable morphological changes, suggesting elasticity as a potential early biomarker of disease[68].

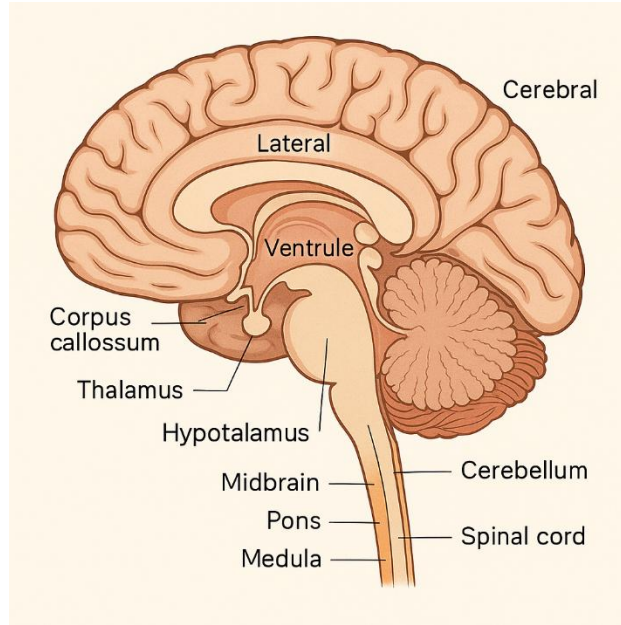


Figure 1.3 Brain anatomy

While magnetic resonance elastography (MRE) has demonstrated the feasibility of *in vivo* brain stiffness imaging, its spatial resolution (millimeter scale) and low-frequency excitation limit sensitivity to microscale heterogeneity [69]. By contrast, OCE achieves micrometer-scale spatial resolution and nanometer displacement sensitivity, enabling high-resolution mapping of local viscoelastic variations in cortical and subcortical regions. Applications of compressional OCE and wave-based OCE have revealed stiffness contrasts between gray and white matter, between hippocampal layers, and within cleared brain tissue volumes [70-72]. The combination of OCE with optical clearing techniques further extends penetration depth and allows correlation between elasticity maps and histological features, facilitating three-dimensional neuro-biomechanical mapping. Such studies are paving the way for quantitative investigation of how cellular architecture and disease-related microstructural remodeling influence mechanical function in the CNS.

1.3.3 The Need for Quantitative OCE

Despite these advances, many OCE implementations remain semi-quantitative, reporting relative phase shifts or displacement amplitudes without rigorous modulus calibration. True quantitative OCE requires precise control of excitation, phase stabilization, and accurate modeling of tissue mechanics to convert measured motion into absolute elasticity values. Standardization across excitation modes—such as air-coupled ultrasound, piezo-compression, or

acoustic radiation force—is essential to ensure reproducibility and comparability of measurements. Advanced inversion algorithms, incorporating wave dispersion analysis, viscoelastic modeling, and finite-element simulation, enable reliable reconstruction of Young’s and shear moduli from observed wavefields [73]. Quantitative calibration also allows for longitudinal and cross-study comparison, an essential step for establishing mechanical biomarkers in clinical research.

In ocular and neural imaging, these quantitative capabilities are transformative. In the anterior eye, quantitative OCE can map spatial stiffness variations to distinguish biomechanical softening from thinning, assess crosslinking efficacy, and evaluate the influence of IOP on corneoscleral strain. In the posterior eye, it can quantify ONH deformation and scleral remodeling related to glaucoma susceptibility. In the brain, quantitative OCE enables high-resolution measurement of stiffness gradients across cortical layers or lesion boundaries, providing mechanistic insight into tissue remodeling, trauma, and degeneration. Together, these applications highlight the unique role of OCE as a bridge between optical imaging and biomechanical metrology—an emerging frontier that this dissertation aims to advance.

CHAPTER 2: Principle of Optical Coherence Elastography

Optical Coherence Elastography (OCE) is fundamentally rooted in the physics and signal processing of Optical Coherence Tomography (OCT). While the prior section provided a broad conceptual introduction to OCT and its role in biomechanical imaging, this chapter examines the principles in greater technical depth. Understanding OCT's mathematical framework, imaging performance metrics, and essential post-processing steps is critical for developing high-precision elastography systems. Accordingly, the sections that follow present the theoretical foundations of OCT and describe the system parameters and computational techniques that enable nanometer-scale displacement sensitivity and quantitative mechanical measurements in OCE.

2.1 Mathematical Framework for OCT

Optical Coherence Tomography (OCT) is fundamentally an interferometric depth-gated imaging modality that reconstructs spatial information by analyzing the interference of broadband optical fields. Since Optical Coherence Elastography (OCE) derives nanoscale mechanical displacements from phase variations in the OCT signal, a rigorous formulation of the OCT detection process—beginning directly from the optical field—is essential for understanding how mechanical motion is encoded in the measured interferogram. In this section, we develop the OCT forward model from first principles: we introduce the broadband illuminating field, derive the reflected fields from the sample and reference arms, calculate the detected optical intensity by evaluating the interference cross terms, and show how the resulting spectral modulation encodes optical path length and enables depth reconstruction through Fourier transformation.

2.1.1 Time-domain OCT

Time-domain optical coherence tomography (TD-OCT) represents the original and conceptually clearest formulation of OCT, in which depth information is acquired by mechanically scanning the optical path length in the reference arm of a Michelson interferometer. Although modern OCT systems overwhelmingly employ Fourier-domain detection, the TD formulation provides the most direct illustration of how low-coherence interferometry isolates depth information, how reflectors within a scattering medium produce interferometric modulation, and how coherence theory governs axial resolution. Because all elastography measurements ultimately rely on the phase properties of the OCT interference

signal, a rigorous understanding of TD-OCT is indispensable for appreciating the physics underlying OCE.

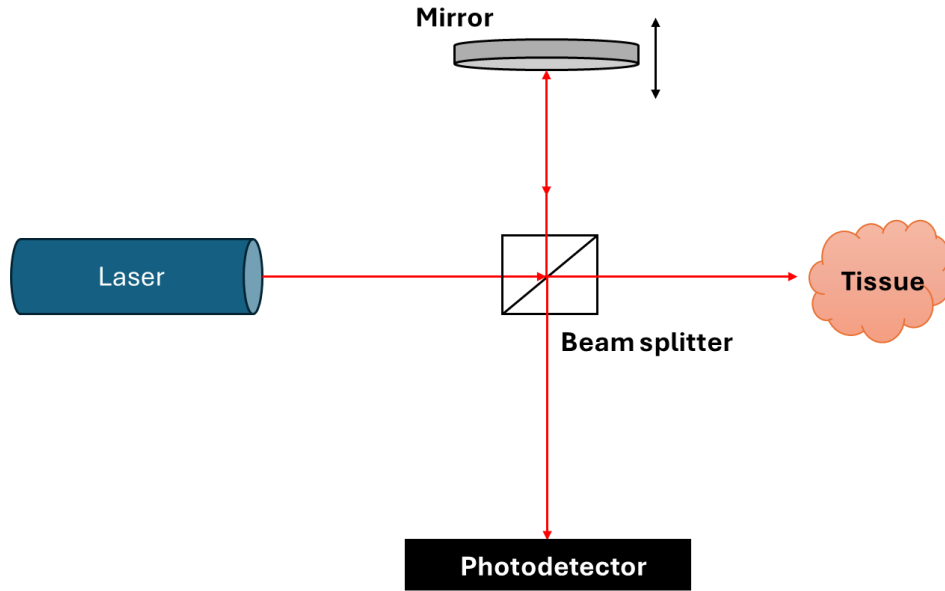


Figure 2.1. Time-domain optical coherence tomography (TD-OCT). Schematic illustration of the TD-OCT architecture. Depth-resolved imaging is achieved by mechanically scanning the reference arm to match the optical path length of sample reflections. Interference occurs only when the reference delay falls within the coherence gate, producing localized axial reflectivity peaks. Sequential scanning of the reference mirror generates an A-scan, and lateral beam translation forms B-scan cross-sectional images. Although historically important, TD-OCT is limited by slow mechanical delay scanning and reduced sensitivity compared to Fourier-domain methods. []

Illuminating Field and Spectral Decomposition

To derive the TD-OCT signal, we begin by describing the broadband illumination field in terms of its spectral components. For a source with spectral amplitude $s(k, \omega)$, representing both the distribution of optical frequencies and the relative phase of each component, the incident field can be written as

$$E_i(k, \omega) = s(k, \omega) e^{i(kz - \omega t)}. \quad (2.1)$$

This expression is not simply a mathematical convenience; it reflects the fundamental fact that OCT relies on low-coherence light whose coherence properties arise from the superposition of many frequency components. The coherence length of the light—which determines axial resolution—is directly related to the bandwidth of $s(k, \omega)$. For a broadband source, the short coherence length ensures that interference only occurs over a narrow range of optical delays, creating a natural depth gate.

Reflected Fields From Sample and Reference Arms

After the incident field passes through the 50/50 beam splitter, the amplitude is divided equally between the sample and reference arms. Suppose the sample contains a single reflective interface at depth z_s , characterized by a complex reflectivity coefficient r_s , while the reference arm contains a mirror at depth z_r with coefficient r_r . The back-reflected fields are then

$$E_s(k, \omega) = \frac{s(k, \omega)}{\sqrt{2}} r_s e^{i(2kz_s - \omega t)}, \quad (2.2)$$

$$E_r(k, \omega) = \frac{s(k, \omega)}{\sqrt{2}} r_r e^{i(2kz_r - \omega t)}. \quad (2.3)$$

The exponential terms incorporate a **round-trip phase delay**, since the optical path is traversed twice—once traveling toward the reflector and once returning. The reflectivity coefficients may themselves be complex, accounting for possible phase shifts at the reflecting boundaries.

Although a single reflector is considered here for clarity, biological tissues generally contain many scattering sites. In such cases, the sample field is a coherent sum of multiple reflections, each producing its own phase-modulated term.

Field Superposition and Detector Response

At the output of the interferometer, the returning fields superpose coherently:

$$E_D(k, \omega) = E_s(k, \omega) + E_r(k, \omega). \quad (2.4)$$

A photodetector does not measure the instantaneous electric field but rather the **time-averaged intensity**, proportional to the square of the field magnitude. With detector responsivity ρ , the measured signal is

$$I_D(k, \omega) = \frac{\rho}{2} \langle |E_s + E_r|^2 \rangle = \frac{\rho}{2} (|E_s|^2 + |E_r|^2 + E_s E_r^* + E_s^* E_r). \quad (2.5)$$

A key step in understanding Fourier-domain OCT is recognizing how the detected optical power decomposes into distinct physical contributions. When the sample and reference fields interfere at the detector, the resulting intensity contains two DC terms—one proportional to the sample-arm power $|E_s|^2$ and the other to the reference-arm power $|E_r|^2$. These components represent stationary background signals that do not carry depth-dependent information. In

contrast, the cross-terms $E_s E_r^* + E_s^* E_r$ arise from coherent interference between the two arms and are the only terms that encode the relative phase difference induced by optical path length variations within the sample. Because this phase difference varies with depth, the interference terms are solely responsible for depth localization in OCT and therefore constitute the fundamental signal from which structural and elastographic information is extracted.

Derivation of the Interference Term

Substituting Eqs. (2.2)–(2.3) into the interference expression yields

$$E_s E_r^* = \frac{|s(k, \omega)|^2}{2} r_s r_r^* e^{i2k(z_s - z_r)}, \quad (2.2)$$

$$E_s^* E_r = \frac{|s(k, \omega)|^2}{2} r_s^* r_r e^{-i2k(z_s - z_r)}. \quad (2.3)$$

Adding these terms gives the real-valued modulation

$$E_s E_r^* + E_s^* E_r = |s(k, \omega)|^2 r_s r_r \cos [2k(z_s - z_r)]. \quad (2.6)$$

Thus, the interferometric component oscillates with spatial frequency proportional to the optical path difference $\Delta z = z_s - z_r$.

This is the fundamental physical principle enabling OCT: the depth of a reflector determines the frequency of oscillation in the detected interferogram.

As the reference mirror is scanned, the path difference changes, modulating the interference signal. Interference appears only when the delay is within the coherence length of the source—illustrating why broadband illumination is essential for axial sectioning.

Coherence Envelope and Axial Resolution

Because the source is broadband, the measured interference is not a pure cosine wave but is enveloped by the coherence function of the light. The interference visibility is modulated by the complex degree of coherence

$$\gamma(\tau) = \frac{\int S(k) e^{i2k\tau} dk}{\int S(k) dk}, \quad (2.7)$$

where $S(k) = |s(k)|^2$ is the source power spectrum. For a Gaussian spectrum, the coherence envelope is Gaussian, and the axial resolution is determined by the coherence length:

$$l_c = \frac{2 \ln 2}{\pi} \frac{\lambda_0^2}{\Delta \lambda}. \quad (2.8)$$

Reflectors separated by more than the coherence length do not interfere, enabling the system to isolate different tissue depths.

Formation of the TD-OCT A-Scan

By mechanically varying the reference arm position, the optical delay τ is scanned through the coherence gate. Peaks in the detected intensity occur whenever the optical path difference matches the depth of a real scatterer. The resulting time-varying interferogram encodes depth information and forms the raw data for reconstructing the TD-OCT A-scan.

Mathematically, the envelope of the interference signal provides reflectivity amplitude, while the oscillatory component carries phase information.

Limitations and Relevance to OCE

While TD-OCT is foundational, several intrinsic limitations prevent its use in optical coherence elastography:

- **Mechanical scanning** restricts acquisition speed to the kilohertz range.
- **Vibration sensitivity** reduces phase stability, making nanometer-scale displacement detection difficult.
- **Low duty cycle** results in reduced sensitivity compared to Fourier-domain methods.

Modern OCE requires rapid acquisition (tens to hundreds of kHz A-scan rates) and highly stable phase measurements, which TD-OCT cannot provide. Although no longer used in practice, TD-OCT remains central to understanding the coherence principles that make phase-resolved elastography possible.

2.1.2 Fourier Domian OCT

While time-domain OCT provides the clearest conceptual picture of coherence gating, the practical limitations imposed by mechanical reference-arm scanning—slow acquisition speed,

low phase stability, and reduced sensitivity—led to the development of Fourier-domain OCT (FD-OCT). FD-OCT represents a transformative theoretical and technological advancement: instead of scanning the optical delay in time, the system records the spectral interference pattern generated by the superposition of the sample and reference fields, and then numerically recovers depth information through Fourier transformation. This approach eliminates the need for moving parts in the reference arm and simultaneously improves sensitivity, acquisition speed, and phase stability, making it the de facto standard for modern OCT and the only viable framework for phase-resolved OCE.

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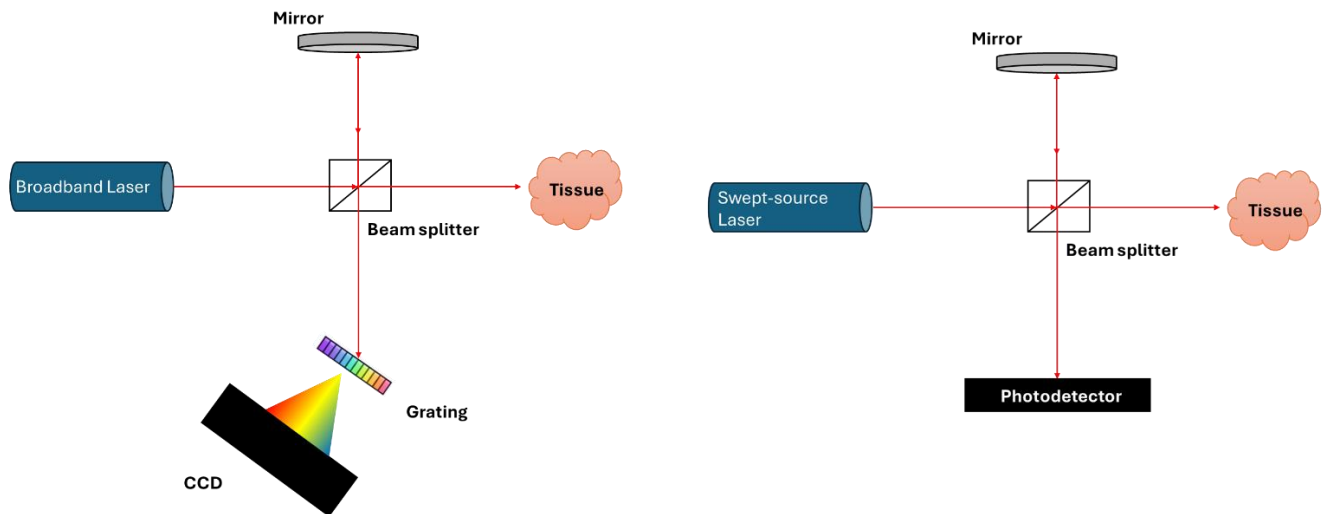


Figure 2.2. Fourier-domain OCT modalities. Schematic comparison of the two major Fourier-domain OCT implementations. Left: Spectral-domain OCT (SD-OCT), in which the interferometric signal is dispersed by a diffraction grating and detected by a line-scan camera to obtain the spectrum simultaneously. Right: Swept-source OCT (SS-OCT), where a rapidly tuning narrowband laser sequentially samples the spectrum and the interference is detected by a high-speed photodetector. Both modalities use Fourier transformation to reconstruct depth-resolved reflectivity profiles, but differ in their detection architecture, imaging speed, and sensitivity roll-off characteristics.

Field Description in Wavenumber Space

The mathematical description of FD-OCT begins by expressing the sample and reference fields directly in the wavenumber domain. Using the same notation introduced in Section 2.1.1, the broadband source with spectral amplitude $s(k)$ generates reflected fields

$$E_s(k) = \frac{s(k)}{\sqrt{2}} r_s e^{i2kz_s}, E_r(k) = \frac{s(k)}{\sqrt{2}} r_r e^{i2kz_r}, \quad (2.2 \ 2.3)$$

where z_s and z_r denote the optical depths of the sample reflector and reference mirror, respectively. The factor e^{i2kz} again represents the two-way propagation phase. Unlike TD-OCT, where delay is scanned in time, FD-OCT measures the **spectral dependence** of the interference pattern directly.

The detected spectral field is

$$E_D(k) = E_s(k) + E_r(k). \quad (2.9)$$

Spectral Interferogram and Detector Intensity

A photodetector measures optical intensity integrated over time, so the detected signal at wavenumber k is

$$I_D(k) = \frac{\rho}{2} (|E_s|^2 + |E_r|^2 + E_s E_r^* + E_s^* E_r), \quad (2.10)$$

where ρ is the detector responsivity. Evaluating the terms yields

$$|E_s|^2 = \frac{|s(k)|^2}{2} |r_s|^2, |E_r|^2 = \frac{|s(k)|^2}{2} |r_r|^2,$$

and the interference terms become

$$E_s E_r^* + E_s^* E_r = |s(k)|^2 r_s r_r \cos [2k(z_s - z_r)]. \quad (2.11)$$

Thus, the FD-OCT spectral interferogram is

$$I_D(k) = \frac{\rho}{2} |s(k)|^2 [r_s^2 + r_r^2 + 2r_s r_r \cos (2k(z_s - z_r))]. \quad (2.12)$$

This expression reveals the central insight of FD-OCT:

Depth information is encoded as sinusoidal modulation of the interferogram in wavenumber space.

Reflectors at greater depth produce more rapidly oscillating fringes. A tissue with many scattering sites produces a superposition of cosine terms, each at a different spatial frequency.

Coherence Envelope in the Spectral Domain

Although Eq. (2.9) is expressed purely in terms of wavenumber, the envelope of the detected signal is implicitly shaped by the spectral power distribution $S(k) = |s(k)|^2$ of the source. For a Gaussian spectrum centered at k_0 ,

$$S(k) = S_0 \exp \left[-\frac{(k - k_0)^2}{2\sigma_k^2} \right], \quad (2.13)$$

the depth-domain point spread function (obtained via inverse Fourier transform) is also Gaussian, with axial resolution determined by the spectral bandwidth:

$$\Delta z = \frac{2 \ln 2}{\pi} \frac{\lambda_0^2}{\Delta \lambda}. \quad (2.14)$$

Thus, axial resolution in FD-OCT is fundamentally governed by the coherence properties of the source, just as in TD-OCT.

Depth Reconstruction via Fourier Transformation

The most important consequence of the spectral interferogram is that the depth profile of the sample can be recovered through an **inverse Fourier transform**. Writing the interferogram generically as

$$I_D(k) = A_0(k) + A_1(k) \cos [2k(z_s - z_r)], \quad (2.15)$$

where $A_0(k)$ contains the DC terms and $A_1(k)$ contains the modulation amplitude, the depth-resolved reflectivity is given by

$$A(z) = \mathcal{F}^{-1}[I_D(k)]. \quad (2.16)$$

Each cosine term in the spectrum produces a localized peak at depth $z = z_s$ in the spatial domain. For a biological tissue, the final A-scan includes hundreds or thousands of such peaks corresponding to microstructural scattering sites.

In practice, the Fourier transform is computed numerically, and the spectrum must be resampled to be linear in wavenumber—a critical calibration step discussed later in Section 2.3.

Advantages of Fourier-Domain Detection

Fourier-domain OCT provides several decisive advantages over time-domain OCT that make it uniquely well suited for quantitative elastography. First, FD-OCT achieves a dramatic sensitivity gain because all depth information contributes simultaneously to the detected interference signal, producing a multiplexing advantage on the order of 20–30 dB. Second, the reference arm remains stationary, eliminating the need for mechanical delay scanning and enabling acquisition rates in the tens to hundreds of kilohertz. Third, the absence of moving components confers exceptional phase stability, reducing delay-induced fluctuations and allowing displacement sensitivity at the nanometer or even sub-nanometer scale. Collectively, these features distinguish Fourier-domain detection not merely as a faster alternative to time-domain OCT, but as a qualitatively superior approach capable of capturing rapid biological dynamics and resolving the minute motions required for high-precision OCE.

Relevance to Optical Coherence Elastography

For OCE, Fourier-domain detection is indispensable. The phase of the reconstructed complex OCT signal contains direct information about axial displacement. A small physical displacement Δz at depth z_s produces a phase shift

$$\Delta\phi = 2k\Delta z, \quad (2.17)$$

leading to the elastography displacement sensitivity relation

$$\Delta z = \frac{\Delta\phi}{2k}. \quad (2.18)$$

Because the phase variance in FD-OCT can be as small as 10^{-3} – 10^{-4} radians, the corresponding displacement sensitivity often reaches the sub-nanometer regime, enabling

measurement of subtle mechanical responses such as elastic wave propagation, microstructural deformation, and viscoelastic relaxation.

For this reason, essentially all modern OCE systems rely on Fourier-domain OCT as their detection framework. TD-OCT, although conceptually foundational, does not provide the speed or phase stability necessary for elastography.

2.2 OCT parameters

The performance of an Optical Coherence Tomography system—and consequently the fidelity of Optical Coherence Elastography measurements—is governed by a set of interdependent optical and detection parameters. These parameters determine the spatial resolution, imaging range, signal-to-noise ratio, and phase stability, each of which places fundamental limits on the accuracy of displacement and strain measurements in OCE. Although the theoretical formulation of OCT, presented in Section 2.1, reveals how the interferometric signal encodes depth information, the practical realization of an OCT system requires careful optimization of its optical bandwidth, numerical aperture, detection sensitivity, and spectral sampling. In this section, we examine the physical origins and mathematical definitions of these parameters and discuss their implications for elastographic imaging.

2.2.1 Axial Resolution and Coherence Length

The axial resolution of an OCT system is determined exclusively by the temporal coherence properties of its light source. From the coherence theory developed in Section 2.1, the axial point spread function (PSF) is the Fourier transform of the source spectrum $S(k)$. For a Gaussian spectrum centered at wavelength λ_0 with full-width at half-maximum (FWHM) bandwidth $\Delta\lambda$, the coherence envelope is also Gaussian, and the axial resolution is given by

$$\Delta z = \frac{2 \ln 2}{\pi} \frac{\lambda_0^2}{\Delta \lambda}. \quad (2.19)$$

This expression reveals a quadratic dependence on the center wavelength and an inverse dependence on the spectral bandwidth. Shorter wavelengths and broader bandwidths therefore improve axial resolution. For example, an 850 nm source with $\Delta\lambda = 100\text{nm}$ yields axial resolution near 3–4 μm in tissue, while a 1300 nm source with the same fractional bandwidth yields slightly lower resolution due to the longer central wavelength.

Axial resolution is of particular importance for OCE because deformation often varies significantly through depth. The ability to resolve small axial gradients in displacement and strain hinges on maintaining a narrow coherence gate. Broader coherence functions blur depth-dependent contrast, reduce strain estimation accuracy, and make it more challenging to detect depth-varying elastic wave propagation.

2.2.2 Lateral Resolution and Numerical Aperture

While axial resolution is determined by coherence, lateral resolution is governed by the focusing properties of the sample arm optics. Under the paraxial approximation, the lateral PSF is given by the Airy pattern, and the lateral resolution (defined as the radius of the first zero) can be approximated as

$$\Delta x \approx 0.37 \frac{\lambda_0}{\text{NA}}, \quad (2.20)$$

where NA is the numerical aperture of the sample arm objective. Increasing NA improves lateral resolution but reduces depth-of-focus (DOF), which scales as

$$\text{DOF} \approx \frac{2\lambda_0}{\text{NA}^2}. \quad (2.21)$$

For elastography, the trade-off between lateral resolution and DOF is nontrivial. In tissues with substantial thickness variation, such as sclera or brain, maintaining adequate DOF ensures uniform illumination and minimizes the need for repeated refocusing. Conversely, studies requiring fine lateral feature discrimination—such as mapping microstructural anisotropy or fine elastic gradients—may benefit from higher NA despite the reduced DOF.

Additionally, lateral resolution directly influences the spatial sampling density of the displacement field. Undersampling can obscure shear-wave propagation patterns or produce aliasing artifacts in quantitative stiffness reconstruction.

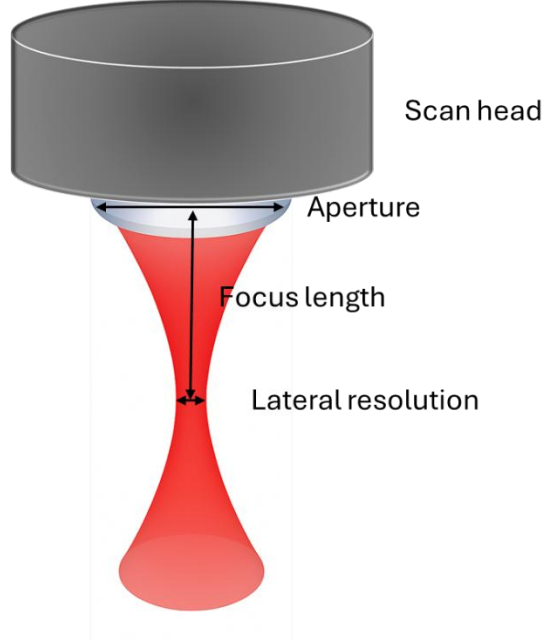


Figure 2.3. Relationship between lateral resolution and numerical aperture (NA). Illustration of how the lateral resolution of an OCT system is determined by the focusing optics. A higher numerical aperture produces a tighter focal spot, improving lateral resolution but reducing the depth of focus. Conversely, a lower NA results in a larger focal spot and degraded transverse resolution, but with an extended depth of field. The balance between NA, beam waist w_0 , and Rayleigh range z_R defines the system's transverse imaging performance and determines the optimal design for specific biological applications.

2.2.3 Sensitivity, Noise, and Displacement Precision

Sensitivity defines the minimum detectable backscattered power and is a critical parameter in OCT, especially for OCE where phase stability determines displacement precision. The sensitivity of an OCT system is influenced by reference arm power, detector noise, digitization depth, and A-scan rate. Ultimately, the signal-to-noise ratio (SNR) sets the limit on phase precision:

$$\sigma_\phi \approx \frac{1}{\sqrt{\text{SNR}}}, \quad (2.22)$$

and the corresponding displacement sensitivity is

$$\delta z_{\min} = \frac{\sigma_\phi}{2k} = \frac{1}{2k \sqrt{\text{SNR}}}. \quad (2.23)$$

For a typical Fourier-domain OCT system with $\text{SNR} \approx 50$ dB and $\lambda_0 \approx 900$ nm, the displacement sensitivity lies in the 0.5–2 nm range. This is several orders of magnitude smaller

than the imaging resolution itself, enabling OCE to detect minute mechanical deformations long before they become optically resolvable.

The sensitivity advantage of Fourier-domain OCT over time-domain OCT arises from the parallel detection of all spectral components, a multiplexing benefit sometimes referred to as the "Jacquinot advantage." This enhanced sensitivity is crucial for OCE because low-amplitude shear waves or subtle axial motions require high SNR to ensure accurate phase demodulation.

2.2.4 Imaging Range and Spectral Sampling

In Fourier-domain OCT, the imaging range is governed by the spectral sampling interval. If the interferogram is sampled uniformly in wavenumber with interval Δk , the maximum depth without aliasing is

$$z_{\max} = \frac{\pi}{\Delta k}. \quad (2.24)$$

This relationship arises directly from the Nyquist sampling theorem: deeper reflectors produce higher frequencies in the spectral interferogram and require finer sampling to avoid folding into the near-depth region.

Conversely, the depth resolution in Fourier-domain OCT is determined by the total spectral bandwidth Δk_{tot} :

$$\Delta z_{\text{PSF}} \approx \frac{1}{\Delta k_{\text{tot}}}. \quad (2.25)$$

Thus, there is an intrinsic trade-off between imaging range and axial resolution. Increasing the depth range by reducing Δk necessitates longer spectral sweeps or higher spectrometer resolution, both of which may increase system complexity or reduce sweep speed.

In the context of OCE, imaging range must be sufficient to fully capture the propagation path of elastic waves, especially in thick tissues such as sclera or brain cortex. Insufficient imaging range truncates the displacement field, leading to biased or erroneous stiffness estimation. Moreover, insufficient spectral linearization or nonuniform sampling introduces depth-dependent phase distortions that directly degrade elastographic accuracy.

2.3 OCT Post processing

The interferometric signal measured by an OCT system must undergo several computational steps before it can be interpreted as a depth-resolved structural image or used for phase-sensitive elastography. These steps include converting the raw camera or swept-source data into a uniformly sampled wavenumber grid, correcting dispersion mismatches between sample and reference arms, suppressing noise and artifacts, and extracting complex-valued depth profiles via Fourier transformation. Because OCE relies on the phase of the complex OCT signal to quantify nanometer-scale displacements, the accuracy of each post-processing stage directly affects the stability and fidelity of subsequent elastographic measurements. In this section, we describe the essential numerical operations that convert raw spectral measurements into calibrated, phase-stable OCT data.

2.3.1 k calibration

Fourier-domain OCT requires that the interferogram be sampled uniformly in wavenumber k . In practice, however, neither spectral-domain OCT spectrometers nor swept-source lasers naturally provide linear sampling in k . A line-scan camera samples wavelength uniformly, not wavenumber, and swept-source lasers typically sweep in time with nonlinear tuning curves. Because the Fourier transform assumes uniformly spaced samples, any nonlinearity in k causes depth-dependent distortions, broadening of the axial point-spread function, and instability in phase measurements.

To correct this, a **k -space calibration** is performed. A common method uses the interference signal produced by a mirror placed at a known reference position. The resulting spectral fringes have a phase

$$\phi(k_i) = 2k_i z_r, \quad (2.26)$$

and the phase derivative $d\phi/dk$ should be constant for linear k spacing. Deviations from linearity reveal the mapping between the acquired camera pixels (or sweep time points) and their true wavenumber positions. A polynomial or spline-based interpolation is then used to resample the measured interferogram onto a uniform k -grid:

$$I_D(k) \leftarrow I_D(\lambda(k)). \quad (2.27)$$

This calibration is one of the most critical operations for OCE, since phase errors introduced by incorrect k -spacing accumulate over depth and manifest as artificial phase gradients—indistinguishable from mechanical displacement. Elastography systems therefore require highly stable and precise k -linearization to ensure that observed phase variations originate solely from tissue motion.

2.3.2 Numerical Dispersion compensation

Even with perfect spectral sampling, biological imaging is degraded by **chromatic dispersion mismatch** between the sample and reference arms. Dispersion causes different wavelengths to accumulate different phase delays, leading to broadening of the axial PSF and depth-dependent phase distortions. Mathematically, dispersion introduces higher-order phase terms:

$$\Phi(k) = \alpha_2(k - k_0)^2 + \alpha_3(k - k_0)^3 + \dots, \quad (2.28)$$

where α_2 and α_3 represent second- and third-order dispersion differences. To compensate for this, a numerical phase correction is applied to the interferogram:

$$I_D^{\text{corr}}(k) = I_D(k) e^{[-i(\alpha_2(k-k_0)^2 + \alpha_3(k-k_0)^3)]}. \quad (2.29)$$

Optimal dispersion parameters may be determined by minimizing the axial width of a point reflector or by optimizing image sharpness across the field of view.

For OCE, dispersion compensation has even greater importance. Residual dispersion produces depth-dependent phase curvature that can mimic mechanical strain. Correcting dispersion ensures that observed phase gradients truly represent tissue deformation or elastic wave propagation rather than optical artifacts.

2.3.3 Background Removal, Windowing, and Fourier Transformation

After k -linearization and dispersion compensation, the calibrated interferogram still contains a strong DC term and fixed-pattern spectral contributions from the light source and spectrometer. These are removed by subtracting either a time-averaged background spectrum or a reference frame acquired without sample reflectivity.

A spectral window function $W(k)$, such as a Hanning or Kaiser-Bessel window, is applied to suppress sidelobes associated with finite sampling:

$$I_W(k) = I_D^{\text{corr}}(k) W(k). \quad (2.30)$$

Finally, the depth-resolved complex OCT signal is obtained through the Fourier transform:

$$A(z) = \mathcal{F}^{-1}\{I_W(k)\}, \quad (2.31)$$

in which the magnitude $|A(z)|$ provides structural contrast and the phase $\phi(z) = \arg A(z)$ encodes the optical path length.

Because OCE relies on the complex phase rather than the amplitude, it is essential that the Fourier transform and all preceding steps preserve phase fidelity. Even small numerical inconsistencies—such as improper zero-padding, window asymmetry, or discretization artifacts—can degrade phase stability and thus limit displacement sensitivity.

2.3.4 Phase Extraction and Phase Stability Considerations

The complex-valued A-scan obtained from the Fourier transform can be written as

$$A(z) = |A(z)| e^{i\phi(z)}, \quad (2.32)$$

where $\phi(z)$ is the depth-dependent phase. For a reflector at depth z , a small displacement Δz produces a phase shift of

$$\Delta\phi = 2k_0\Delta z. \quad (2.33)$$

Accurate phase extraction requires the OCT system to maintain phase stability within only a few milliradians. Achieving this degree of stability necessitates precise temporal coordination between successive acquisitions, rigorous suppression of mechanical vibration, stable and low-noise light-source operation, and careful management of electronic noise throughout the detection chain. In swept-source systems, additional attention must be given to minimizing trigger jitter, as even small timing inconsistencies introduce phase fluctuations that can mimic or obscure true tissue motion. Because phase noise directly determines the minimum detectable displacement, it fundamentally limits elastography sensitivity. As demonstrated in Chapters 3 and 4, even modest phase instabilities can distort wave-speed estimation, bias elasticity calculations, and degrade spatial strain reconstructions, underscoring the need for stringent phase-stability control in quantitative OCE.

2.3.5 Importance of Post-Processing for Elastography

Post-processing converts raw interferograms into clean, phase-stable OCT data suitable for quantitative elastography, and each computational step plays a critical role in preserving displacement fidelity. k-linearization enforces uniform sampling in wavenumber space, ensuring depth accuracy and eliminating artificial phase curvature. Numerical dispersion compensation maintains both axial resolution and phase linearity by correcting wavelength-dependent phase delays between the sample and reference arms. Background subtraction and spectral windowing suppress fixed-pattern noise and sidelobe artifacts, improving the signal-to-noise ratio of the reconstructed profiles. Finally, complex Fourier transformation provides the depth-resolved analytic signal from which displacement fields are derived. Because OCE interprets phase changes directly as mechanical motion, inaccuracies introduced at any stage of post-processing propagate into displacement, strain, and elasticity estimates. Consequently, post-processing is not a peripheral computational task but a foundational element of OCE methodology, governing the precision, stability, and quantitative reliability of the resulting elastograms.

2.4 Mathematical Framework for OCE

Optical Coherence Elastography (OCE) extends the interferometric principles of Fourier-domain OCT to enable quantitative measurements of tissue deformation at nanometer-scale sensitivity. While conventional OCT interprets the magnitude of the reconstructed complex signal for structural imaging, OCE leverages the phase component, whose exquisite stability directly encodes minute physical displacements. The ability to resolve displacements far below the OCT axial resolution allows OCE to detect microscopic strains, map stress distributions, and track the propagation of elastic waves with high temporal and spatial fidelity.

This section develops the formal mathematical framework that links OCT phase to mechanical displacement, establishes how displacement evolves in time under external or intrinsic excitation, and introduces the filtering strategies required to isolate meaningful elastographic motion. These principles form the foundation for the dynamic and compressional OCE methods described in later sections.

2.4.1 Phase-Resolved Displacement Measurement

In Fourier-domain OCT, each depth location is represented by a complex analytic signal

$$A(z, t) = |A(z, t)| e^{i\phi(z, t)}, \quad (2.32)$$

where $|A(z, t)|$ encodes scattering amplitude and $\phi(z, t)$ is the interferometric phase. Mechanical deformation of tissue changes the optical path length between the sample and reference arms, which alters $\phi(z, t)$ in time. Because OCT detects *round-trip* optical phase, an axial displacement $\Delta z(z, t)$ produces a phase shift

$$\Delta\phi(z, t) = 2k_0\Delta z(z, t), \quad (2.33)$$

where $k_0 = 2\pi/\lambda_0$ is the center wavenumber of the OCT illumination. Solving this expression yields the classical OCE displacement relation:

$$\Delta z(z, t) = \frac{\Delta\phi(z, t)}{2k_0}. \quad (2.34)$$

This linear relationship is exact under the assumption of small axial motion—a condition overwhelmingly satisfied in OCE, where displacements typically range from tenths of a nanometer to a few micrometers.

Complex Domain Derivation

An alternative derivation emphasizes the complex OCT signal. Let

$$A(z, t_1) = |A_1| e^{i\phi_1}, A(z, t_2) = |A_2| e^{i\phi_2}.$$

The phase difference is

$$\Delta\phi = \arg [A(z, t_2)A^*(z, t_1)]. \quad (2.35)$$

If $|A_1| \approx |A_2|$, which holds when scattering amplitude changes are slow relative to phase, the measured phase difference depends solely on the optical path length change. This forms the basis of **phase-difference OCT**, the core algorithmic operation in OCE.

To reconstruct displacement relative to a baseline time t_0 , we define

$$u(z, t) = \frac{\phi(z, t) - \phi(z, t_0)}{2k_0}. \quad (2.36)$$

Phase wrapping occurs whenever $\Delta\phi$ exceeds π . However, in most OCE applications displacements are sufficiently small that unwrapping is rarely required; when necessary, standard 1D or temporal phase-unwrapping algorithms ensure continuity.

Displacement Sensitivity

The minimum detectable displacement is linked to phase noise σ_ϕ by

$$\sigma_u = \frac{\sigma_\phi}{2k_0}. \quad (2.37)$$

With milliradian phase stability, displacement sensitivity easily reaches 0.3–1nm. This sensitivity is what allows OCE to measure subtle shear-wave fields, local microindentation responses, and texture-dependent mechanical signatures invisible in OCT amplitude images.

2.4.2 Temporal Displacement Field and Motion Filtering

Once axial displacement is extracted from the OCT phase, the resulting measurements represent the time-varying motion of individual scattering structures within the tissue. This temporal evolution of displacement contains the mechanical response of interest but is also unavoidably influenced by non-elastographic sources of motion. The measured displacement field can therefore be expressed as a superposition of multiple components,

$$u(z, t) = u_{\text{mech}}(z, t) + u_{\text{phys}}(z, t) + u_{\text{drift}}(z, t) + n(z, t), \quad (2.38)$$

where $u_{\text{mech}}(z, t)$ denotes the deformation caused directly by the externally applied mechanical excitation, $u_{\text{phys}}(z, t)$ arises from low-frequency physiological fluctuations such as ocular pulsation or vascular motion, $u_{\text{drift}}(z, t)$ captures instrument- or environment-induced bulk shifts, and $n(z, t)$ represents stochastic noise inherited from the OCT phase measurement. Each of these components has distinct spectral characteristics; however, within the raw displacement field they are inseparably intertwined.

A principal objective of OCE post-processing is therefore to isolate $u_{\text{mech}}(z, t)$, the elastographically meaningful component, while suppressing the remaining terms that obscure or distort the mechanical response. This is achieved by applying temporal filtering to the displacement field. If $\mathcal{F}_t[\cdot]$ denotes a filtering operator acting along the temporal dimension, the elastographically relevant displacement can be written as

$$u_f(z, t) = \mathcal{F}_t[u(z, t)]. \quad (2.39)$$

The design of this filter depends on both the excitation modality and the frequency content of the mechanical response. In compressional OCE, where the mechanical loading often induces quasi-static or slowly varying deformation, high-pass filtering is used to eliminate low-frequency drift and bulk-motion artifacts, thereby revealing the true deformation profile. In dynamic OCE, where the response is dominated by oscillatory motion induced by harmonic or broadband excitations, a narrow band-pass filter centered at the excitation frequency effectively isolates the shear-wave displacement while rejecting unrelated motion and system noise. Importantly, the filtering process must preserve the temporal phase relationships within the mechanical waveform; poor filter design may alter the apparent wave arrival times or attenuate spatially dependent amplitude variations, thereby biasing subsequent mechanical property estimation.

Once temporal filtering has isolated the displacement component of interest, the displacement field is typically examined not only as a function of depth but also across lateral dimensions. By extending the displacement into a two- or three-dimensional spatiotemporal representation $u(x, z, t)$, OCE enables the visualization of full-field mechanical dynamics. Such maps provide the basis for shear-wave tracking, propagation analysis, strain computation, and the identification of spatial gradients in tissue stiffness or anisotropy. The clarity and interpretability of these spatiotemporal maps depend critically on the fidelity of the filtered displacement field, making temporal filtering a foundational step in the overall OCE processing pipeline.

In summary, temporal displacement analysis serves as the bridge between raw OCT phase measurements and the higher-level mechanical metrics that define elastography. It transforms the inherently noisy, multi-component phase-derived displacement into a cleaned, mechanically interpretable field from which wave propagation, strain, and elastic moduli can be quantitatively extracted. Proper construction of this displacement field—and careful suppression of confounding motion—are indispensable for achieving accurate and reproducible OCE measurements.

2.5 Dynamic OCE

Dynamic Optical Coherence Elastography (Dynamic OCE) measures the propagation of mechanically induced elastic waves—typically shear waves—within biological tissues. Unlike

compressional OCE, which analyzes quasi-static deformation under applied load, dynamic OCE interrogates the tissue's *dynamic* mechanical response to time-varying excitations. When a transient or harmonic force is applied to the tissue, mechanical waves propagate through the medium with velocities determined by its viscoelastic properties. OCT, with its high-speed acquisition and nanometer-scale displacement precision, can track these waves in space and time, enabling quantitative estimation of stiffness, viscosity, anisotropy, and dispersion behaviors.

Dynamic OCE has become especially important in applications where wave-based elasticity measurements provide superior sensitivity to local mechanical variations or where deformation must be measured rapidly without sustained loading. The mathematical foundations of dynamic OCE rely on interpreting the spatiotemporal displacement field $u(x, z, t)$ in terms of the governing equations of wave propagation in elastic media.

2.5.1 Mechanical Wave Generation and Propagation

In dynamic OCE systems, mechanical waves may be generated by a variety of excitation mechanisms, including air-coupled ultrasound, piezoelectric transducers, acoustic radiation force, or localized mechanical impact. Regardless of the source, the resulting displacement field within the tissue satisfies the elastodynamic wave equation. For an isotropic, linear, elastic medium, shear waves obey

$$\frac{\partial^2 u}{\partial t^2} = c_s^2 \nabla^2 u, \quad (2.40)$$

where u is the displacement, c_s is the shear wave speed, and ∇^2 is the Laplacian operator. The wave speed is related to mechanical stiffness via

$$c_s = \sqrt{\frac{G}{\rho}}, \quad (2.41)$$

with G the shear modulus and ρ the mass density of the medium. Tissues with higher stiffness produce faster shear-wave propagation, while softer tissues yield slower waves. This relationship underpins all wave-based elastography techniques.

In the presence of viscoelasticity—ubiquitous in biological tissues—the wave equation includes damping, and the shear modulus becomes frequency-dependent:

$$G^*(\omega) = G'(\omega) + iG''(\omega), \quad (2.42)$$

where G' is the storage modulus and G'' the loss modulus. In these cases, the shear-wave speed and attenuation depend on frequency, and dynamic OCE can provide access to the dispersion characteristics of tissue.

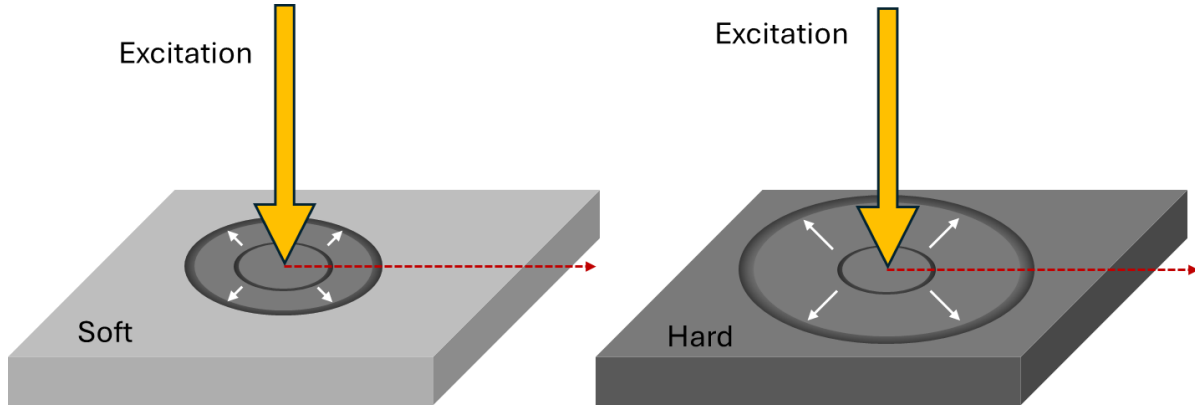


Figure 2.4. Wave propagation behavior in soft versus stiff materials. Schematic illustration showing how mechanical waves respond to localized loading in tissues of different stiffness. In the soft material (left), the applied impulse generates a larger deformation region and slower radial wave propagation, indicated by the broader displacement field. In contrast, the stiff material (right) exhibits a smaller deformation zone and faster wave propagation due to higher elastic modulus. These differences in deformation profile and propagation speed form the basis for quantitative elasticity estimation in optical coherence elastography (OCE).

2.5.2 Spatiotemporal Displacement Field Measurement

To analyze wave propagation, OCT is used to measure the displacement field along spatial coordinates and over time:

$$u(x, z, t) = \frac{\phi(x, z, t) - \phi(x, z, t_0)}{2k_0}. \quad (2.43)$$

This spatiotemporal mapping yields a four-dimensional dataset comprising lateral position, axial depth, time, and displacement amplitude. High-speed OCT systems (50–500 kHz A-scan rates) allow acquisition of densely sampled displacement fields that capture wave propagation with minimal temporal aliasing.

The wavefronts appear in the displacement map as diagonal ridges in the (x, t) plane, whose slope corresponds to wave speed:

$$c_s = \frac{\Delta x}{\Delta t}. \quad (2.44)$$

However, due to noise, dispersion, and boundary interactions, direct ridge tracking is often insufficient, necessitating more sophisticated spectral or model-based methods.

2.5.3 Wave Speed Estimation via 2D Fourier Analysis

One of the most widely used approaches for extracting shear-wave speed is two-dimensional Fourier analysis. The displacement field is transformed into frequency–wave number space:

$$U(k_x, \omega) = \mathcal{F}_{x,t}[u(x, t)], \quad (2.45)$$

where k_x is the spatial wave number and ω is the temporal frequency. In this representation, propagating shear waves appear as energy concentrated along a dispersion ridge satisfying

$$\omega = c_s k_x. \quad (2.46)$$

By estimating the slope of this ridge, the shear-wave speed c_s can be computed with high precision. Because the frequency and wave number domains inherently suppress noise and isolate coherent propagation modes, 2D Fourier analysis provides robustness against measurement noise and tissue heterogeneity.

For harmonic excitations, the displacement can be modeled as

$$u(x, t) = A e^{-\alpha x} \sin(k_s x - \omega t), \quad (2.47)$$

from which both the amplitude attenuation α and the wave number k_s can be recovered, enabling estimation of viscoelastic moduli through models such as Kelvin–Voigt or Maxwell.

2.5.4 Time-of-Flight and Phase-Delay Methods

Time-of-flight methods estimate wave speed by measuring peak or zero-crossing travel times between adjacent positions:

$$c_s = \frac{x_2 - x_1}{t_2 - t_1}. \quad (2.48)$$

Alternatively, phase-delay methods compute the phase difference at a fixed frequency:

$$c_s = \frac{\omega(x_2 - x_1)}{\Delta\phi(x_2, x_1)}, \quad (2.49)$$

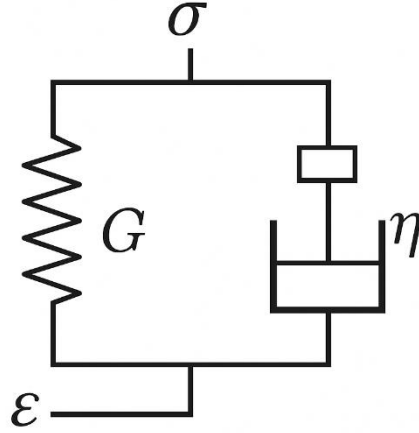
where $\Delta\phi$ is the phase shift between two lateral locations. These methods require high SNR and accurate temporal filtering but can provide excellent spatial localization of stiffness changes.

2.5.5 Mechanical Property Reconstruction

Biological tissues rarely behave as perfectly elastic solids; instead, they exhibit viscoelastic behavior characterized by both elastic energy storage and viscous energy dissipation. Among the constitutive models used to describe this dual response, the Kelvin–Voigt (Voigt) model is one of the most widely applied in OCE because of its simplicity and its ability to capture the frequency-dependent propagation characteristics of shear waves. In this model, tissue is represented by a purely elastic spring with shear modulus G operating in parallel with a viscous dashpot of viscosity η . For a material subjected to shear strain $\varepsilon(t)$, the total stress $\sigma(t)$ is the sum of the elastic and viscous contributions,

$$\sigma(t) = G \varepsilon(t) + \eta \frac{d\varepsilon(t)}{dt}, \quad (2.50)$$

where the first term represents the instantaneous elastic restoring force and the second term accounts for damping due to viscous fluid flow, fiber sliding, and other dissipative mechanisms.



Kelvin-Voigt model

Figure 2.5 Voigt Model: spring (G) and dashpot (η)

Under harmonic excitation at angular frequency ω , the strain is expressed as $\varepsilon(t) = \varepsilon_0 e^{i\omega t}$. Substitution into the constitutive law yields

$$\sigma(t) = (G + i\omega\eta) \varepsilon_0 e^{i\omega t}, \quad (2.51)$$

revealing the complex shear modulus

$$G^*(\omega) = G'(\omega) + iG''(\omega), \quad (2.52)$$

with

$$G' = G, G'' = \omega\eta. \quad (2.53)$$

Here, G' denotes the storage modulus, reflecting elastic stiffness, whereas G'' represents the loss modulus, quantifying viscous dissipation. A key characteristic of the Voigt model is that G' is frequency independent, while G'' increases linearly with frequency—behavior consistent with the empirically observed dispersion and attenuation of shear waves in soft tissues.

Substituting the complex modulus into the shear-wave solution $u(x, t) = u_0 e^{i(kx - \omega t)}$ yields a complex wave number,

$$k(\omega) = \omega \sqrt{\frac{\rho}{G + i\omega\eta}}, \quad (2.54)$$

where the real and imaginary parts correspond to the shear-wave speed and attenuation, respectively. The resulting shear-wave speed is

$$c_s(\omega) = \sqrt{\frac{2(G^2 + \omega^2\eta^2)}{\rho[G + \sqrt{G^2 + \omega^2\eta^2}]}} \quad (2.55)$$

which approaches the purely elastic limit $c_s \approx \sqrt{G/\rho}$ at low frequencies ($\omega\eta \ll G$), but increases with frequency as viscous effects become significant. This dispersion behavior is commonly observed in dynamic OCE measurements and provides a practical pathway for estimating the viscoelastic parameters G and η .

The Voigt model captures several hallmark features of soft-tissue biomechanics, including elastic stiffness arising from collagen and extracellular matrix structure, viscous damping from interstitial fluid and fiber interactions, frequency-dependent wave propagation, shear-wave attenuation, and phase delays between stress and strain. Because dynamic OCE often employs harmonic or narrowband excitations, the Kelvin–Voigt framework offers a tractable and robust means of interpreting measured wave speeds and attenuation profiles in terms of intrinsic viscoelasticity. Once shear-wave speed is determined, stiffness can be approximated under isotropic assumptions using

$$G = \rho c_s^2, \quad (2.56)$$

and Young's modulus follows from

$$E = 2G(1 + \nu), \quad (2.57)$$

with Poisson's ratio $\nu \approx 0.5$ for nearly incompressible soft tissues. In anisotropic tissues such as the sclera or brain white matter, wave speed depends on propagation direction, and dynamic OCE can be extended to reconstruct directional stiffness or even full stiffness tensors using multi-directional wave measurements.

Dynamic OCE offers several important advantages for biomechanical imaging. Because shear-wave speed depends quadratically on shear modulus, small changes in stiffness generate detectable changes in wave propagation, enabling high sensitivity to mechanical contrast. The required acquisition times are short, making real-time or near-real-time stiffness evaluation

feasible. Dynamic OCE also permits measurement of viscoelasticity, dispersion, and anisotropy, and is compatible with noncontact excitation methods such as air-coupled ultrasound. Moreover, because displacement is induced mechanically rather than optically, the optical load on the sample remains minimal. Collectively, these strengths make dynamic OCE particularly well suited for ophthalmic biomechanics, neural tissue characterization, and other applications where high spatial resolution and depth-resolved mechanical assessment are essential.

2.5.6 Advantages of Dynamic OCE

Dynamic OCE offers several compelling advantages that make it particularly powerful for biomechanical imaging of soft tissues. Because shear-wave speed depends quadratically on the shear modulus, the technique exhibits high sensitivity to stiffness, enabling detection of subtle mechanical variations that may be difficult to resolve using other modalities. The rapid acquisition rates achievable with modern OCT systems allow shear-wave propagation to be captured on millisecond timescales, supporting real-time or near-real-time estimation of tissue elasticity. Dynamic OCE can also characterize viscoelastic behavior, frequency-dependent dispersion, and material anisotropy, providing a richer mechanical description than static methods. Its compatibility with noncontact excitation strategies—such as air-coupled ultrasound—further reduces experimental complexity and eliminates the need for physical coupling media. Moreover, because tissue motion is induced mechanically rather than through modulated optical forces, the optical loading on the sample remains minimal. Collectively, these advantages make dynamic OCE exceptionally well suited for ophthalmic biomechanics, neural tissue studies, and broader investigations where localized, depth-resolved, and high-sensitivity mechanical measurements are required.

2.6 Compressional OCE

Compressional Optical Coherence Elastography (OCE) encompasses a class of techniques in which tissue mechanics are inferred from quasi-static or slowly varying deformation produced by an externally applied compressive load. Unlike dynamic OCE—which infers stiffness from shear-wave propagation—compressional OCE directly relates local displacement and strain to material stiffness through controlled perturbation. Because OCT provides nanometer-scale sensitivity to axial motion, even modest mechanical loads produce measurable deformation fields, enabling high-resolution assessment of spatially heterogeneous

tissue properties. Many biological tissues, particularly in ophthalmology and soft neural systems, respond predictably to low-amplitude compressive loading, making compressional OCE a natural framework for quantitative biomechanical evaluation.

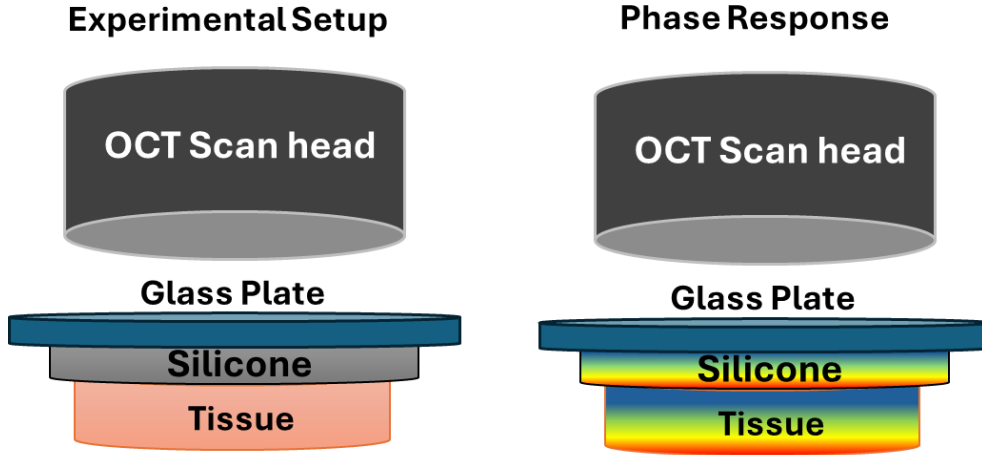


Figure 2.6. Experimental configuration and phase-response illustration for silicone–tissue phantoms. (Left) Schematic showing the layered sample geometry used for calibration and validation experiments, consisting of a silicone layer placed above the biological tissue and interrogated by the OCT probe. (Right) Corresponding phase-variation map acquired with phase-resolved OCT, demonstrating distinct phase responses in the silicone and tissue layers. The color bar represents phase shifts from $-\pi$ to π , highlighting the depth-dependent displacement behavior needed for strain and elasticity quantification.

2.6.1 Mechanical Loading and Phase-Resolved Displacement Measurement

In compressional OCE, the tissue is subjected to a known or well-controlled compressive load. The load may be imparted through various modalities—such as piezoelectric actuation, controlled preload perturbation, micro-indentation, or pneumatic excitation—so long as the resulting deformation evolves slowly enough to be captured as quasi-static. Let t_0 denote the reference (unloaded) state and t_1 the compressed state. The OCT phase at depth z changes from $\phi(z, t_0)$ to $\phi(z, t_1)$, yielding an axial displacement

$$u(z) = \frac{\phi(z, t_1) - \phi(z, t_0)}{2k_0}. \quad (2.58)$$

Because the deformation progresses over tens to hundreds of milliseconds rather than microseconds, temporal instability such as bulk drift must be mitigated, but high temporal resolution is not essential. Indeed, the quasi-static nature of loading confers several advantages: the displacement field is typically smooth, dominated by compressive strain rather than oscillatory motion, and often easier to interpret mechanically.

2.6.2 Strain Reconstruction

With the displacement field $u(z)$ measured along depth, the next step is to compute axial strain. In continuum mechanics, strain represents the spatial gradient of displacement:

$$\varepsilon(z) = \frac{\partial u(z)}{\partial z}. \quad (2.59)$$

Direct finite-difference differentiation, however, amplifies phase noise. For this reason, strain is almost universally estimated through spatial regression. Over an axial window centered at z , the least-squares estimate of strain is

$$\varepsilon(z) = \frac{\sum_{i=-N}^N (z_i - \bar{z}) u(z_i)}{\sum_{i=-N}^N (z_i - \bar{z})^2}, \quad (2.60)$$

where $2N + 1$ is the window size and $\bar{z}$ is the local mean depth. This formulation acts as a spatial low-pass filter, suppressing noise at the cost of modest smoothing. The window width must therefore balance noise reduction against axial resolution; smaller windows preserve fine mechanical features, whereas larger windows yield lower-variance strain estimates.

The resulting strain maps provide a depth-resolved depiction of how the tissue deforms under the applied load. Regions with lower stiffness deform more and thus exhibit higher strain, whereas stiffer regions deform less. This contrast mechanism underlies the mechanical imaging capability of compressional OCE.

2.6.3 Stress–Strain Relationships and Effective Stiffness Estimation

In principle, if the local stress field σ is known or controlled, compressional OCE enables direct recovery of the elastic modulus through the uniaxial constitutive law

$$\sigma = E \varepsilon. \quad (2.61)$$

When the applied stress is spatially uniform—such as during controlled indentation or distributed preload—Young’s modulus can be estimated as

$$E(z) = \frac{\sigma}{\varepsilon(z)}. \quad (2.62)$$

However, biological tissues are heterogeneous and often layered; stresses may not be perfectly uniform, and deformation fields may be influenced by sample geometry, boundary conditions, or out-of-plane effects. For this reason, compressional OCE frequently reports **relative** mechanical contrast or **effective local stiffness**, rather than absolute moduli. Nevertheless, the technique reliably captures spatial heterogeneity in stiffness, and with careful calibration, absolute quantification is achievable.

In many applications—such as corneal biomechanics, optic nerve head assessment, or brain tissue evaluation—the applied stress profile can be approximated or measured with sufficient accuracy to yield meaningful modulus values, particularly when loading conditions are repeatable and controlled.

2.6.4 Localized Compression and Microstructural Mechanical Contrast

A key strength of compressional OCE is its ability to interrogate mechanical properties at spatial scales comparable to OCT's intrinsic resolution. By confining the applied load to a narrowly defined region—through micro-indentation, focal preload modulation, or other localized compression approaches—compressional OCE isolates the mechanical response of small tissue volumes. This capability enables detailed micromechanical mapping of laminar microarchitecture in the sclera and optic nerve head, stromal layer variations within the cornea, heterogeneous elasticity in developing or pathological neural tissue, and mechanical alterations associated with tumor infiltration or fibrotic remodeling. It also facilitates the characterization of depth-resolved modulus gradients in layered biological structures. Because all regions experience the same applied load but deform according to their intrinsic stiffness, localized differences in strain reveal microstructural mechanical contrasts that are invisible in conventional OCT intensity images.

2.6.5 Advantages, Limitations, and Complementarity with Dynamic OCE

Compressional OCE offers several practical and conceptual advantages that make it a powerful tool for quantitative biomechanical imaging. Because it directly relates measured strain to the applied load, compressional OCE enables straightforward mechanical interpretation through the stress–strain relationship. Its stiffness contrast is governed primarily by OCT's intrinsic spatial resolution and the selection of the strain-estimation window, allowing high-resolution mapping of localized mechanical variations. Unlike dynamic methods, compressional OCE does not rely on the propagation of shear waves or on extremely high imaging speeds, and

its instrumentation can therefore be simpler, often requiring only controlled mechanical loading without additional acoustic sources. These features make compressional OCE particularly effective for assessing fine-scale mechanical heterogeneity in thin, layered, or slowly varying biological structures.

Despite these strengths, the approach has notable limitations. Accurate recovery of absolute moduli depends on precise knowledge of the applied stress, and deviations from idealized loading—arising from sample geometry, boundary conditions, or localized stress concentrations—can introduce nonlinearities in the measured response. Quasi-static measurements are also susceptible to bulk drift, creep, and viscoelastic relaxation, which may obscure true tissue deformation. Strain estimation amplifies high-frequency noise, necessitating carefully optimized spatial filtering to balance precision and spatial resolution. Furthermore, because compressional OCE lacks temporal information about wave dynamics, it is less informative regarding viscoelastic behavior unless combined with dynamic excitation.

For these reasons, compressional OCE and dynamic OCE are best regarded as complementary modalities. Compressional OCE excels at generating high-resolution stiffness maps under quasi-static or slowly varying conditions, whereas dynamic OCE probes frequency-dependent phenomena such as viscoelasticity, dispersion, and anisotropy through shear-wave propagation. When implemented together within a single imaging framework, these two approaches provide a comprehensive biomechanical characterization spanning multiple temporal and spatial scales.

2.7 Noise Propagation and Precision Limits

The ability of Optical Coherence Elastography (OCE) to detect nanometer-scale mechanical motion is ultimately constrained by noise sources that affect OCT phase stability. Because OCE derives displacement, strain, and mechanical parameters directly from variations in the complex OCT signal, understanding how noise propagates through the OCE processing pipeline is essential for evaluating measurement precision, optimizing system design, and interpreting quantitative biomechanical results. This section examines the principal noise sources in Fourier-domain OCT, derives their impact on displacement and strain measurements, and discusses fundamental limits that govern OCE sensitivity.

2.7.1 Phase Noise in Fourier-Domain OCT

In Fourier-domain OCT, each depth-resolved measurement can be expressed as a complex analytic signal

$$A(z, t) = |A(z, t)| e^{i\phi(z, t)},$$

where the magnitude encodes structural reflectivity and the phase $\phi(z, t)$ carries information about sub-wavelength path-length changes. Several noise sources perturb this complex signal. Shot noise, arising from the quantum statistics of photon detection, imposes a fundamental limit on sensitivity. Thermal and electronic noise originating from the spectrometer and detector further contribute to amplitude and phase fluctuations. In swept-source systems, laser sweep nonlinearity and timing jitter introduce additional phase instability, while mechanical vibrations and environmental disturbances modulate the optical path length and produce low-frequency phase drift.

For high-SNR OCT systems, these effects are commonly modeled as additive complex Gaussian noise, allowing a compact description of their influence on phase stability. Under this assumption, the variance of the measured phase is inversely proportional to the signal-to-noise ratio (SNR):

$$\sigma_\phi^2 \approx \frac{1}{\text{SNR}},$$

where SNR is expressed in linear units. This relationship establishes the fundamental phase-noise floor and therefore sets the lower bound on displacement resolution in OCE. All subsequent elastographic measurements—including displacement, strain, and modulus estimation—are constrained by this phase stability, making noise control essential for achieving reliable quantitative performance.

2.7.2 Displacement Noise Floor

Phase changes in the OCT signal are converted to axial displacement through

$$u = \frac{\Delta\phi}{2k_0},$$

where $k_0 = 2\pi/\lambda_0$ is the central optical wavenumber. The corresponding displacement noise level follows directly from the phase variance,

$$\sigma_u = \frac{\sigma_\phi}{2k_0} = \frac{1}{2k_0\sqrt{\text{SNR}}},$$

indicating that displacement sensitivity improves with both higher SNR and shorter optical wavelengths. For typical OCT systems operating between 800 and 1300 nm, $2k_0$ is on the order of 10^7 m^{-1} . As a result, even moderate SNR values (40–50 dB) enable sub-nanometer displacement resolution. This level of precision allows OCE to resolve subtle biomechanical signatures—including shear-wave oscillations, micro-strains, and boundary-layer deformations—that remain inaccessible to most other imaging modalities.

Because displacement estimation is commonly performed on a frame-to-frame basis or relative to a reference state, additional temporal noise sources may arise. Phase drift can accumulate over long acquisition intervals, physiological motion (such as blood flow or respiration) may overlap with elastographically induced motion, and mechanical or thermal variations can alter the reference-arm path length. These factors introduce low-frequency fluctuations that contaminate displacement measurements. Consequently, temporal filtering (Section 2.4.2) is essential to suppress non-elastographic motion and isolate the displacement fields that accurately reflect underlying mechanical behavior.

2.7.3 Noise Amplification in Strain Estimation

Strain is obtained by differentiating the axial displacement field,

$$\varepsilon(z) = \frac{\partial u(z)}{\partial z}.$$

Because spatial differentiation amplifies high-frequency noise, strain estimation is intrinsically more sensitive to noise than displacement. For depth sampling interval Δz , a simple finite-difference approximation yields a strain noise level of

$$\sigma_\varepsilon \approx \frac{\sigma_u}{\Delta z}.$$

Given that Δz is typically 2–10 μm in broadband OCT systems, even nanometer-scale displacement noise can produce detectable strain fluctuations on the order of 10^{-4} – 10^{-3} , even before any filtering is applied.

To mitigate this noise amplification, strain is typically estimated using linear regression over a small axial window,

$$\varepsilon(z) = \frac{\sum_{i=-N}^N (z_i - \bar{z}) u(z_i)}{\sum_{i=-N}^N (z_i - \bar{z})^2},$$

which effectively acts as a low-pass filter on the displacement field. By averaging over neighboring axial samples, this approach suppresses uncorrelated noise while preserving the underlying strain trend. The choice of window size, defined by the number of samples N , governs the trade-off between precision and spatial resolution: larger windows reduce noise but blur fine-scale mechanical variations, while smaller windows preserve sharp features at the cost of higher variance. Because compressional OCE depends critically on accurate, noise-robust strain mapping, careful window selection is a central element of experimental and algorithmic optimization.

2.7.4 Noise Limits in Dynamic OCE

Dynamic OCE infers tissue stiffness from the propagation characteristics of shear waves, making the accuracy of wave-number and frequency estimation directly dependent on the quality of the measured displacement field. For harmonic waves of the form

$$u(x, t) = A \sin(kx - \omega t) + n(x, t),$$

noise perturbs both the amplitude and phase of the waveform. In the two-dimensional Fourier (k – ω) domain, displacement noise elevates the background level of the spectrum, reduces the contrast of the dispersion ridge, and introduces bias into the extracted wave-number or frequency components. The resulting uncertainty in shear-wave speed c_s scales approximately as

$$\sigma_{c_s} \propto \frac{\sigma_u}{A f},$$

where A denotes wave amplitude, f is the excitation frequency, and σ_u represents displacement noise. This relationship highlights several important considerations: larger wave amplitudes improve measurement precision, higher frequencies increase phase sensitivity, and low-SNR displacement fields can significantly distort stiffness estimates. The scaling also explains why broadband or low-amplitude excitations often require ensemble averaging or spatial–spectral filtering to recover stable dispersion curves.

2.7.5 System-Level Factors Affecting Precision

Several hardware and system-design parameters strongly influence the precision of OCE measurements. Laser phase stability is fundamental, as swept-source jitter, timing nonlinearities, and sampling-clock errors introduce phase fluctuations that directly compromise displacement accuracy. Spectrometer sensitivity also plays a major role in spectral-domain OCT, where detector noise, grating efficiency, and readout electronics determine the achievable SNR and thus the underlying phase-noise floor. Interferometer stability is equally important; mechanical vibration, temperature drift, or other environmental perturbations modulate the optical path length and produce slow phase variations that can contaminate elastographic measurements. Sampling density in the axial direction constrains both strain precision and the degree of noise amplification during numerical differentiation. Additionally, numerical processing—including filtering, phase unwrapping, resampling, and interpolation—can introduce secondary noise artifacts unless implemented with care. Optimizing each of these factors is essential for achieving high-fidelity displacement, strain, and modulus estimates in quantitative OCE.

2.7.6 Fundamental Sensitivity Limits

When all noise sources are taken into account, the minimum detectable displacement in an ideal Fourier-domain OCT system is determined by the phase-noise floor,

$$\sigma_u^{\min} = \frac{1}{2k_0\sqrt{\text{SNR}}}.$$

For modern OCT instrumentation, this limit typically lies in the range of 0.1–1 nm, representing one of the highest displacement sensitivities achievable in biomedical imaging. This fundamental constraint has important implications for OCE system design and experimental optimization. In dynamic OCE, shear-wave amplitudes must exceed the displacement noise floor

to enable accurate phase tracking and reliable wave-speed estimation. In compressional OCE, temporal drift and cumulative noise between pre- and post-compression states must be minimized to preserve strain accuracy. Higher excitation frequencies improve displacement precision by producing larger phase excursions per unit displacement, while increased SNR directly reduces phase noise and enhances all downstream elastographic metrics. Collectively, these considerations define the precision envelope within which quantitative OCE must operate and guide the design of hardware, excitation strategies, and processing algorithms to achieve robust biomechanical measurements.

CHAPTER 3: In Vivo Quantitative OCE of the Optic Nerve Head

3.1 Introduction

Glaucoma is the second leading cause of blindness worldwide, affecting more than 60 million people, with prevalence expected to rise as populations age [74]. It is a chronic, progressive optic neuropathy that disrupts visual function through a cascade of structural and cellular changes at multiple levels of the visual pathway, including the photoreceptors and retinal ganglion cells (RGCs) [75]. Although its exact biological mechanisms remain incompletely understood, substantial evidence indicates that damage to RGC axons within the optic nerve head (ONH) represents a central pathological event in glaucoma [76]. The ONH occupies a structurally and mechanically distinct region where the corneoscleral shell transitions into the porous, collagenous lamina cribrosa. This anatomical discontinuity focuses IOP-related stresses, making the ONH especially vulnerable to deformation and strain-induced axonal injury [77, 78].

Elevated intraocular pressure (IOP) is widely recognized as the primary and most modifiable risk factor for glaucoma progression. Increased IOP imposes additional mechanical loading on the ONH, causing connective tissues to stiffen, distort, and remodel. These alterations can impede axonal transport, disrupt laminar microarchitecture, and ultimately contribute to RGC degeneration and optic nerve atrophy [79, 80]. A deeper understanding of how IOP influences ONH biomechanics is therefore essential for elucidating disease mechanisms, informing computational models, and advancing clinical risk assessment.

Multiple techniques have been employed to study the mechanical properties of the ONH and peripapillary sclera, including tensile testing [81], OCT-based deformation tracking [82], and ultrasound speckle imaging [83]. However, each method has significant limitations for in vivo assessment. Tensile testing is inherently ex vivo and cannot replicate physiological conditions. Ultrasound imaging lacks the spatial resolution needed to resolve the fine structure of the posterior eye. Conventional OCT provides excellent anatomical detail but does not yield quantitative biomechanical information. These gaps highlight the need for a noninvasive, high-resolution imaging method capable of quantifying ONH mechanics in vivo.

Elastography offers such a capability by measuring tissue deformation or elastic-wave propagation to infer material properties. Both ultrasonic elastography and optical coherence elastography (OCE) have been applied to quantify ONH stiffness under varying IOP conditions

[84, 85]. While these approaches have demonstrated feasibility, most studies have been limited to ex vivo tissues. Compared with ultrasound elastography, OCE provides superior micrometer-scale resolution, making it particularly suitable for ocular structures. Although imaging depth is typically constrained by optical scattering, the natural transparency of the cornea, aqueous humor, crystalline lens, and vitreous humor allows OCE to access the posterior eye with minimal attenuation, providing a distinct advantage in ophthalmic applications [64].

Our group has previously demonstrated the utility of OCE for quantifying the biomechanics of multiple ocular tissues, including the cornea [86, 87], crystalline lens [64], and retina [88, 89] in vivo. A key requirement for OCE is a mechanical excitation source to induce tissue deformation or shear-wave propagation. Common excitation modalities include air-puff devices [90], acoustic radiation force (ARF) [88], and mechanical shakers [91]. Air-puff stimuli attenuate rapidly and are ineffective for deep structures such as the ONH. ARF raises safety concerns due to ultrasound exposure limits in the eye [92]. Mechanical shakers, by contrast, generate broadband, high-amplitude excitations that propagate efficiently through the eye and have been used successfully in human studies without reported complications [93].

In this study, we introduce a high-resolution, high-speed shaker-based OCE system designed specifically for in vivo imaging of ONH biomechanics under controlled IOP conditions. Using a rabbit model, we demonstrate— to our knowledge for the first time—robust in vivo reconstruction of ONH elastic properties across physiologically relevant pressure levels. This chapter presents the system configuration, experimental methods, and quantitative findings, establishing a new foundation for studying ONH biomechanics and their role in glaucoma pathophysiology.

3.2 System Settings and synchronization

In this study, we introduce a high-resolution, high-speed shaker-based OCE system designed specifically for in vivo imaging of ONH biomechanics under controlled IOP conditions. Using a rabbit model, we demonstrate— to our knowledge for the first time—robust in vivo reconstruction of ONH elastic properties across physiologically relevant pressure levels. This chapter presents the system configuration, experimental methods, and quantitative findings,

establishing a new foundation for studying ONH biomechanics and their role in glaucoma pathophysiology.

A custom phase-resolved spectral-domain OCT (SD-OCT) system was developed to visualize shear-wave propagation at the optic nerve head (ONH). The system employed a broadband superluminescent diode (SLED; Superlum, USA) centered at 890 nm with a 144-nm bandwidth, providing high axial resolution and excellent phase stability. The total output power of the light source was approximately 9 mW. Before entering the interferometer, the source beam passed through an optical isolator to suppress back-reflections that could destabilize the SLED. The beam was then routed to a 20:80 fiber coupler that divided it into the reference and sample arms. The optical power delivered to the sample arm was maintained at ~2 mW, well within ANSI ophthalmic safety limits.

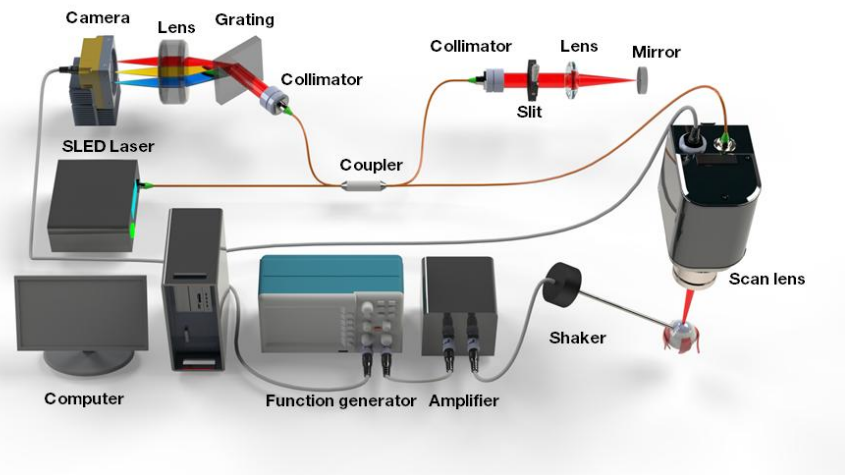


Figure 3.1: Schematics of the shaker-based OCE system

In the sample arm, the beam was collimated and directed onto a dual-axis galvanometer scanning system capable of both MB-mode acquisition and three-dimensional volumetric imaging. A scan lens focused the beam onto the ocular tissues, and backscattered light was collected and recombined with the reference arm signal to form spectral interference fringes. These fringes were recorded by a custom-built

spectrometer optimized for high-speed, low-noise detection, enabling the phase sensitivity required for nanometer-scale displacement measurements.

To generate elastic waves for OCE imaging, a miniature mechanical shaker (Type 4810; Bruel & Kjaer, Duluth, Georgia, USA) was used as the excitation source. For in vivo experiments, the shaker's rod tip was gently positioned at the corneal limbus and aligned parallel to the OCT beam to ensure efficient coupling of mechanical energy into the globe. The rod had a radius of 0.4 mm, defining a consistent contact area while minimizing localized loading effects on the cornea.

The galvanometer scanner was programmed with a fine lateral step size of 3 μm , yielding high-density sampling across the imaging field. A total of 1000 lateral positions were sequentially acquired during MB-mode OCE scans, providing a sufficiently wide field of view to capture wave propagation across the ONH. The location of the ONH was identified and verified in real time using structural OCT B-scans.

Optical exposure during OCT imaging remained well within established ophthalmic safety limits. The incident power at the corneal surface was maintained at $< 0.8 \text{ mW}$, corresponding to an irradiance of $< 0.5 \text{ mW/mm}^2$ for the 1.5-mm diameter sample beam. These values are substantially below the **ANSI Z136.1-2022 Maximum Permissible Exposure (MPE)** for continuous retinal illumination at 890 nm, ensuring safe operation even during extended acquisitions. The spectral-domain OCT system operated with short coherence length and nonfocused retinal delivery, further reducing the effective retinal hazard. Because the imaging beam was centered on the posterior pole, the optical power distribution remained uniform across the ONH with no localized hotspots or scanning-induced intensity buildup. No photothermal or photochemical risks were expected given the low irradiance, short acquisition duty cycle, and near-infrared wavelength. Consistent with these safety considerations, **post-imaging slit-lamp evaluation revealed no signs of corneal epithelial disruption, iris transillumination defects, or retinal whitening**, confirming that the optical imaging parameters posed no acute risk to ocular tissues.

The mechanical excitation delivered by the electromechanical shaker was carefully regulated to ensure safe interaction with the ocular surface. The shaker produced **submillimeter**

vibrational amplitudes (typically 50–200 μm peak-to-peak at 400 Hz) and **dynamic forces below 0.1 N**, transmitted through a compliant silicone tip placed gently against the closed eyelid. This configuration ensured that the applied load remained purely dynamic, with negligible static compression on the globe and **no measurable alteration of intraocular pressure (IOP)**. The eyelid acted as a natural mechanical buffer, distributing the dynamic load broadly across the anterior scleral surface and minimizing localized stress concentrations. Owing to the low-frequency, low-amplitude nature of the excitation, the induced vibrations generated only mild shear waves without imposing significant indentation or altering corneal curvature. **Post-imaging slit-lamp examinations** consistently showed **no evidence of corneal abrasion, conjunctival irritation, epithelial compromise, or scleral injury**, and the anterior chamber remained clear and quiet after all trials. These observations confirm that the chosen mechanical exposure parameters were well within safe limits and did not introduce any detectable acute ocular trauma during or after elastography imaging.

IOP manipulation and monitoring were performed using a custom control system. A reservoir filled with sterile phosphate-buffered saline (PBS) was connected to the anterior chamber through a 23-gauge needle. By adjusting the height of the reservoir, IOP could be increased or decreased in a controlled manner according to the hydrostatic pressure relationship. To enable continuous pressure monitoring, a second small scleral port was created near the posterior pole, through which an intracameral pressure probe was inserted. Prior to each OCE measurement, IOP was allowed to stabilize for at least three minutes to eliminate transient fluctuations and ensure consistent biomechanical loading.

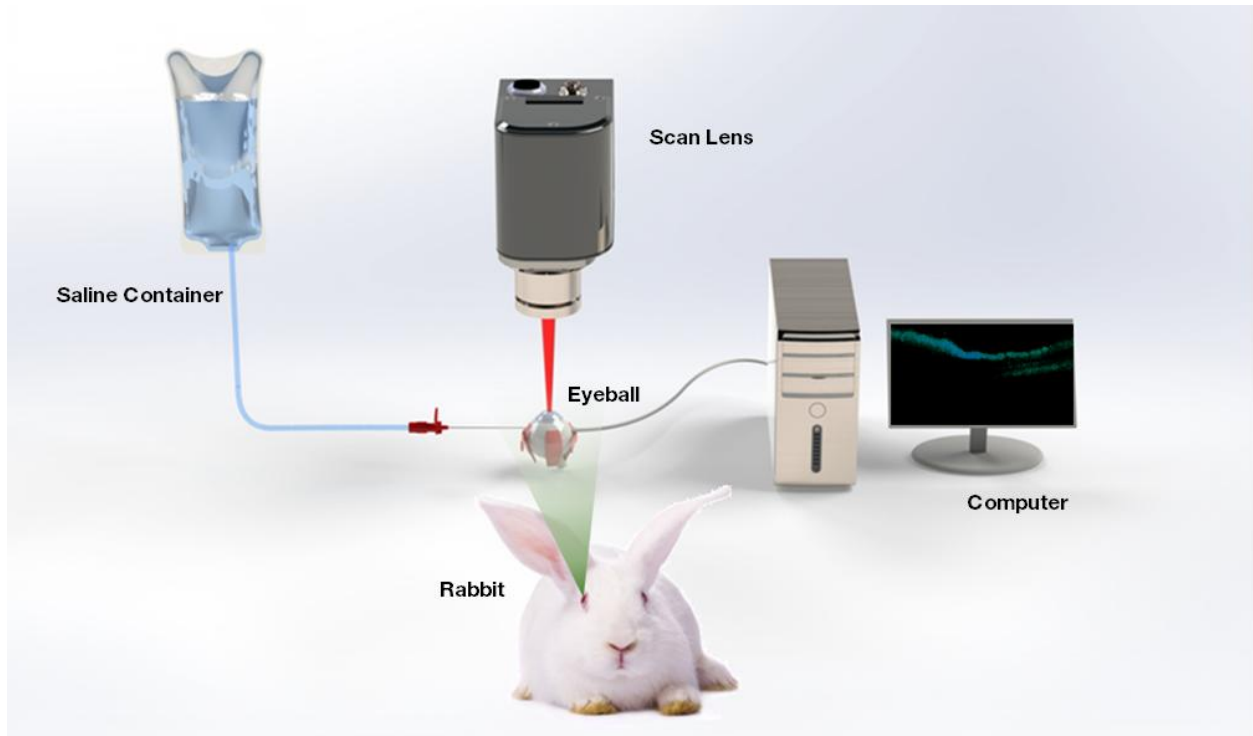


Figure 3.2 IOP control and detection system

Accurate visualization of shear-wave propagation requires precise temporal coordination between mechanical excitation and OCT data acquisition. To achieve this, the system employed a customized MB-mode scanning protocol designed to capture the temporal evolution of tissue motion at each spatial location with high phase stability. The protocol began with an M-mode acquisition in which 400 consecutive A-lines were recorded at a single lateral position. These A-lines were acquired at a sampling rate of 50 kHz, providing sufficient temporal resolution to resolve the rapid passage of the induced elastic wave.

Mechanical excitation from the shaker was triggered during each M-mode sequence. Specifically, the computer-controlled synchronization module initiated the shaker pulse at the 100th A-line, ensuring that the onset of the elastic wave occurred at a consistent time point across all lateral positions. This precise temporal alignment is essential for phase-resolved elastography, where even small timing discrepancies can introduce phase noise or distort the recovered displacement fields.

After completing one M-mode acquisition, the galvanometer scanner advanced the OCT beam laterally by $3\text{ }\mu\text{m}$ to the next measurement location. The same M-mode protocol—consisting of 400 A-lines with synchronized mechanical excitation—was then repeated. This process continued sequentially across all lateral positions until the entire scanning range was covered. By combining densely spaced M-mode recordings along the lateral axis, a full MB-mode dataset was constructed, capturing both spatial and temporal information of the shear-wave propagation.

A set of digital trigger signals, generated by the data acquisition computer, ensured precise synchronization between the line-scan camera and the mechanical shaker. This timing accuracy was critical for maintaining the phase stability required by Doppler and phase-resolved OCT techniques, which rely on detecting subtle, nanometer-scale changes in optical path length. The resulting MB-mode dataset enabled construction of spatiotemporal displacement maps that clearly depicted the trajectory and velocity of the elastic waves as they traversed the ONH.

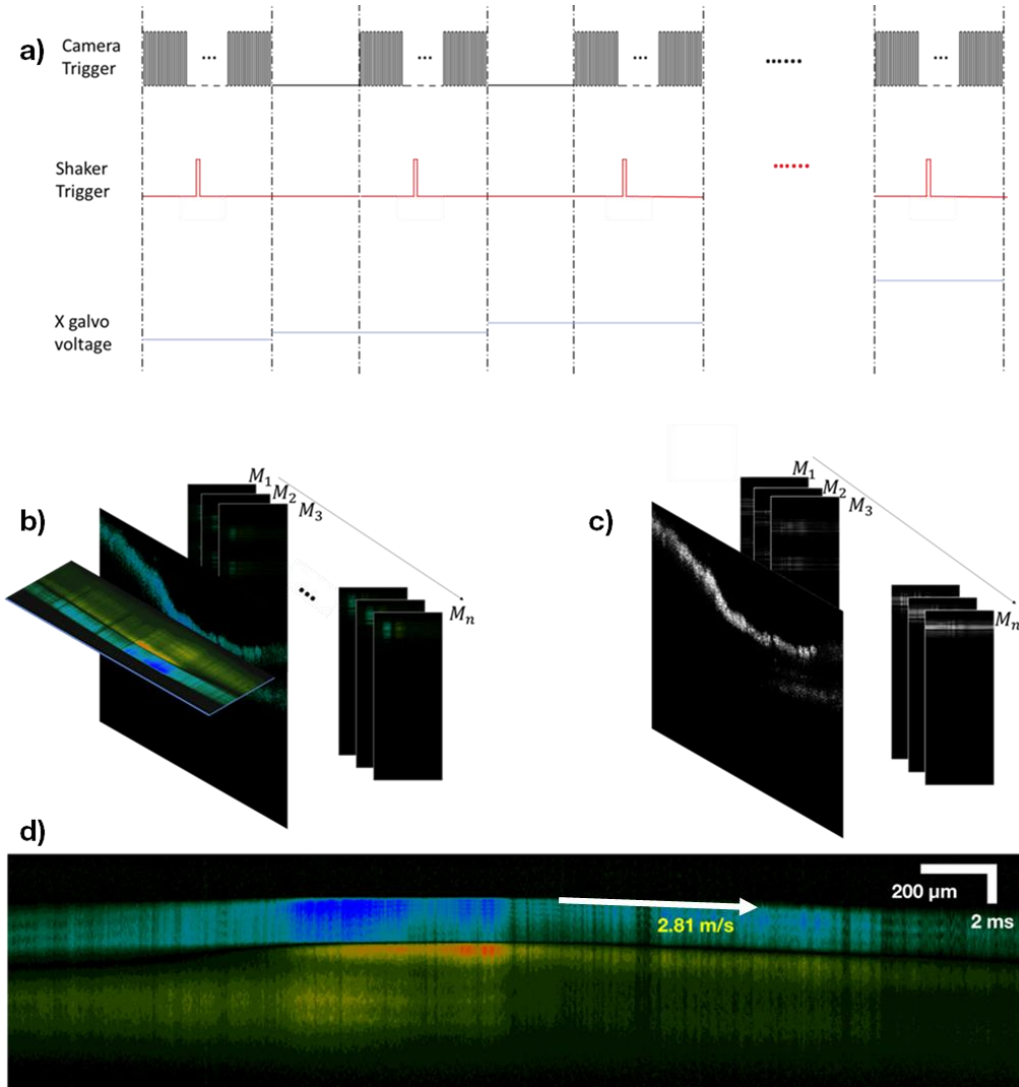


Figure 3.3 Timing diagram of the MB scanning mode and the OCT images (a) Timing diagram of the MB-mode scanning protocol (b) Doppler OCT sequences of MB-mode (c) OCT sequences of MB-mode (d) Spatiotemporal Doppler OCT image

3.3 Results

The New Zealand white rabbit eye serves as an ideal experimental model because its ocular dimensions and posterior segment architecture closely approximate those of the human eye. This anatomical similarity enables evaluation of posterior biomechanics under conditions that are relevant to potential future clinical applications. Although a mechanical shaker was used in this study to ensure strong and controllable wave excitation, the same OCE framework could be translated clinically using noncontact methods, such as an ultrasonic transducer coupled with ocular gel to generate acoustic radiation force. Such an approach would allow safe in vivo biomechanical assessment without direct corneal contact and could serve as a foundation for developing a noninvasive “optical biopsy” technique for ONH elasticity.

To validate the feasibility of measuring ONH mechanics and to characterize baseline tissue behavior without physiological motion, ex vivo OCE imaging was first performed on freshly enucleated rabbit eyes. High-resolution structural OCT imaging successfully captured the anatomy of the posterior pole, revealing the ONH as a distinct depression situated significantly deeper than the surrounding peripheral retina. Following structural acquisition, Doppler phase-resolved OCT was applied to these ex vivo samples to quantify the elasticity of the ONH. The system produced a clear three-dimensional reconstruction of the ONH region and enabled reliable detection of wave-induced displacements within the tissue. These preliminary ex vivo results confirmed that the proposed OCE system could penetrate to the depth of the ONH, resolve its structural morphology, and detect wave propagation with sufficient sensitivity for meaningful biomechanical analysis.

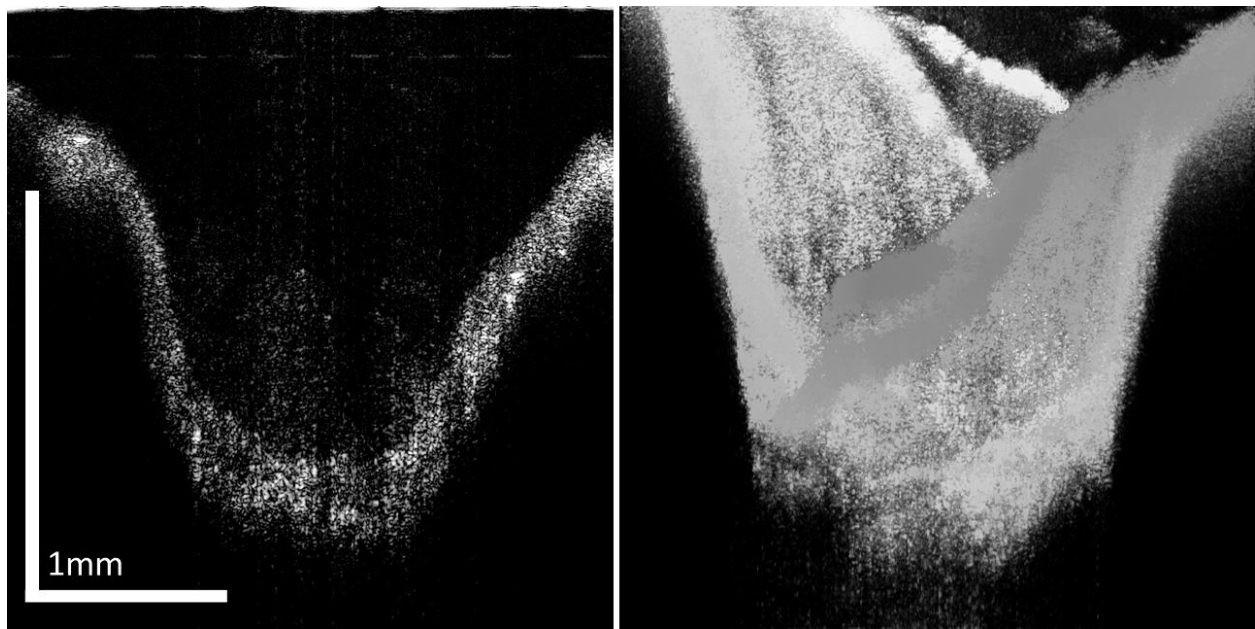


Figure.3.4 OCT images and 3D reconstruction of ex-vivo rabbit ONH

3.3.1 Ex Vivo Experiment

To characterize wave behavior in the ONH ex vivo, structural OCT imaging was first used to identify an optically accessible and relatively flat region of the ONH suitable for visualizing shear-wave propagation. In the time-resolved structural OCT B-scans, only subtle brightness fluctuations were visible, and no appreciable morphological changes could be observed during the excitation sequence. However, once Doppler phase analysis was applied, the nanometer-scale displacements induced by the external excitation became clearly detectable. At

approximately 0.08 ms after excitation, a pronounced phase shift appeared near the center of the ONH, corresponding to the initial location of shear-wave generation. As time progressed, the wavefront propagated radially outward toward the periphery of the ONH, demonstrating the ability of the system to follow dynamic mechanical disturbances with high temporal and spatial resolution.

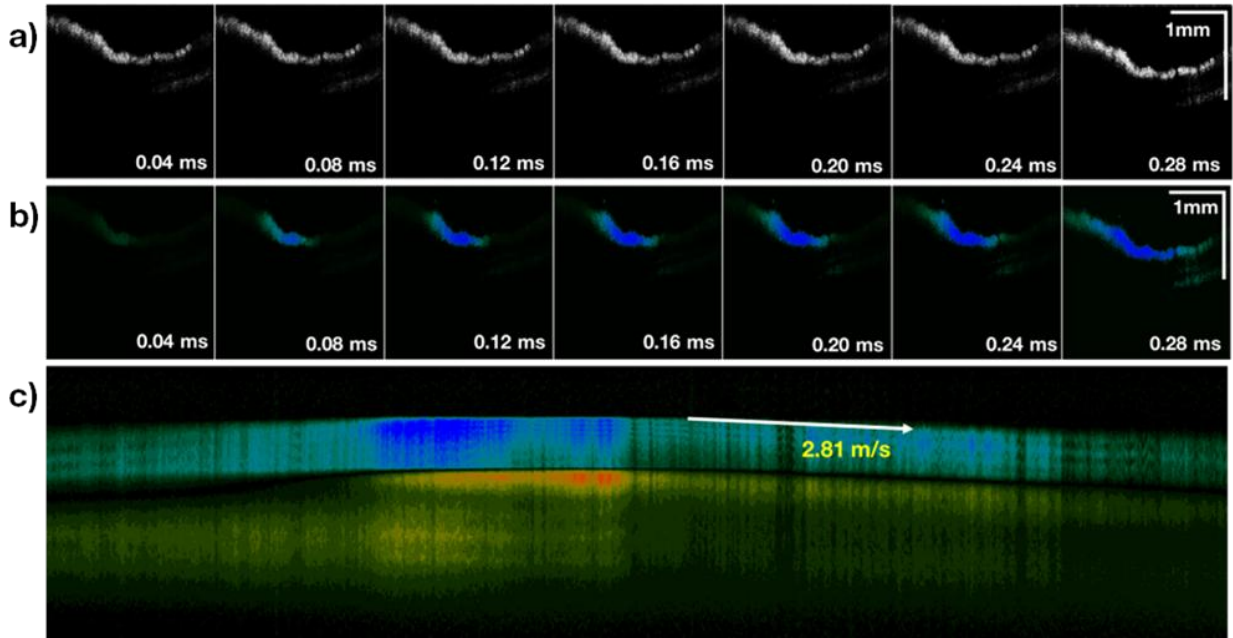


Figure 3.5 Ex-vivo ONH OCT and elastography. (a) Time-lapse OCT B-scan images of an ONH. (b) Time-lapse Doppler OCT B-scans of an ONH. (c) Spatiotemporal Doppler OCT of the ONH

From these displacement image sequences, shear-wave velocity could be quantified by measuring the distance traveled by the wavefront over a known time interval. Although direct measurement from individual frames is feasible, a more intuitive and robust approach involves examining the displacement of a fixed depth position as a function of time. Constructing a spatiotemporal displacement map enables visualization of the wave trajectory as a diagonal ridge, with the vertical axis representing time and the horizontal axis representing spatial position across the ONH. The slope of this ridge provides a direct measurement of shear-wave velocity. Using this method, the velocity of shear-wave propagation in the ex vivo rabbit ONH was determined to be approximately 2.81 m/s. Applying the Voigt-model-based relationship between wave velocity and material stiffness, the corresponding Young's modulus of the ONH was calculated to be 23.69 kPa.

To further investigate how mechanical loading influences ONH elasticity, the same measurement procedure was repeated while incrementally elevating the intraocular pressure. Under each IOP condition, Doppler OCE imaging exhibited clear displacement wavefronts propagating across the ONH, confirming that wave visualization remained stable across a physiologically relevant pressure range. Spatiotemporal displacement maps again provided a straightforward method for extracting shear-wave velocity, and a consistent trend emerged: wave speed increased with higher IOP. Because shear-wave velocity scales with the square root of tissue stiffness, this increase directly translated to higher reconstructed Young's modulus values. Specifically, the ONH exhibited stiffness values of approximately 21.39 kPa, 27.18 kPa, and 36.96 kPa at IOP levels of 15, 20, and 25 mmHg, respectively. These results demonstrate a clear positive relationship between ONH elasticity and intraocular pressure, consistent with the expected nonlinear stiffening response of posterior ocular tissues under increased mechanical load.

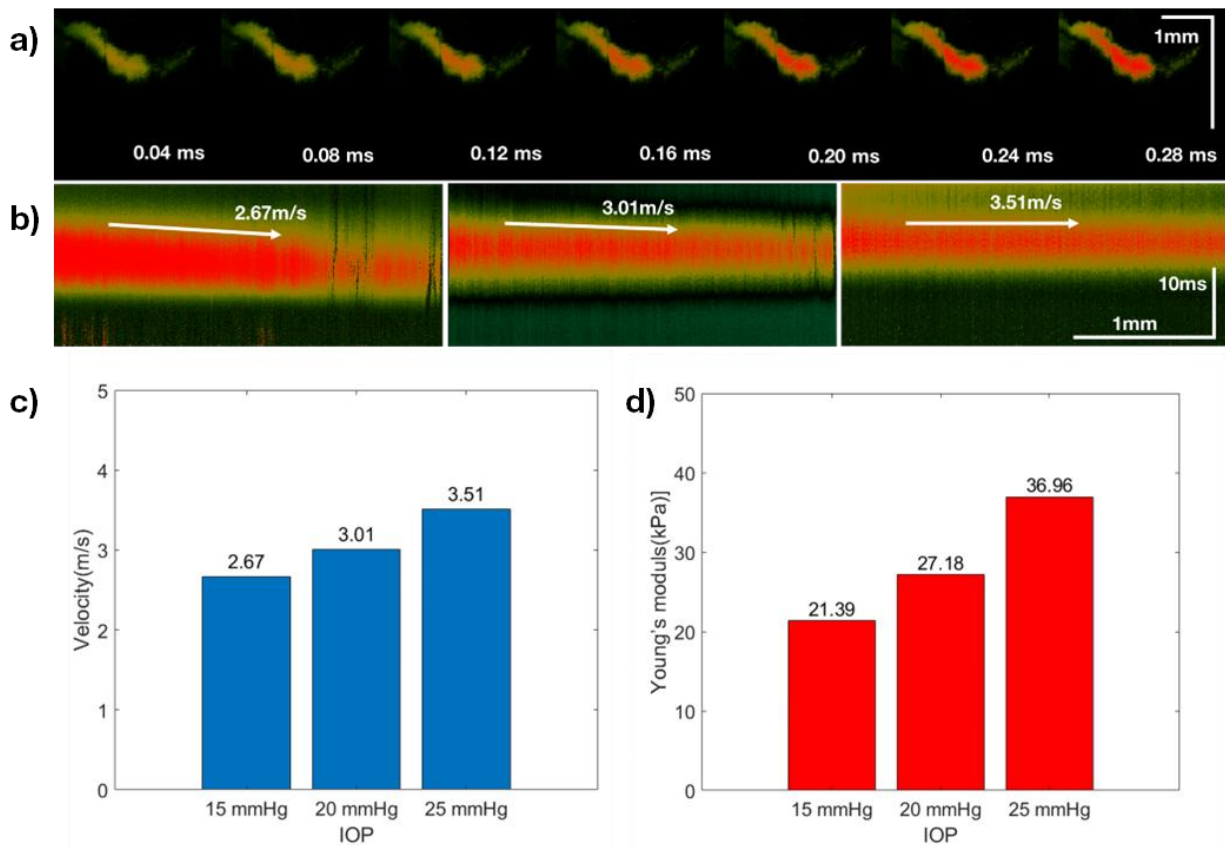


Figure 3.6 Elasticity of the ex-vivo ONH. (a) Time-lapse OCT B-scan images of an ONH. (b) Spatiotemporal Doppler OCT of the ONH under different IOP (c) Shear wave velocities in the ONH and corresponding Young's modulus at various IOP

3.3.2 In Vivo Experiment

To evaluate the capability of the Doppler OCT system to measure elastic properties in vivo, additional experiments were performed using live anesthetized rabbits under controlled intraocular pressures. These measurements introduced several physiological factors—such as blood flow, ocular pulsation, and respiratory motion—that are absent in ex vivo experiments but must be managed for reliable in vivo elastography. Time-resolved Doppler OCT B-scans revealed distinct phase shifts at both the surface and deeper regions of the ONH, arising from blood flow within the retinal and laminar vasculature. Although these flow-related phase fluctuations were expected, they introduced localized noise that, if unaccounted for, could distort displacement measurements.

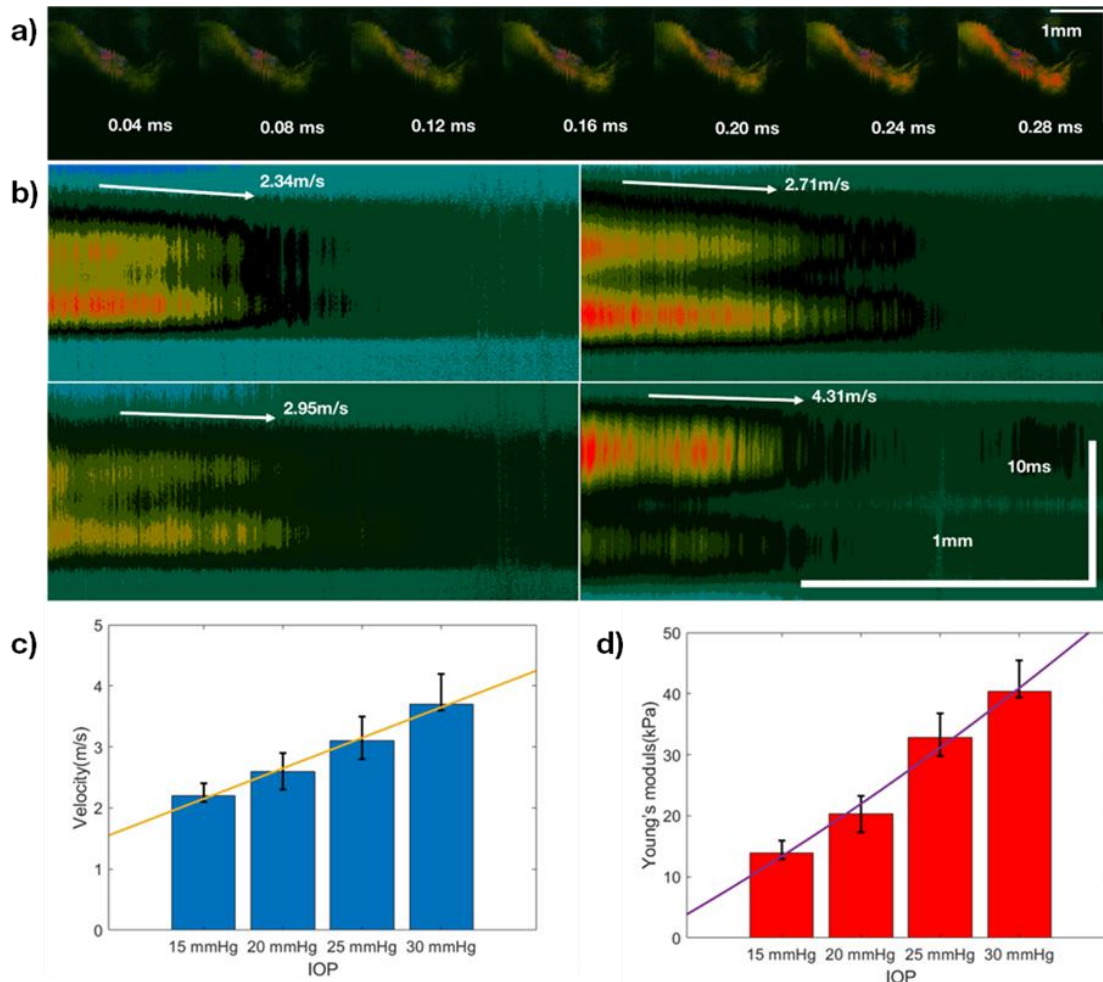


Figure 3.7 Elasticity of the in-vivo ONH. (a) Time-lapse OCT B-scan images of an ONH. (b) Spatiotemporal Doppler OCT of the ONH under different IOP (c) Shear wave velocities in the ONH and (d) corresponding Young's modulus as a function of IOP.

Despite the presence of blood flow, shear-wave propagation could still be clearly visualized across the ONH. By assembling displacement traces from successive A-lines into a spatiotemporal map, the shear wave appeared as an oblique trajectory similar to that observed in ex vivo experiments. To ensure that blood-flow-induced artifacts did not bias the velocity estimation, wave speed was extracted from regions at the periphery of the wavefront slope, where vascular contributions were minimal and the displacement signal was dominated by mechanical excitation. This approach provided a reliable estimate of the true shear-wave velocity.

As in the ex vivo case, shear-wave velocity increased systematically with elevated IOP. The velocity–pressure relationship demonstrated a strong positive correlation, confirming that ONH tissue stiffens under increased mechanical loading even under physiological conditions. A linear regression applied to the measured velocities showed consistent pressure-dependent trends across multiple animals. Using the established Voigt-model-based relationship between wave velocity and material stiffness, Young’s modulus values were then calculated for each IOP level. The resulting stiffness measurements followed a nonlinear—approximately quadratic—trend, with Young’s modulus increasing more rapidly at higher pressures. This behavior aligns with the well-known nonlinear stiffening response of collagenous ocular tissues, in which fiber recruitment and load redistribution progressively enhance tissue rigidity as IOP rises.

Together, these in vivo results demonstrate that the proposed Doppler OCE system is capable of detecting shear-wave propagation at the ONH with sufficient sensitivity and robustness to physiological noise. They further confirm that ONH elasticity is strongly dependent on IOP, validating the findings observed in the ex vivo experiments and establishing the feasibility of in vivo biomechanical assessment of the posterior eye.

The in vivo mechanical characterization of the ONH remains a major challenge, yet it offers substantial value for understanding glaucoma pathogenesis. The results of this study demonstrate that phase-resolved OCE provides a sensitive and repeatable approach for quantifying ONH stiffness under controlled physiological conditions. Both ex vivo and in vivo experiments revealed a strong positive relationship between IOP and ONH elasticity, consistent with computational models and prior ex vivo mechanical testing studies.

The mechanical shaker employed here offers several advantages over other excitation methods. Unlike air-puff or acoustic radiation force approaches, the shaker delivers high-amplitude, broadband mechanical stimulation that penetrates through the anterior and posterior segments. Although the excitation is non-confocal with the OCT beam and does not generate purely shear waves, prior work has shown that the resulting wave velocity differences are negligible for practical elastography. The simplicity and robustness of shaker-based excitation also support potential future translation into clinical systems.

Several challenges remain for further development. Imaging speed limits the temporal resolution and may restrict the ability to probe faster or weaker mechanical waves, though emerging MHz-rate swept sources may help overcome this. Image quality at high IOP can degrade due to corneal swelling and optical aberrations, suggesting that improved pressure control interfaces or wavefront correction strategies may be beneficial. Furthermore, the ONH is structurally complex and biomechanically heterogeneous; future work combining 3D elastography with anatomical modeling may enable more detailed assessment of regional variations relevant to glaucoma.

3.4 Summary

This chapter presented the first in vivo quantification of optic nerve head elasticity using phase-resolved optical coherence elastography. A shaker-based excitation strategy enabled robust wave generation, and a high-speed SD-OCT system provided the phase sensitivity necessary to track nanometer-scale displacements at the ONH. Both ex vivo and in vivo results demonstrated a positive relationship between IOP and ONH stiffness, supporting the biomechanical hypothesis of glaucoma vulnerability. These findings establish a foundation for future clinical translation of ONH elastography and highlight the potential of OCE for advancing biomechanical assessment in ophthalmology.

CHAPTER 4: Multiscale Analysis of Scleral Anisotropy

4.1 Introduction

The sclera, a dense collagenous shell encasing the eye, is the principal structure responsible for preserving globe shape and resisting deformation under intraocular pressure (IOP) [94] [95]. Its biomechanical properties influence not only global ocular stability but also the stresses transmitted to sensitive structures such as the optic nerve head. While extensive research has been dedicated to the posterior sclera and peripapillary region—motivated largely by glaucoma pathophysiology—the **equatorial sclera** has received far less attention. This is surprising given that the equatorial region is intimately involved in several clinically significant behaviors, including **axial elongation in myopia**, **pressure-induced shape changes**, and **globe deformation during blunt trauma**. A rigorous biomechanical characterization of the equatorial sclera, particularly its directional stiffness and pressure-dependent mechanical response, is therefore critical for understanding ocular health and disease progression.

Soft biological tissues are inherently **anisotropic**, exhibiting direction-dependent mechanical behavior due to variations in collagen fiber alignment, lamellar organization, and extracellular matrix interactions[96-99]. Traditional interpretations of scleral mechanics often assume that material anisotropy arises directly from collagen fiber orientation—i.e., that tissues are stiffer along the predominant fiber axis. However, recent work has revealed a more complex structure–function relationship. Fiber waviness, branching, interweaving between layers, and depth-dependent inclination can all modulate mechanical response in ways not directly predicted by planar orientation alone[100]. These findings underscore the need for **direct mechanical measurement**, rather than reliance on microstructural imaging alone, to understand how the equatorial sclera responds to loading.

Optical coherence elastography (OCE) provides a uniquely powerful approach for this purpose. OCE detects micrometer-scale displacements induced by propagating elastic waves, enabling high-resolution, depth-sensitive mapping of tissue stiffness. Its combination of optical-scale resolution and sensitivity to small elastic deformations makes it particularly well suited for thin ocular structures, where conventional elastography modalities may lack precision. Although OCE has been successfully applied to the cornea, crystalline lens, retina, and optic nerve head, its use for quantifying directional biomechanics of the **equatorial sclera** has not been previously

established. In this chapter, OCE serves as the **primary quantitative method** for assessing scleral anisotropy under controlled IOP loading.

To strengthen and contextualize OCE findings, we incorporated complementary multimodal techniques:

Ultrasonic Elastography (UE).

UE measures elastic wave propagation using an ultrasound transducer array, providing a wider field of view and deeper penetration than OCT-based detection. While UE lacks the micrometer-scale sensitivity of OCE, its ability to visualize larger regions and validate directional trends makes it an ideal complementary technique for confirming global anisotropy patterns across quadrants.

Polarized Light Microscopy (PLM).

PLM visualizes collagen fiber orientation by exploiting birefringence, generating high-resolution mapping of in-plane collagen alignment. PLM has been widely used to characterize scleral and corneal architecture and offers valuable structural insight into the alignment, dispersion, and organization of collagen fibers across the equatorial region.

Scanning and Transmission Electron Microscopy (SEM/TEM).

These high-resolution imaging modalities provide sub-micrometer detail of collagen fibril topology, including interfibrillar spacing, cross-sectional arrangement, fiber waviness, and interweaving across lamellae. SEM and TEM help illustrate the **multi-scale complexity** of scleral collagen architecture, clarifying why macroscopic mechanical behavior may diverge from simple planar fiber orientation.

By integrating OCE's high-resolution mechanical quantification with UE's wide-field perspective and PLM/SEM/TEM structural analyses, this chapter presents a **hybrid, multi-scale examination** of equatorial scleral anisotropy. Specifically, we aim to:

1. **Quantify direction-dependent wave speeds** in the equatorial sclera using OCE across multiple IOP levels.
2. **Compare mechanical anisotropy across ocular quadrants**, identifying regional differences in stiffness.
3. **Examine the relationship between mechanical anisotropy and collagen organization**, evaluating whether functional stiffness follows or diverges from structural alignment.

4. **Elucidate how IOP influences both stiffness and anisotropy**, providing insight into pressure-dependent recruitment of collagen and matrix elements.

The results presented in this chapter advance our understanding of equatorial scleral biomechanics and offer implications for ocular diseases—including myopia and glaucoma—as well as ocular trauma biomechanics. Furthermore, this work establishes OCE as a robust tool for probing direction-dependent mechanical properties in thin, anisotropic ocular tissues, laying the groundwork for future **in vivo** applications.

4.2 method

This section describes the multimodal experimental framework used to quantify the anisotropic mechanical properties of the equatorial sclera. Optical coherence elastography (OCE) was implemented as the primary modality for measuring elastic wave propagation with high spatial sensitivity, while ultrasonic elastography (UE) provided complementary wide-field-of-view validation. To contextualize the mechanical findings within the underlying tissue architecture, polarized light microscopy (PLM) and electron microscopy (SEM and TEM) were employed to characterize collagen fiber orientations and microstructural features at multiple scales. The following subsections detail the experimental design, tissue preparation, OCE acquisition and analysis, validation procedures, and structural imaging methods.

4.2.1 Overview of Experimental Approach

The overarching objective of this study was to quantitatively characterize the anisotropic mechanical properties of the equatorial sclera using a rigorously controlled, multi-instrument experimental design. Because the sclera is a thin, highly heterogeneous, and directionally dependent collagenous tissue, a single imaging or elastography modality is insufficient to capture its full mechanical and structural complexity. To address this challenge, we implemented a **multi-scale, multi-modal experimental framework centered on optical coherence elastography (OCE) and ultrasound elastography (UE), supported by complementary validation and structural imaging techniques.**

Optical coherence elastography (OCE) served as the primary measurement modality due to its ability to detect nanometer- to micrometer-scale displacements with high axial sensitivity and depth resolution. The confocal OCT/ultrasound architecture used in this study enabled the induction of localized elastic waves through acoustic radiation force (ARF) and

subsequent detection of wave propagation through phase-resolved OCT. The combination of high temporal sampling rate, wide spectral bandwidth, and precise synchronization between excitation and detection permitted accurate reconstruction of spatial–temporal displacement fields within the thin equatorial sclera. This made OCE ideally suited for resolving directional differences in wave speed at the scale of tens of micrometers—an essential capability for quantifying anisotropy in ocular tissues.

Because elastography-derived wave speeds can depend on several experimental and tissue parameters—including sample geometry, boundary conditions, and excitation configuration—**ultrasonic elastography (UE)** was incorporated as an independent mechanical validation modality. UE employs ultrafast plane-wave imaging to track shear or guided wave propagation over a larger field of view than OCT-based methods. Although its displacement sensitivity is lower than that of OCE, UE offers advantages in penetration depth, imaging coverage, and robustness to optical scattering. By performing UE and OCE on matched quadrants under identical IOP loading protocols, we verified that observed directional stiffness trends were inherent to the tissue and not an artifact of a particular instrumentation approach. Furthermore, UE allowed us to confirm that elastic wave propagation in the equatorial sclera was sufficiently coherent and directional to permit quantitative wave-speed estimation.

To contextualize the mechanical measurements within the underlying collagen architecture, two complementary structural imaging techniques were used. **Polarized light microscopy (PLM)** was implemented to quantify in-plane collagen fiber orientation distributions throughout the full thickness of the equatorial sclera. PLM relies on tissue birefringence and enables high-throughput mapping of fiber orientation with micrometer-scale lateral resolution. By registering serial 30- μm cryosections and computing orientation histograms across sections, PLM provided quadrant-specific, statistically robust measurements of bulk fiber alignment. These data allowed us to assess whether OCE- and UE-derived mechanical anisotropy aligned with predominant collagen orientation, and to identify cases where structural and mechanical anisotropy diverged.

Because birefringence-based analyses capture only in-plane orientation and lack sensitivity to out-of-plane fiber inclination, **scanning electron microscopy (SEM)** and **transmission electron microscopy (TEM)** were used to examine the mesoscale and nanoscale architecture of collagen fibrils. SEM offered visualization of fiber interweaving, lamellar

transitions, and spatial variations in collagen bundle density at scales relevant to mechanical load transfer. TEM allowed characterization of fibril diameter, packing, undulation, and three-dimensional orientation dispersion at sub-micrometer resolution. These structural data were critical for interpreting mechanical anisotropy that could not be explained solely by planar fiber alignment, such as cases involving fiber crimp, twisted bundles, and multi-angle lamellar arrangement in the scleral stroma.

Experiments were performed *ex vivo* using fresh porcine eyes, which possess scleral microstructure, thickness profiles, and mechanical properties comparable to those of the human globe. The equatorial region of each eye was divided into four anatomical quadrants—Nasal, Superior, Temporal, and Inferior—and elastic wave propagation was measured along two orthogonal anatomical axes: the **meridional direction** (anterior–posterior) and the **equatorial direction** (circumferential). To assess pressure-dependent biomechanical behavior, intraocular pressure (IOP) was controlled in real time and incremented systematically from **10 to 30 mmHg** in steps of 5 mmHg. This allowed evaluation of mechanical anisotropy as a function of load, consistent with physiological ranges of IOP.

Together, these elements formed a deeply integrated experimental platform in which **OCE provided high-resolution mechanical quantification**, **UE validated directional stiffness at larger spatial scales**, and **PLM/SEM/TEM supplied multiscale structural correlates**. This hybrid methodological design was essential for disentangling the relationships between collagen architecture, IOP, and direction-dependent mechanical response in the equatorial sclera. The following subsections detail the implementation of each component, including sample preparation, elastography acquisition protocols, displacement reconstruction algorithms, structural imaging workflows, and statistical analysis methods.

4.2.2 Tissue Preparation and Intraocular Pressure Regulation

Fresh porcine eyes were obtained from a research-grade supplier and used within twelve hours of enucleation to minimize post-mortem alterations in hydration, collagen cross-linking, and stromal integrity. Immediately upon receipt, each globe was carefully cleaned using microsurgical tools to remove extraocular muscles, fascial tissue, and residual orbital fat while preserving the native geometry of the scleral shell. Particular care was taken to avoid compressive loading or distortion of the limbal region and posterior pole during preparation, as even small deformations can influence subsequent elastographic measurements. Following

dissection, the eyes were mounted in a custom-engineered holder designed to stabilize the globe without constraining physiological deformation under pressure. The holder incorporated a recessed cavity to support the optic nerve stump and a broad limbal support surface that prevented rotation during imaging, ensuring that quadrant registration and directional alignment remained consistent across all modalities.

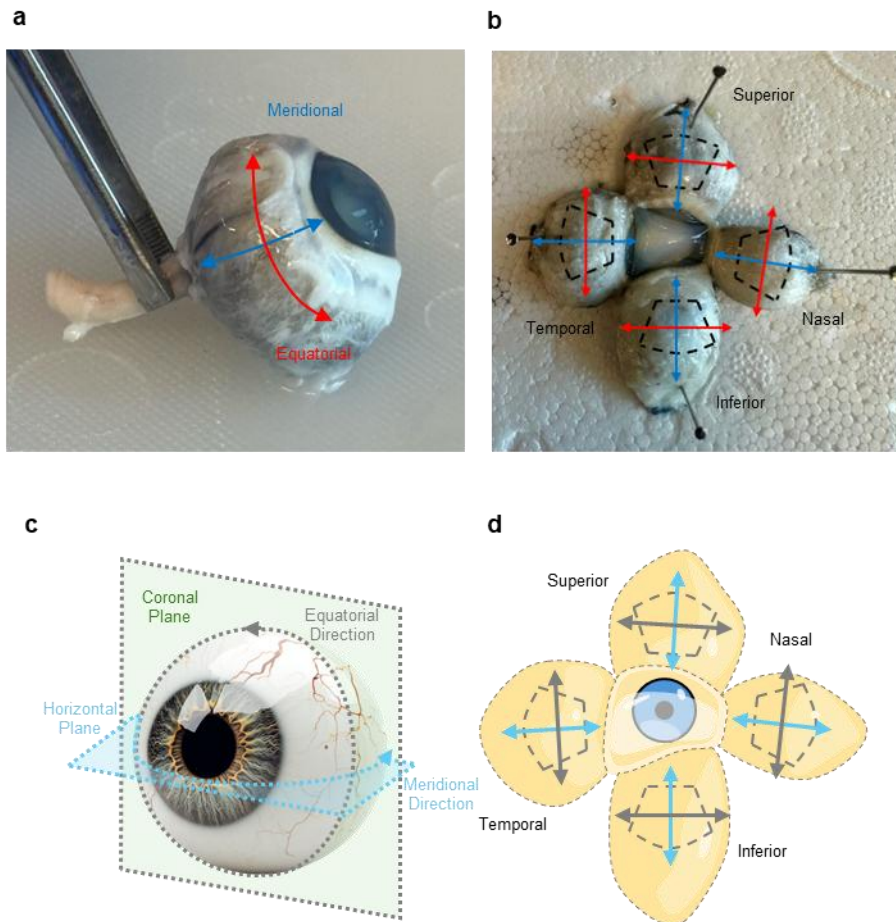


Figure 4.1 Scleral orientation, quadrant dissection, and directional analysis. (a) Intact globe showing the meridional (blue) and equatorial (red) directions. (b) The eye is divided into superior, inferior, nasal, and temporal quadrants, with each flap pinned flat while preserving orientation. Directional axes are marked to guide OCE measurements. (c) Anatomical diagram illustrating how meridional and equatorial axes correspond to longitudinal and circumferential orientations on the corneal plane. (d) Schematic of the four flattened quadrants, showing dual-axis (meridional/equatorial) measurement directions used to assess regional scleral anisotropy.

While blue arrow indicates meridional direction, grey arrow indicates equatorial direction.

Intraocular pressure (IOP) was controlled using a closed-loop fluidic regulation system consisting of a precision pressure controller, a digital transducer, and a saline reservoir connected through microfluidic tubing to a 30-gauge needle inserted at the peripheral cornea. The needle

was introduced tangentially to reduce corneal deformation and to minimize leakage around the insertion site. Before each experiment, the fluidic system was fully flushed with saline to remove air bubbles, and the pressure controller was calibrated against a NIST-traceable manometer to ensure measurement accuracy. During imaging, the controller operated in constant-pressure mode, dynamically adjusting reservoir height and flow to maintain the designated pressure set-point. Continuous feedback from the pressure transducer enabled stabilization within ± 0.5 mmHg, which was essential for accurately assessing pressure-dependent changes in elastic wave speed.

The experimental protocol involved incrementally increasing IOP from 10 to 30 mmHg in 5-mmHg steps. At each pressure level, the system was allowed to equilibrate for approximately one minute to account for viscoelastic creep and fluid redistribution within the scleral shell. Prior to data acquisition, the equatorial region was inspected using B-mode OCT or ultrasound to confirm the absence of trapped air, localized detachment, or structural compromises that could interfere with wave propagation. For each quadrant, elastographic measurements were performed in both the meridional and equatorial directions while maintaining the same pressure level, after which the IOP was temporarily returned to 10 mmHg to minimize cumulative loading effects. This pseudo-baseline step helped reduce the influence of preconditioning and ensured that stiffness measurements reflected the instantaneous mechanical state at each load rather than a progressive pressure-induced history.

To maintain anatomical consistency between measurements and modalities, the limbus of each eye was marked at four standardized positions corresponding to the nasal, superior, temporal, and inferior quadrants. These fiducial marks served as reference points throughout the elastography sessions and later during structural imaging with PLM, SEM, and TEM, enabling accurate spatial registration between mechanical measurements and collagen microstructural assessments. Experiments were terminated and repeated if pressure instability, fluid leakage, scleral buckling, or motion artifacts were observed. Typically, a minority of datasets required exclusion based on these criteria, ensuring that only high-quality, physiologically representative data were included in subsequent analyses.

4.2.3 Optical Coherence Elastography System

The primary modality used to quantify elastic wave propagation in the equatorial sclera was a custom-built phase-resolved optical coherence elastography (OCE) system integrated with

an acoustic radiation force (ARF) excitation source. The system was based on a broadband spectral-domain OCT platform centered at 890 nm with a 144-nm bandwidth, providing an axial resolution of approximately $2.4\ \mu\text{m}$ in tissue and a lateral resolution of $15.3\ \mu\text{m}$ at the focal plane. This high-resolution optical subsystem enabled precise mapping of the scleral surface and ensured that the propagation of small-amplitude elastic waves could be monitored with micrometer-scale sensitivity across the thin equatorial region.

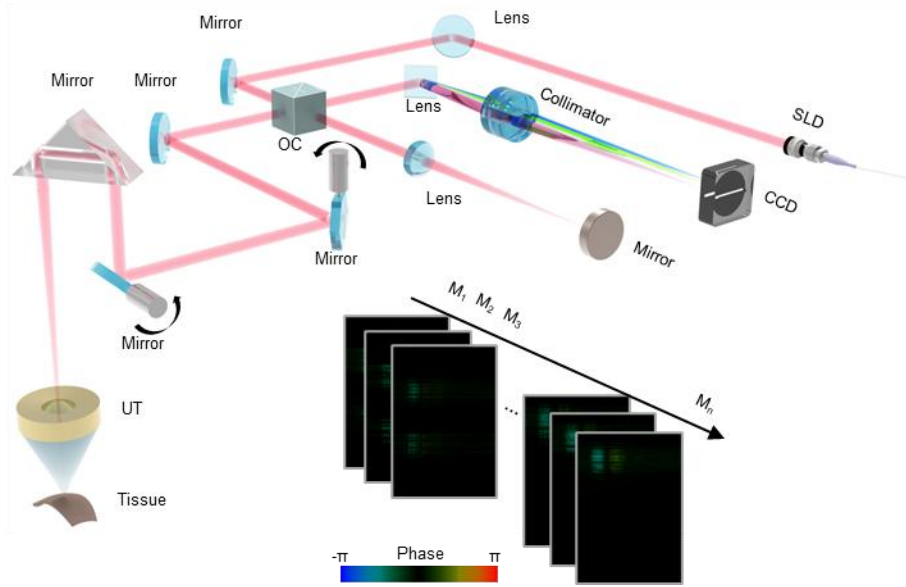


Figure 4.2 Schematic of the OCE system. At each lateral position, *M*-mode images were acquired by measuring phase shifts using Doppler OCT. These *M*-mode images were then used to reconstruct 3D axial displacement data. Scale bars, $50\ \mu\text{m}$ (z) and $500\ \mu\text{s}$ (t).

Elastic wave generation was achieved using a 6.8-MHz ring-shaped ultrasound transducer positioned such that the OCT sample beam passed through the central aperture of the transducer in a confocal configuration. This geometry ensured that ARF excitation and OCT detection were spatially co-aligned, thereby improving wave coherence and minimizing lateral misregistration between the induced mechanical perturbation and the detection beam. Each ARF burst generated a localized transient force normal to the tissue surface, launching a guided elastic wave that propagated tangentially through the scleral shell. The excitation amplitude and pulse duration were carefully selected to produce waveforms within the dynamic range of the OCT phase detection without causing tissue damage or nonlinear distortion.

During OCE acquisition, the system operated in an M-B mode sequence in which multiple A-lines were repeatedly acquired at each lateral position before the beam advanced to the next location in the scan. At each position, 400 A-lines were recorded at a $20\text{-}\mu\text{s}$ inter-A-line

interval, allowing a temporal sampling rate sufficient to capture the propagation of waves traveling at several meters per second. The lateral scan consisted of 300 positions spaced by 10 μm , yielding a three-dimensional dataset in which each voxel contained depth-resolved phase information tied to a specific time point. The acquisition strategy ensured that the temporal evolution of the elastic wave could be reconstructed without violating the Nyquist sampling requirements dictated by the anticipated wave speeds.

Phase-resolved detection was used to convert the interferometric OCT signal into an axial displacement trace for each A-line. The complex OCT signal at each depth was compared between successive A-lines to compute the incremental phase shift, which was then transformed into physical displacement using the standard relation $\Delta d = (\lambda_0 / 4\pi n) \Delta \phi$, where λ_0 is the center wavelength, n is the refractive index of scleral tissue, and $\Delta \phi$ is the measured phase change. This approach provided sub-nanometer sensitivity to induced motion, enabling accurate detection of the very small axial displacements associated with guided-wave propagation in sclera. The resulting displacement data were assembled into spatial–temporal maps plotting lateral position against time. Within these maps, the propagating elastic wave appeared as an obliquely oriented trajectory whose slope corresponded to the local wave speed.

To extract wave speed, linear regression was performed along the wavefront trajectory across the region of interest. Depth-averaging was applied within the scleral layer to improve the signal-to-noise ratio and account for small variations in reflectivity across the tissue thickness. The final wave speed estimates represented the group velocity of the propagating guided wave, which has been shown to correlate strongly with local tissue stiffness under the boundary conditions exhibited by thin ocular tissues. Because the excitation geometry and tissue thickness satisfied the assumptions commonly associated with low-order Lamb wave propagation, the measured wave speeds were interpreted as relative stiffness indicators rather than absolute elastic moduli.

The system maintained sub-micrometer displacement sensitivity throughout the full imaging field, and calibration measurements performed on a homogeneous tissue-mimicking phantom confirmed that the OCE platform produced consistent wave-speed measurements across the full lateral range. The high coherence of the induced waves, coupled with the spatial precision afforded by the confocal ARF–OCT configuration, allowed the system to resolve

directional differences in elastic wave propagation with sufficient accuracy to quantify anisotropy in the equatorial sclera across all quadrants and pressure levels.

4.2.4 Ultrasonic Elastography Validation

To corroborate the wave-speed measurements obtained with optical coherence elastography and to ensure that the observed mechanical behavior of the equatorial sclera was not modality-specific, ultrasonic elastography (UE) was employed as an independent validation technique. UE offers a fundamentally different detection mechanism from OCE, relying on the tracking of acoustic backscatter rather than optical phase changes, and thus provides an important cross-modality confirmation of mechanical trends, particularly in the presence of optical scattering or microstructural inhomogeneities that may influence OCT-based displacement sensitivity.

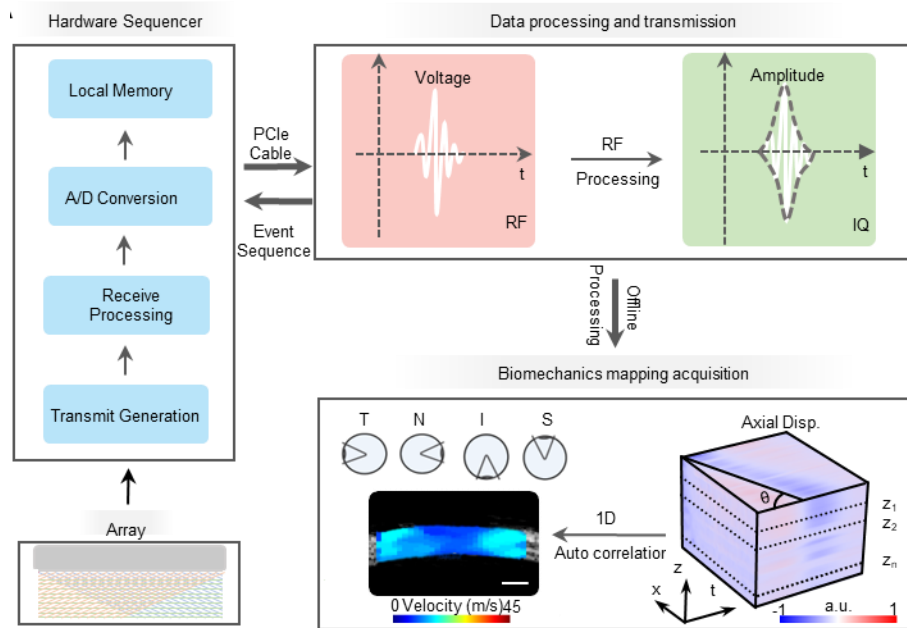


Figure 4.3 Workflow of UE. rf data were acquired, transmitted, and processed into IQ data using the hardware sequencer and host PC. During offline processing, 3D axial displacement data—comprising depth (z), lateral position (x), and time (t)—were reconstructed (dimension: 20 mm by 12.8 mm by 14 ms). Spatial-temporal maps at various depths were generated, where the slope angle (θ) represented the elastic wave speed in the target. The color map indicates normalized axial displacement. Final elastic wave maps were reconstructed using a 1D autocorrelation algorithm across four quadrants: temporal (T), nasal (N), inferior (I), and superior (S). Scale bar, 2 mm.

The UE system was built on a Verasonics Vantage 256 research ultrasound platform equipped with a 128-element, 18-MHz linear array transducer. This array was selected to balance lateral resolution, penetration depth, and field-of-view coverage across the equatorial sclera. Although ultrasound axial resolution is inherently lower than that of OCT, the ultrasonic

transducer provided a substantially wider imaging aperture, enabling the visualization of larger tissue regions within a single frame and permitting robust assessment of global wave propagation patterns in each quadrant. By combining a relatively high probe frequency with ultrafast plane-wave imaging, the system achieved sufficient temporal and spatial sampling to track small displacements induced by guided elastic waves.

Elastic wave generation in UE was achieved using a mechanical shaker positioned orthogonally to the scleral surface. A single, brief 1-kHz sinusoidal burst was applied during each measurement, generating a shear-dominant wave that propagated laterally across the equatorial region. This excitation frequency was chosen to produce measurable displacements without inducing resonant behaviors or nonlinear deformation. Because the sclera is thin relative to the wavelength of the induced disturbance at this driving frequency, the resulting waves exhibited mixed-mode behavior consistent with low-order Lamb or quasi-surface waves. The ultrafast imaging sequence, implemented via plane-wave compounding at a pulse repetition frequency of 7 kHz, captured the propagation of these waves as high-frame-rate radiofrequency (RF) sequences across the entire insonified field.

To reconstruct displacement fields, the RF data were demodulated into in-phase and quadrature (I/Q) components, and axial motion was estimated using a one-dimensional autocorrelation approach applied between successive A-lines. This estimator provided robust phase-sensitive displacement measurements while remaining relatively insensitive to amplitude fluctuations caused by local variations in acoustic reflectivity. The displacement traces for all lateral positions were compiled into spatial–temporal maps analogous to those used in OCE. Because ultrasound imaging provides a larger depth of field, wavefronts could be visualized across a wider scleral thickness, though only the displacement within the equatorial layer was used in subsequent analysis to preserve correspondence with OCE-derived measurements.

Wave speed estimation in UE followed procedures parallel to those used for OCE: a linear fit was applied to the dominant wavefront trajectory in the spatial–temporal map, and wave speeds were computed from the slope of this regression line. Although UE exhibits lower sensitivity than OCE—particularly for the small displacement amplitudes associated with stiff ocular tissues—it provided consistent wave-speed estimates in both the meridional and equatorial directions across all quadrants. Importantly, the anisotropy trends obtained from UE matched those observed with OCE, confirming that the directional stiffness differences measured

optically reflected true biomechanical properties rather than artifacts of optical sensitivity, beam alignment, or tissue scattering.

The complementary nature of UE and OCE was especially important in regions where OCT penetration was locally reduced due to curvature or superficial reflectivity changes. In such cases, UE's deeper acoustic penetration provided continuous measurement across the equatorial arc, ensuring that the mechanical characterization was spatially comprehensive. The agreement between the two modalities across all quadrants and pressure levels established OCE as a reliable and highly sensitive technique for probing biomechanical anisotropy in thin ocular tissues, while UE provided confidence that the measured trends were robust, modality-independent, and preserved over larger spatial domains.

4.2.5 Structural Imaging: Polarized Light Microscopy, SEM, and TEM

To relate the elastography measurements to the sclera's underlying architecture, collagen microstructure in each equatorial quadrant was examined using polarized light microscopy (PLM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). These techniques provided complementary information at different spatial scales and were essential for interpreting whether the observed mechanical anisotropy reflected simple fiber alignment or more complex lamellar organization.

PLM was used to quantify in-plane collagen fiber orientations throughout the scleral thickness. After elastographic testing, tissue samples were excised from the nasal, superior, temporal, and inferior quadrants, cryoprotected, and sectioned into 30- μm slices. Each section was imaged under four crossed-polarizer orientations, allowing computation of local birefringence phase retardance and fiber direction. Serial sections were registered using fiducial marks to reconstruct a volumetric orientation map for each quadrant. Orientation distributions were calculated using energy-weighted histograms, providing a robust descriptor of the dominant fiber angles and their dispersion.

Because PLM is limited to in-plane architecture, SEM and TEM were used to examine mesoscale and nanoscale features that influence mechanical stiffness. SEM imaging of critical-point-dried tissues revealed the surface lamellar morphology, including fiber interweaving, branching, and variations in bundle thickness—features that PLM cannot resolve but that strongly affect load transfer. TEM provided higher-resolution characterization of collagen fibrils, including diameter distribution, packing density, crimp, and out-of-plane inclination. These

ultrastructural details helped explain cases where mechanical anisotropy diverged from PLM-predicted orientation, indicating that the sclera's functional behavior arises from both planar alignment and three-dimensional fiber organization.

Together, PLM, SEM, and TEM supplied a multi-scale structural context that was directly compared with OCE- and UE-derived stiffness measurements, enabling a more complete assessment of how collagen architecture contributes to anisotropic mechanics in the equatorial sclera.

4.2.6 Data Processing and Statistical Analysis

All elastography data were processed offline to extract displacement fields, reconstruct spatial-temporal wave propagation maps, and quantify elastic wave speed in each quadrant and direction. For OCE, the phase-resolved OCT signal was first converted into axial displacement using the standard phase-displacement relationship. Displacement traces were then assembled into two-dimensional spatial-temporal maps representing lateral position as a function of time. Because the sclera is thin relative to the OCT axial imaging range, displacement values across the scleral thickness were averaged to improve the signal-to-noise ratio while preserving the integrity of the dominant guided-wave trajectory. The elastic wave speed was obtained by performing a linear regression along the wavefront in the spatial-temporal map, where the slope of this trajectory corresponded to the group velocity of the propagating wave. Only regions exhibiting coherent wave propagation were included in the fit to ensure reliability of the estimates.

UE data were analyzed in a parallel manner. The demodulated in-phase and quadrature signals were used to compute axial displacements via a one-dimensional autocorrelation estimator applied between successive A-lines. This yielded displacement fields analogous to those obtained with OCE, allowing direct formation of ultrasound-based spatial-temporal maps. Wave speed was extracted using the same regression approach, and the resulting values were compared against OCE-derived measurements to confirm consistency across modalities.

For each quadrant and direction, measurements at a given IOP level were repeated three times, and a high-quality dataset free of artifacts was selected for analysis. The anisotropy index for each quadrant was defined as the ratio of wave speed in the equatorial direction to that in the meridional direction. Statistical comparisons across IOP levels and directions were performed using ordinary one-way ANOVA, and linear regression was used to evaluate pressure-dependent

trends when appropriate. Data are reported as mean $\pm$ standard deviation unless otherwise stated. All analyses were conducted in MATLAB and Fiji, and significance thresholds followed standard conventions for biomedical research.

4.3 Result

4.3.1 System Accuracy Validation

Before characterizing the biomechanics of the equatorial sclera, it was essential to establish the accuracy and directional sensitivity of both elastography systems. This validation ensured that any anisotropy observed in the sclera reflected true tissue mechanics rather than limitations of the measurement platforms. To this end, a homogeneous tissue-mimicking phantom was first examined using both UE and OCE. In such an isotropic medium, elastic waves should propagate identically along all directions, providing a stringent test of measurement consistency. Both systems produced nearly identical spatial–temporal wavefronts, and the resulting wave speeds were in close agreement— 2.48 ± 0.04 m/s for UE and 2.58 ± 0.03 m/s for OCE—confirming that each modality was capable of accurately resolving wave propagation in homogeneous tissue.

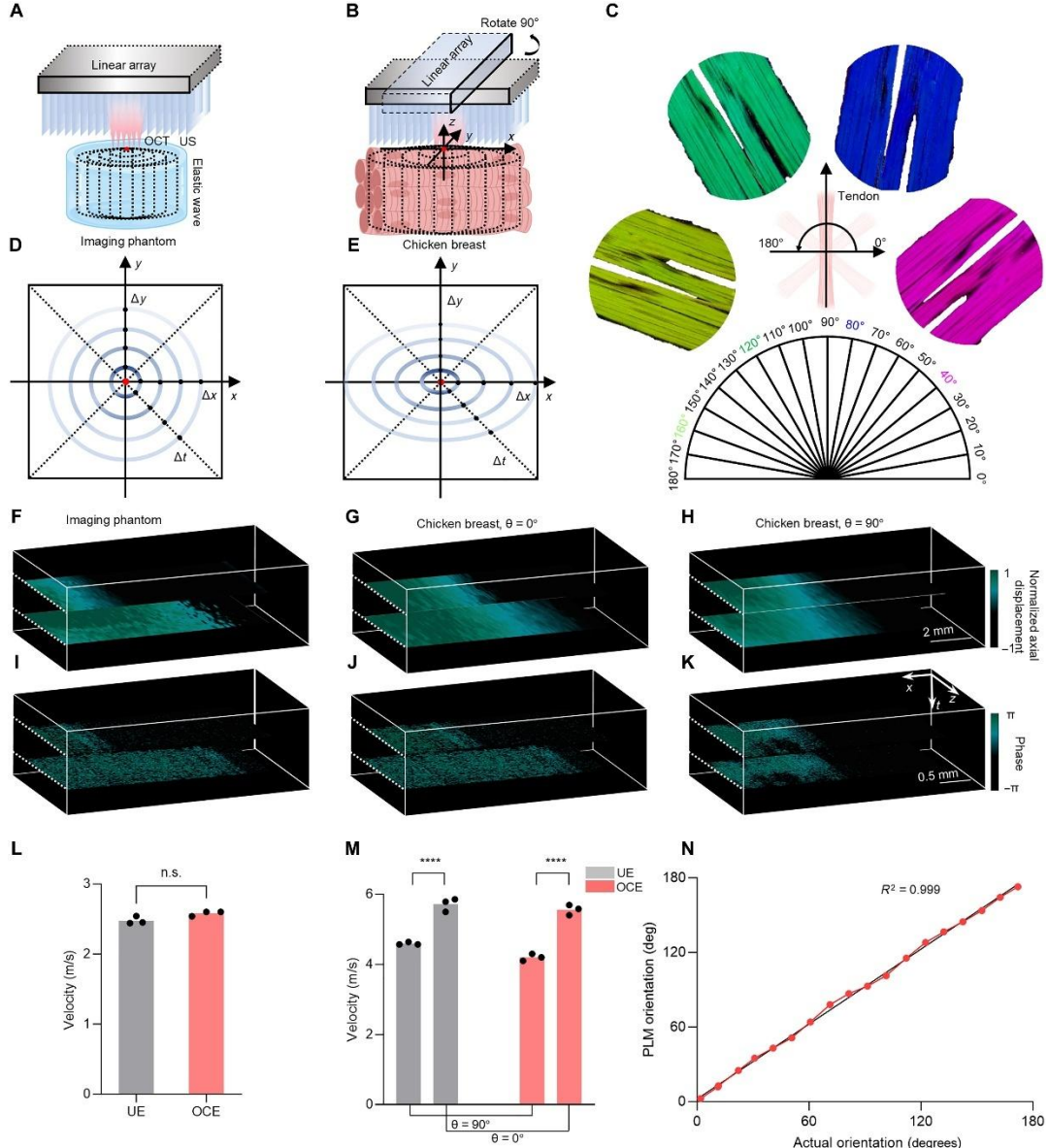


Figure 4.4 Validation of UE, OCE and PLM systems. (A) Accuracy test: UE and OCE measure wave speed in the same isotropic and homogeneous gelatin phantom. (B) Anisotropy test: both modalities probe chicken breast with the scanning axis parallel ($\theta = 0^\circ$) or perpendicular ($\theta = 90^\circ$) to muscle-fiber alignment. (C) PLM calibration: a sheep tendon is imaged every 10° while rotated 0 – 180° to compare measured and true fiber angles. (D) Top view schematic of wavefronts in the same imaging phantom: equal travel distances ($\Delta x = \Delta y$) for a fixed time Δt . (E) Top view schematic of wavefronts in anisotropic chicken breast: farther propagation along fibers ($\Delta x > \Delta y$). (F–H) UE results: induced elastic wave propagation in the phantom (F), chicken breast at $\theta = 0^\circ$ (G) and $\theta = 90^\circ$ (H). (I–K) OCE results: elastic wave propagation in the phantom (I), chicken breast at $\theta = 0^\circ$ (J) and $\theta = 90^\circ$ (K). (L) Elastic wave speeds in the imaging phantom measured by UE and OCE. (M) Elastic wave speeds in the chicken breast along the two directions, measured by UE and OCE. (N) Comparison between PLM-measured and known fiber orientations in a sheep tendon. Scale bars: (F–H), 2 mm; (I–K), 0.5 mm. Time intervals between two elastic wave propagation maps in (F): 1.29 ms; (G and H): 0.43 ms; (I): 0.24 ms; (J, K): 0.1 ms.

Following verification in isotropic media, we next assessed whether the systems could reliably detect mechanical anisotropy. This step was critical because the sclera exhibits direction-dependent fiber architecture, and the elastography platforms must be sensitive enough to

distinguish directional stiffness differences. Chicken breast, a well-established anisotropic tissue with pronounced fiber alignment, was used for this validation. Both UE and OCE demonstrated clear directional contrast: wave speeds were consistently higher when measured parallel to the muscle fibers than perpendicular to them. UE recorded speeds of 5.72 ± 0.16 m/s parallel to the fibers and 4.59 ± 0.03 m/s perpendicular, while OCE measured 5.56 ± 0.03 m/s and 4.20 ± 0.08 m/s under the same respective conditions. These results confirmed that both systems were capable of resolving physiologically meaningful directional differences and were therefore suitable for evaluating anisotropy in the equatorial sclera.

To complement the mechanical validation, PLM accuracy was also assessed because it would later serve as the structural reference for interpreting anisotropy. Tendon samples with highly aligned collagen fibers were imaged across multiple known orientations. The resulting PLM-derived angles closely matched the ground-truth orientations from 0° to 180° in 10° increments, confirming that the PLM workflow could reliably quantify planar fiber orientation. This successful validation across mechanical and structural modalities ensured that the subsequent scleral measurements could be interpreted with confidence.

4.3.2 Ultrasonic Elastography Measurements

Having established the accuracy of the elastography platforms, we next applied UE to the equatorial sclera to obtain wide-field measurements of direction-dependent stiffness. UE served as a complementary modality to OCE, offering a broader field of view and thus providing a useful perspective on the global mechanical behavior of each quadrant. Real-time B-mode imaging confirmed consistent placement of the equatorial sclera within the ultrasonic focal region across IOP conditions, and the limited morphological variation across pressure levels emphasized the need for elastography to reveal mechanical changes not apparent in structural imaging.

Spatial–temporal maps derived from UE demonstrated coherent wave propagation in all quadrants and directions. These maps formed the basis for calculating local wave speeds and revealed a clear and consistent pattern: elastic waves propagated faster in the equatorial direction than in the meridional direction. This directional trend was observed across all IOP levels, indicating inherent circumferential stiffening of the equatorial sclera. Importantly, wave speeds

in both directions increased steadily with IOP, consistent with the expected recruitment and straightening of collagen fibers under elevated load. The temporal and inferior quadrants frequently exhibited the most pronounced directional contrast, suggesting possible regional specialization in collagen organization or load-bearing function.

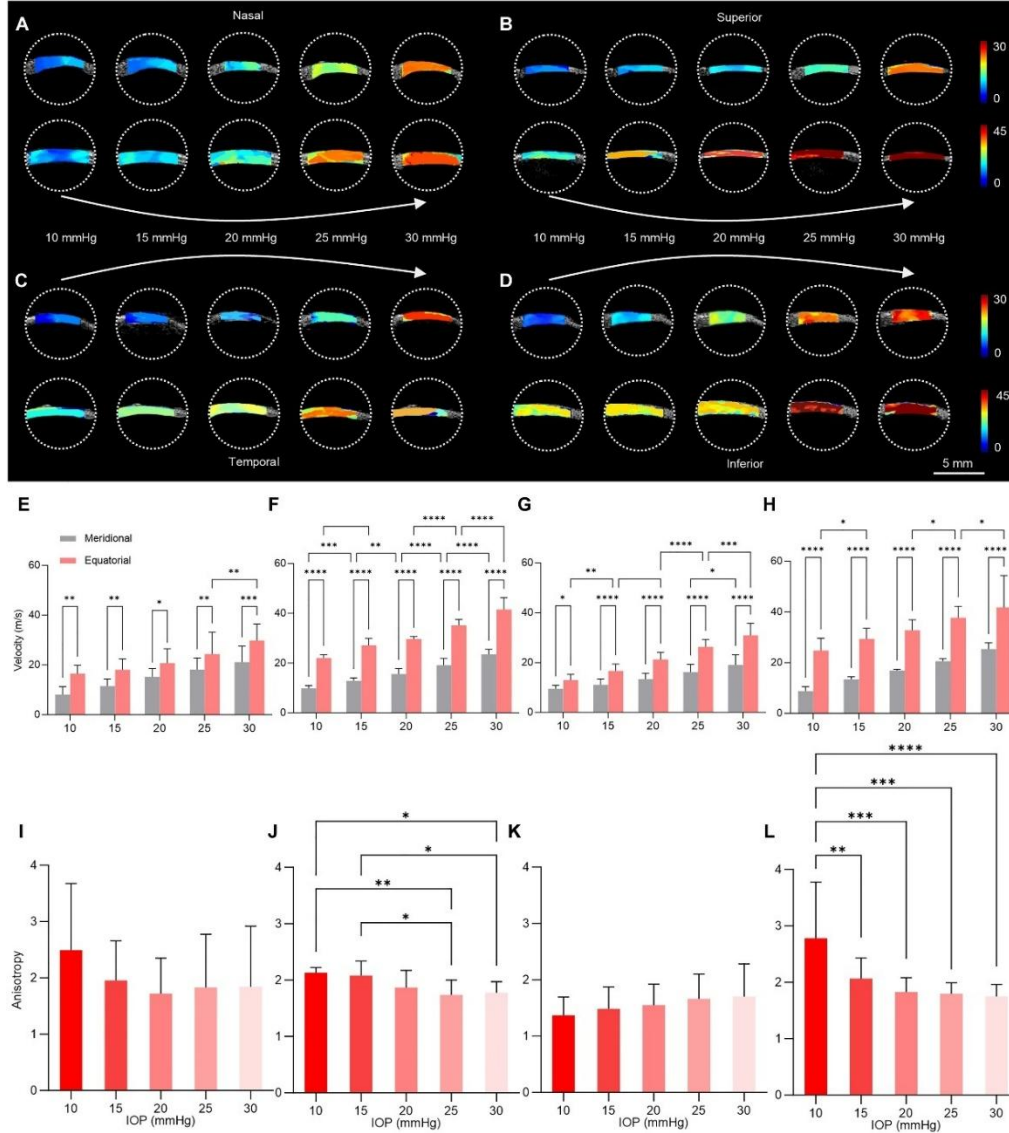


Figure 4.5 Fusion images of the equatorial sclera in different quadrants, directions, and IOPs from UE measurements and statistical analysis. (A to D) Fusion images combining B-mode structural images with elastic wave speed maps in the nasal, superior, temporal, and inferior quadrants, respectively. The top and bottom rows correspond to the meridional and equatorial directions. The color map range is 0 to 30 m/s for the meridional direction and 0 to 45 m/s for the equatorial direction. Color map indicates elastic wave speed (unit: m/s). (E to H) Statistical distributions of elastic wave speeds in the meridional and equatorial directions of the equatorial sclera at varying IOP levels. (I to L) Quantitative analysis of anisotropy between the meridional and equatorial directions in the equatorial sclera at different IOPs. Quadrant mapping: [(E) and (I)] nasal; [(F) and (J)] superior; [(G) and (K)] temporal; and [(H) and (L)] inferior.

To provide additional spatial insight, localized wave-speed maps were generated by correlating neighboring displacement traces. These maps further highlighted regional variations in stiffness and reinforced the directional trends evident in the global wave-speed calculations. The anisotropy index remained greater than unity in every quadrant, confirming that circumferential stiffening is a consistent biomechanical feature of the equatorial sclera. The strong agreement between repeated measurements within each pressure level demonstrated the stability of UE-based characterization and established a reliable baseline for comparison with OCE.

4.3.3 Optical Coherence Elastography Measurements

After characterizing stiffness with UE, we employed OCE to obtain higher-resolution measurements of elastic wave propagation. OCE's enhanced displacement sensitivity and fine lateral sampling enabled a more detailed assessment of scleral anisotropy and provided an opportunity to examine elastic wave behavior with greater spatial precision. Using Doppler OCT guidance, the ARF transducer and OCT beam were aligned confocally, ensuring that wave induction and detection were consistently localized within the intended equatorial region.

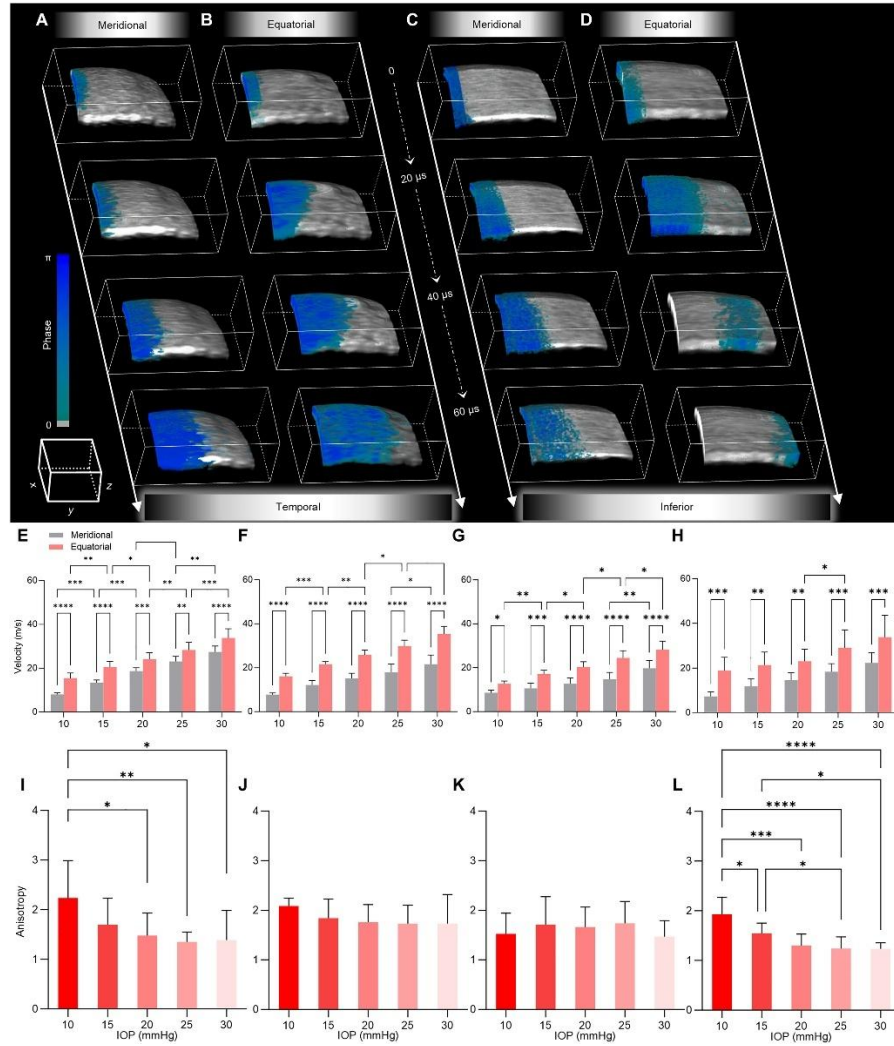


Figure 4.6 3D elastic wave propagation images of the equatorial sclera from OCE measurements and corresponding statistical analyses. 3D elastic wave propagation images of the equatorial sclera acquired using OCE at 20- μ s time intervals in different quadrants and directions. (A and B) Meridional and equatorial directions in the temporal quadrant. (C and D) Meridional and equatorial directions in the inferior quadrant. (E to H) Statistical distributions of elastic wave speeds in the meridional and equatorial directions at varying IOP levels. (I to L) Quantification of anisotropy between the meridional and equatorial directions under different IOPs. [(E) and (I)] Nasal; [(F) and (J)] superior; [(G) and (K)] temporal; [(H) and (L)] inferior. Scale bar, 1 mm³.

The spatial–temporal maps obtained from OCE exhibited highly coherent wavefronts similar in overall form to those observed with UE but with substantially finer spatial resolution. These maps again revealed faster propagation along the equatorial direction compared to the meridional direction, reinforcing the existence of circumferential stiffening. The directional contrast was especially pronounced in the temporal and inferior quadrants, where wavefront slopes in the equatorial direction were noticeably steeper. Consistent with the UE findings, OCE

wave speeds increased monotonically with IOP, demonstrating that pressure-induced stiffening is a robust feature of equatorial scleral biomechanics.

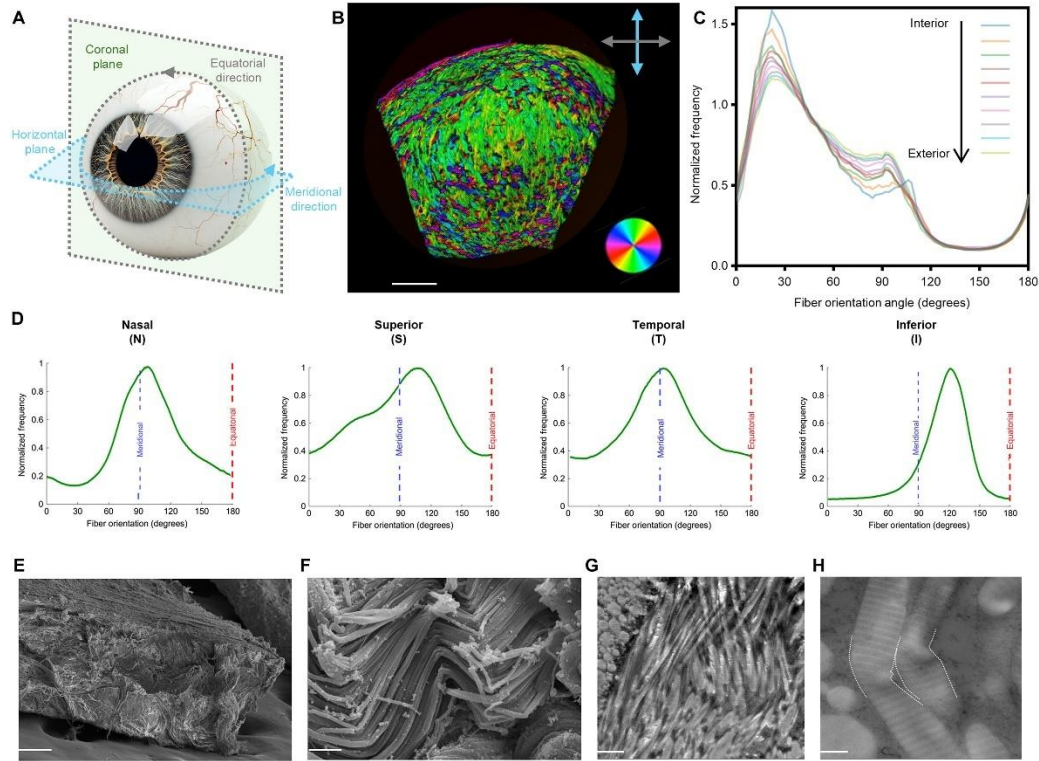
A key advantage of OCE was its ability to acquire volumetric elastography data. By rotating the sample azimuthally and merging displacement fields from multiple positions, we reconstructed three-dimensional wave-propagation volumes. These 3D renderings provided a more complete visualization of how wavefronts evolve over time and highlighted the directional nature of stiffness in a manner not possible with conventional 2D scans. The volumetric data confirmed that axial displacements were consistently smaller in the equatorial direction due to increased stiffness, and these observations aligned quantitatively with the extracted wave speeds.

Importantly, OCE results mirrored the UE findings across all quadrants and pressure levels, indicating that both modalities measured the same underlying biomechanical behavior. This cross-modal agreement provided strong evidence that the observed anisotropy reflects intrinsic material properties rather than artifacts associated with a particular imaging system.

4.3.4 Structural Imaging (PLM, SEM, TEM)

To interpret the mechanical anisotropy revealed by UE and OCE, we next examined the underlying collagen architecture across quadrants using PLM, SEM, and TEM. PLM provided a large-scale view of in-plane collagen orientation and demonstrated that most quadrants displayed predominantly meridional alignment. The nasal, superior, and temporal quadrants showed strong alignment along the anterior–posterior axis, while the inferior quadrant exhibited a similar trend with a modest ($\sim 30^\circ$) offset. These orientation distributions were derived from millions of birefringence-weighted pixel measurements across 8–19 serial sections per quadrant, ensuring that the structural assessment was statistically robust.

Despite these structural patterns, the direction of maximum mechanical stiffness did not coincide with the direction of dominant fiber orientation. While PLM indicated primarily meridional alignment, both elastography modalities consistently showed greater stiffness in the equatorial direction. This discrepancy suggested that planar orientation alone does not fully determine the load-bearing behavior of the equatorial sclera.



SEM imaging offered clues to this divergence by revealing that collagen bundles exhibited significant interweaving and lamellar transitions not detectable with PLM. The scleral stroma contained bundles that crossed at oblique angles, merged between adjacent lamellae, or changed orientation across depth, indicating a far more complex structural organization than a single dominant planar direction. TEM further highlighted nanoscale features such as fibril twisting, out-of-plane inclination, and variations in fibril thickness and crimp amplitude. These ultrastructural characteristics provide potential mechanisms through which circumferential stiffness could emerge even when the bulk planar fiber orientation appears meridional.

Together, the structural imaging results reinforce the conclusion that equatorial scleral anisotropy arises from multi-scale architectural organization rather than simple collagen alignment. The combination of PLM, SEM, and TEM enabled a deeper interpretation of the elastography data and highlighted the importance of three-dimensional fiber architecture in determining ocular tissue mechanics.

4.3.5 Challenges and Limitations

Several practical and methodological challenges were encountered during the multimodal characterization of the equatorial sclera, and these factors are important for contextualizing the results. One challenge stemmed from the intrinsic curvature and heterogeneity of the equatorial

region, which affected both optical and acoustic detection. In OCE, localized curvature occasionally reduced phase stability or OCT penetration, while in UE, variations in coupling and surface geometry sometimes introduced minor distortions in displacement fields. Nevertheless, the directional stiffness trends remained consistent across modalities, indicating that these effects did not alter the primary conclusions.

IOP manipulation introduced additional constraints. Although the closed-loop system maintained pressure within narrow tolerances, short-term fluctuations during the equilibration period could influence early wavefront measurements, necessitating repeated acquisitions to ensure stability. The *ex vivo* nature of the experiments also meant that physiological conditions—such as choroidal perfusion, episcleral pressure, and *in vivo* hydration—were absent, potentially affecting absolute stiffness but not relative trends.

Structural imaging posed its own limitations. Because PLM, SEM, and TEM require extensive tissue processing, they could not be performed on the exact same samples used for elastography, preventing perfect spatial registration between mechanical and microstructural measurements. Furthermore, PLM captures only in-plane birefringence and is not sensitive to out-of-plane fiber inclination or depth-dependent lamellar transitions. SEM and TEM partially addressed these limitations but provide only localized fields of view.

Finally, mixed-mode elastic waves in thin scleral tissue complicate detailed wave-mode identification, and while wave speed offers a reliable relative measure of stiffness, absolute modulus estimation remains model-dependent. For these reasons, the present study focuses on directional trends and anisotropy rather than full mechanical parameter reconstruction.

Despite these constraints, the agreement across OCE, UE, and structural imaging modalities indicates that the principal findings—namely the dominance of circumferential stiffness and the dissociation between planar fiber orientation and mechanical anisotropy—are robust and physiologically meaningful.

4.4 Summary

This chapter presented a multimodal investigation of equatorial scleral biomechanics using optical coherence elastography as the primary measurement tool. Across all quadrants and IOP levels, both OCE and UE consistently demonstrated that elastic waves propagate faster in the equatorial direction than in the meridional direction, indicating a robust circumferential

stiffening of the sclera. Wave speeds increased monotonically with IOP, confirming pressure-dependent collagen recruitment and tissue stiffening. The strong agreement between OCE and UE validated that these trends reflect true biomechanical behavior rather than modality-specific artifacts.

Structural imaging provided essential context for interpreting these findings. Although PLM revealed predominantly meridional in-plane collagen orientation, SEM and TEM exposed substantial three-dimensional complexity—including fiber interweaving and out-of-plane inclination—that likely contributes to the observed discrepancy between structural and mechanical anisotropy. These results emphasize that scleral stiffness cannot be inferred solely from planar collagen orientation and instead arises from a multi-scale architectural organization.

Despite challenges associated with ex vivo pressure control, tissue curvature, and cross-modality registration, the overall conclusions were highly consistent. The equatorial sclera exhibits inherent circumferential reinforcement, and its mechanical response is shaped by the combined contributions of fiber alignment, lamellar connectivity, and depth-dependent collagen architecture. These insights provide a foundation for future in vivo studies and inform modeling efforts aimed at understanding ocular load-bearing behavior.

CHAPTER 5: Wide-Field Optical Coherence Elastography of the Mouse Brain

5.1 Introduction

Understanding the biomechanical properties of the brain is fundamental to advancing both basic neuroscience and clinical neuroimaging. Mechanical cues play a critical role in neuronal development, extracellular matrix organization, axonal integrity, and the progression of neuropathological conditions. Alterations in tissue stiffness have been associated with neurodegeneration, demyelination, inflammation, and age-related remodeling. However, most conventional imaging techniques lack the resolution, contrast mechanisms, or volumetric capabilities needed to characterize these mechanical changes at microscale levels.

Histology, although the gold standard for assessing brain microarchitecture, requires fixation, sectioning, staining, and irreversible tissue destruction[101]. These processes introduce morphological distortions and limit analysis to two-dimensional slices, obscuring the intrinsic three-dimensional continuity of neural structures. Moreover, histology provides no direct information about tissue biomechanics, despite the growing recognition that mechanical signatures may precede overt morphological pathology in diseases such as Alzheimer’s disease.

Optical coherence elastography (OCE), an extension of optical coherence tomography (OCT), has emerged as a powerful modality for probing tissue biomechanics with micrometer-scale spatial resolution[102]. OCE leverages phase-sensitive OCT detection to measure minute deformations under mechanical loading, enabling quantitative mapping of elastic properties. Compared to elastography techniques based on ultrasound[103], MRI[104], or photoacoustics[105], OCE[106] offers superior spatial resolution, high displacement sensitivity, and compatibility with small, delicate tissues such as the mouse brain.

Among OCE implementations, compressional OCE—also referred to as static or quasi-static OCE—provides a simple, robust, and repeatable framework for elasticity assessment. Mechanical loading is applied in a controlled axial manner, and tissue strain is estimated directly from phase-resolved displacement fields. This approach is particularly advantageous for fixed or ex-vivo tissues, where motion-free conditions allow high-fidelity strain reconstruction.

Nevertheless, mapping brain biomechanics remains challenging because stiffness differences between gray and white matter can be subtle, and optical scattering limits imaging penetration. In this chapter, we address these challenges by developing a high-sensitivity, wide-

field compressional OCE system optimized for mouse-brain imaging. The system integrates phase-resolved detection, a calibrated silicone reference layer for stress estimation, and synchronized mechanical loading. Optical clearing is evaluated as a complementary preparation step to enhance visibility and depth resolution without altering intrinsic tissue stiffness.

The following sections describe the system design, strain and elasticity estimation framework, validation with phantoms, wide-field mapping of ex-vivo mouse brains, and quantitative comparisons with histological architecture. Together, these results demonstrate that compressional OCE enables high-resolution, label-free, and quantitative biomechanical characterization of the brain, providing insights into structural organization and potential biomarkers for neuropathology. This chapter details system implementation, strain-estimation strategies, phantom validation, and ex-vivo wide-field brain mapping, concluding with histological correlation across multiple anatomical regions. Together, these results demonstrate that compressional OCE provides high-resolution, label-free, and quantitative assessment of brain biomechanics, enabling detailed differentiation of gray- and white-matter structures.

5.2 System Design and Experimental Methods

Students report increased emotional attachment to their schools through mascots, especially during sports events, pep rallies, and community activities. Table 2.1 summarizes perception scores by grade level.

5.2.1 OCE System Configuration

The compressional OCE system developed for this study was built upon a custom broadband spectral-domain OCT (SD-OCT) platform designed to achieve high phase stability and wide-field imaging capability. A schematic of the system is shown in Fig. 5.1A. The light source was a superluminescent diode (Superlum, Ireland) centered at 890 nm with a 144-nm bandwidth, providing an axial resolution of 2.1 μm in tissue. A 20:80 fiber coupler split the illumination between the reference and sample arms, and approximately 9 mW of optical power was delivered to the sample. Lateral resolution was 15 μm , and the system sensitivity was measured to be 103 dB with a 20- μs exposure time.

Controlled mechanical loading was introduced through a ring-shaped piezoelectric actuator (Thorlabs, USA) mounted coaxially with the OCT imaging path, as shown in Fig. 5.1B. A 3-mm-thick glass plate was bonded to the actuator to achieve uniform compression across the

sample surface. To enable stress calibration and minimize surface-slip artifacts, a $\sim 200\text{-}\mu\text{m}$ -thick silicone reference layer was placed between the glass plate and the sample. This layer served both as a mechanical buffer and as a known stress-sensing element through its independently measured stress–strain response.

A custom electronic synchronization module coordinated OCT acquisition and mechanical loading (Fig. 5.1C). Two sequential B-scans were acquired at distinct loading states: a preload state and an actuated state. Precise temporal alignment allowed accurate phase-difference computation between the two frames, enabling depth-resolved displacement extraction. A representative OCT cross-section and the corresponding OCE strain map from a mouse-brain slice are shown in Fig. 5.1D.

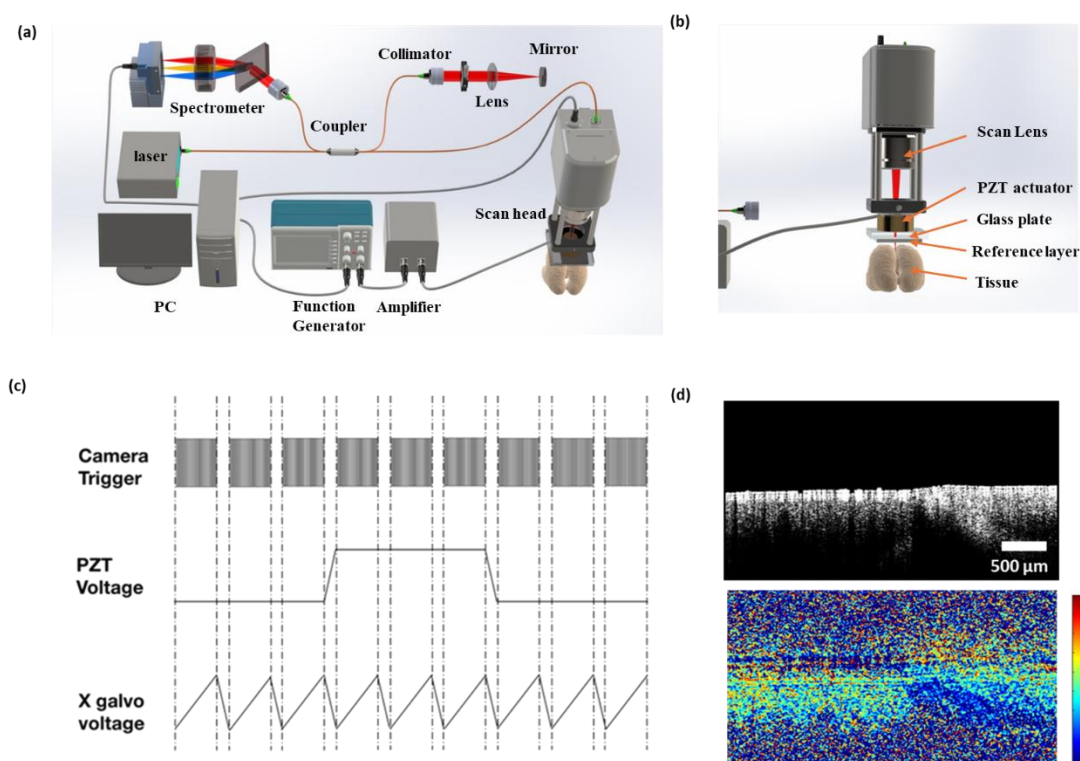


Figure 5.1. Schematic and representative results of the compressional OCE system. a) Schematic of the OCE system setup; b) OCE scan head with compression force unit; c) Timing diagram of system synchronization; d) OCT image and OCE image of brain slice.

5.2.2 Optical Clearing

Optical clearing was incorporated as an optional sample-preparation step to enhance imaging penetration and improve the visibility of subsurface structures during OCE acquisition. Although compressional OCE does not require clearing to generate mechanical contrast, reducing optical scattering can increase phase stability, improve signal uniformity with depth, and allow more accurate delineation of anatomical boundaries—particularly in dense white-matter regions.

In this study, the clearing process followed the OptiMuS protocol, a recently developed single-step, rapid immersion method optimized for preserving structural integrity while improving transparency. The clearing solution consisted of Tris–EDTA buffer, iohexol, D-sorbitol, and urea, prepared according to established formulations. Brain slices with a nominal thickness of 500 μm were immersed in the solution and incubated at 50 °C for 30 minutes. This brief immersion facilitated refractive-index matching between intracellular and extracellular compartments, thereby reducing scattering without inducing swelling, hardening, or other mechanical alterations.

Importantly, no additional chemical treatments or mechanical modifications were performed following clearing. All OCE measurements—before and after optical clearing—were conducted under identical loading conditions using the same silicone reference layer, compression amplitude, and imaging parameters. This ensured that any observed changes in image quality or depth penetration reflected optical improvements rather than biomechanical alterations.

As shown later in Section 5.3, optical clearing resulted in modest improvements in depth sensitivity and structural visibility but preserved the intrinsic relative stiffness patterns across brain regions. These observations support the suitability of clearing as a complementary tool for enhancing the fidelity of wide-field OCE without compromising quantitative biomechanical accuracy.

5.2.3 Sample Preparation

All animal procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) at the University of California, Irvine. Healthy adult B6 mice were euthanized at the conclusion of an approved study, and brain tissues

were harvested within one hour postmortem to minimize degradation of mechanical properties. A total of ten brains were collected for this work.

Immediately after extraction, the brains were briefly fixed in 10% neutral-buffered formalin for two hours to preserve gross structural morphology while avoiding extensive cross-linking that could significantly alter tissue stiffness. Following fixation, each brain was embedded in agarose prepared with phosphate-buffered saline (PBS). The agarose provided mechanical stability and minimized deformation during handling and compression. The embedded tissue blocks were then sectioned coronally into slices approximately 500 μm thick using a vibrating microtome.

Each slice was placed in a cylindrical agarose well to maintain hydration and prevent lateral slippage during compression. Prior to OCE imaging, the surface of the brain slice was gently brought into contact with the silicone reference layer attached to the compression plate. A uniform preload—typically on the order of 150 μm —was applied to ensure consistent contact and eliminate air pockets at the interface.

For experiments involving optical clearing, slices were imaged first in their native state and then immersed in clearing solution as described in Section 5.2.2. After clearing, each slice was carefully repositioned under the OCE system with the same orientation and boundary constraints. This controlled preparation ensured that all comparisons between native and cleared states reflected optical effects rather than changes in tissue mechanics.

5.2.4 Strain Estimation Using the Vector Method

Accurate strain estimation is central to compressional OCE, as the reliability of elasticity reconstruction depends directly on the precision and stability of displacement measurements. Several approaches have been developed for strain estimation in OCT-based elastography, including speckle-tracking algorithms, least-squares phase fitting, and phase-gradient methods. However, the interferometric origin of OCT speckle makes the signal particularly sensitive to decorrelation under mechanical loading, which can degrade the performance of speckle-tracking or least-squares methods. To address these limitations, this study employed the vector method, which has been shown to offer enhanced robustness against noise and phase jitter while avoiding phase-unwrapping errors that often arise in conventional phase-based techniques.

In the vector method, the complex OCT signal at each pixel is represented as a vector in the complex plane. Its amplitude corresponds to the magnitude of the local OCT signal, while its angle encodes the optical phase. For two sequential B-scans acquired at different loading states—referred to as the reference frame and deformed frame—the complex-valued signals are expressed as

$$S_1(x, z) = A_1(x, z)e^{i\phi_1(x, z)}, S_2(x, z) = A_2(x, z)e^{i\phi_2(x, z)}.$$

The interframe phase variation,

$$\Delta\phi(x, z) = \phi_2(x, z) - \phi_1(x, z),$$

is computed by multiplying the reference signal by the complex conjugate of the deformed signal. Unlike least-squares methods, which require explicit phase unwrapping, the vector representation naturally handles local fluctuations in the complex plane and reduces sensitivity to amplitude variations.

To suppress high-frequency noise and improve phase stability, the raw complex signals and phase gradients are averaged over a two-dimensional processing window of size $m \times n_{\text{pixels}}$. This spatial averaging mitigates jitter without oversmoothing structural boundaries. In addition, axial phase gradients are computed using pixel separations greater than one ($k > 1$) when feasible, as increasing the separation improves gradient robustness provided that phase wrapping does not occur across the interval.

The phase variation is converted to a physical displacement field using the standard phase-to-displacement relationship:

$$u(x, z) = \frac{\lambda}{4\pi n} \Delta\phi(x, z),$$

where λ is the OCT center wavelength and n is the refractive index of the tissue. Axial strain is then derived from the spatial derivative of displacement,

$$\varepsilon(x, z) = \frac{\partial u(x, z)}{\partial z},$$

yielding a depth-resolved strain map that reflects local mechanical compliance.

A representative example of the vector-method workflow is shown in Fig. 5.2, including the OCT B-scan, corresponding interframe phase-difference map, and resulting strain distribution. This framework was used consistently for all phantom and brain-tissue experiments throughout this chapter.

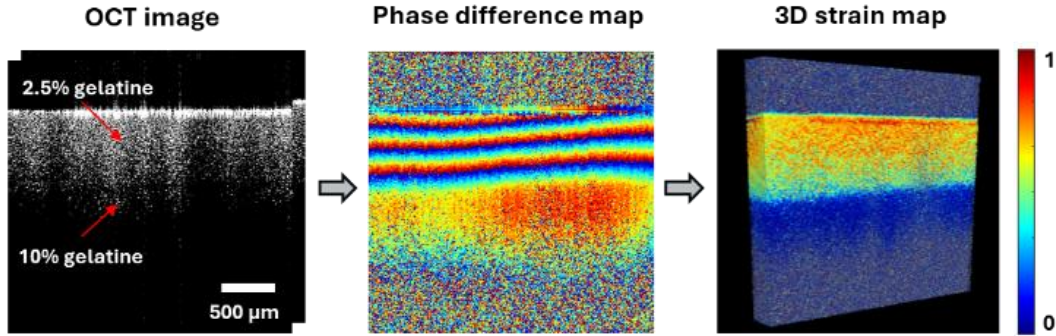


Figure 5.2. Illustration of strain-estimation using the vector method. Left: OCT B-scan image of a two-layer phantom. Middle: phase-difference map between two loading states. Right: strain map revealing mechanical contrast between layers.

5.2.5 Elastography Computation Using Stress–Strain Mapping

To convert measured strain into quantitative elastic modulus values, compressional OCE relies on a calibrated stress–strain relationship that links the applied surface stress to the tissue’s mechanical response. This calibration is enabled through a silicone reference layer placed between the compression plate and the sample. The reference material exhibits a known, stable mechanical behavior, allowing its measured deformation to serve as a proxy for the surface stress transmitted to the tissue.

During OCE acquisition, a uniform preload is first applied to ensure consistent contact between the reference layer and the sample. A second loading state is then introduced by actuating the ring piezoelectric transducer. Two sequential B-scans—corresponding to the preload and actuated states—are used to compute depth-resolved axial displacements in both the reference layer and the tissue using the phase-resolved strain estimation method described in Section 5.2.4.

The strain in the reference layer is defined as

$$\epsilon_{\text{ref}} = \frac{\Delta l}{l_0},$$

where l_0 is the initial thickness and Δl is the measured change in thickness under loading. The corresponding surface stress transmitted to the sample is then obtained from the independently measured stress–strain curve of the reference layer (Fig. 5.3):

$$\sigma = f(\varepsilon_{\text{ref}}).$$

Because the reference layer and tissue are in direct, continuous contact, the normal stress across the interface is conserved. Thus, the same surface stress σ applies to the underlying tissue region being imaged.

The local tissue elasticity (Young’s modulus, E) is then calculated using

$$E(x, z) = \frac{\sigma}{\varepsilon_{\text{tissue}}(x, z)},$$

where $\varepsilon_{\text{tissue}}(x, z)$ is the strain at each spatial location obtained from the depth-resolved displacement field. This formulation enables pixel-wise reconstruction of elastic modulus values and supports generation of quantitative elastograms that reveal spatial variations in stiffness.

Unlike qualitative strain maps, which reflect mechanical compliance under a fixed load, elastograms provide direct comparisons of absolute stiffness across regions and samples. To avoid artifacts associated with noise amplification in low-strain regions, elasticity values exceeding a physiological threshold were masked or excluded from final visualization. Figure 5.3 shows the stress–strain behavior of the silicone reference layer used for calibration.

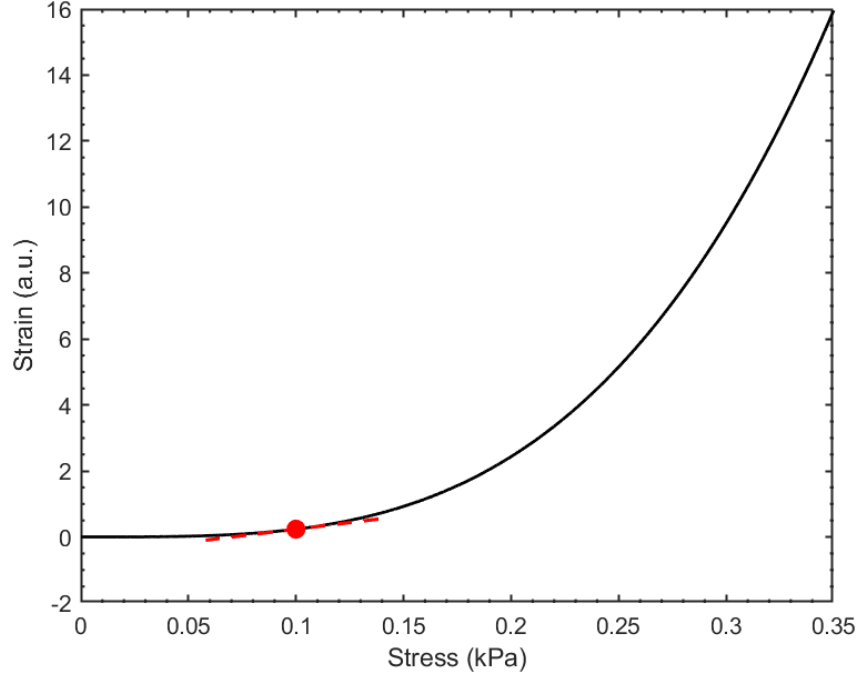


Figure 5.3: Measured stress–strain curve of the silicone reference layer used for converting surface strain into stress for quantitative elasticity reconstruction.

5.3 Result

5.3.1 Phantom Validation

To evaluate the performance, sensitivity, and robustness of the compressional OCE system, we first conducted validation experiments using a tissue-mimicking agarose phantom with a well-defined elastic contrast. The phantom consisted of a soft 2% agarose inclusion embedded within a stiffer 6% agarose background. Both regions were mixed with milk to provide optical scattering levels comparable to biological tissue, with slightly higher milk concentration in the inclusion to enhance structural visibility in OCT.

Prior to imaging, a uniform preload of approximately 150 μm was applied to ensure stable contact between the phantom surface and the silicone reference layer. The ring piezoelectric actuator generated an axial displacement of approximately 10 μm in air, which produced a measurable strain response within both the soft inclusion and the surrounding stiffer matrix.

Representative en-face and cross-sectional images of the phantom are shown in Fig. 5.4. The OCT images (Fig. 5.4A) clearly delineate the inclusion boundary, confirming adequate optical contrast between the two regions. The corresponding OCE strain maps (Fig. 5.4B) reveal pronounced mechanical contrast: the soft 2% agarose inclusion exhibits significantly higher

strain values, while the stiffer 6% agarose matrix displays lower strain, consistent with its higher elastic modulus.

Sharp transitions in strain across the inclusion boundary highlight the system's ability to detect fine spatial variations in stiffness. The absence of significant speckle decorrelation or phase artifacts further demonstrates the stability of the vector-method strain estimation described earlier in Section 5.2.4.

These results confirm that the OCE system provides reliable mechanical discrimination and can accurately resolve localized stiffness differences, establishing a strong foundation for subsequent experiments on more complex biological tissues.

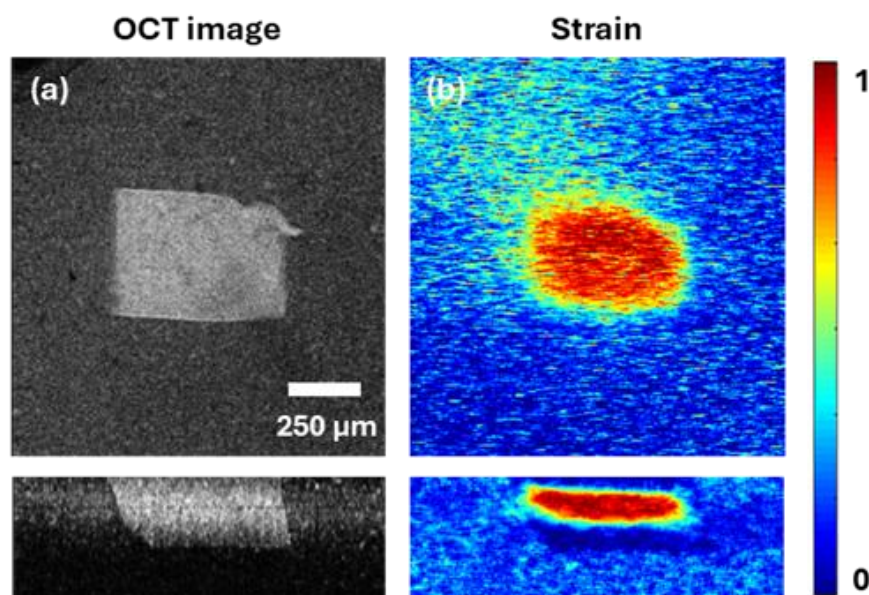


Figure 5.4. OCE imaging of an agarose phantom containing a soft inclusion. (A) OCT structural images showing the embedded 2% agarose region within the 6% matrix. (B) Corresponding strain maps revealing clear mechanical contrast, with higher strain in the softer inclusion and lower strain in the surrounding stiffer gel.

5.3.2 OCE Imaging of Mouse-Brain Slices

After validating the system with tissue-mimicking phantoms, we next applied compressional OCE to ex-vivo mouse-brain slices to assess its capability for high-resolution biomechanical mapping of complex neural tissues. Figure 5.5 presents representative OCT and OCE images acquired under identical loading and imaging conditions.

The native-state strain maps clearly reveal distinct spatial variations in mechanical response across major anatomical regions. Gray-matter structures—such as the cortex and hippocampus—exhibit relatively higher strain values, reflecting their softer mechanical properties. In contrast, white-matter tracts, including the corpus callosum, display consistently

lower strain values corresponding to higher intrinsic stiffness. These findings align with well-established differences in myelination, axonal density, and extracellular matrix composition between gray and white matter.

To investigate the influence of optical clearing on OCE imaging performance, brain slices were imaged before and after immersion in the OptiMuS clearing solution for 10, 20, and 30 minutes. Although optical clearing was not expected to alter the underlying mechanical properties of fixed tissue, it offered a practical means of improving light penetration and reducing scattering.

As shown in Fig. 5.5, optical clearing yielded progressive improvements in structural visibility and signal uniformity with increasing clearing duration. Deeper features—particularly those within white-matter regions—became more sharply defined, and phase stability improved across larger depths. Importantly, however, the relative strain distribution patterns remained consistent across all clearing durations: gray-matter regions remained softer, and white-matter regions remained stiffer.

These observations indicate that optical clearing functions primarily as an optical enhancement rather than a biomechanical modifier. Its primary benefit is improved visualization of deep or densely myelinated structures, enabling more accurate and stable strain estimation throughout the tissue thickness.

Collectively, the mouse-brain slice experiments demonstrate that compressional OCE can reliably quantify regional biomechanical differences in neural tissue and that modest optical clearing can further refine contrast and depth fidelity without altering the intrinsic stiffness relationships between brain regions.

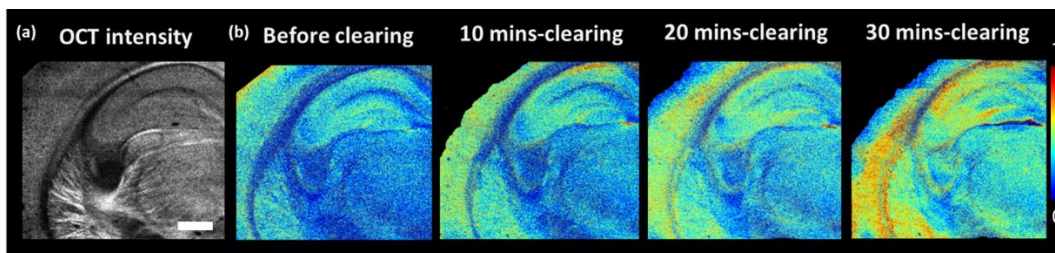


Figure 5.5. OCT and OCE strain mapping of ex-vivo mouse-brain slices before and after optical clearing. Optical clearing enhances structural visibility and depth penetration while preserving the intrinsic stiffness contrast between gray- and white-matter regions.

5.3.3 Wide-Field Brain Mapping and Histological Correlation (Rewritten in Dissertation Paragraph Style)

To assess the capability of compressional OCE for large-scale biomechanical characterization, wide-field imaging was performed across multiple coronal levels of the mouse brain. This analysis enabled evaluation of both the consistency of OCE-derived mechanical contrast and its correspondence with established histological architecture. For each coronal location, a comprehensive set of images was acquired, including the OCT structural image, the OCE elastogram before clearing, the elastogram after clearing, and the corresponding hematoxylin and eosin (H&E) histological section. Together, these datasets allowed direct comparison of structural, mechanical, and histological information across the brain.

Across all examined regions, the OCE strain maps revealed spatially heterogeneous mechanical behavior that closely aligned with known neuroanatomical features. Gray-matter regions, including the cerebral cortex and hippocampus, consistently exhibited higher strain values under uniform compressive loading, reflecting their softer tissue composition. The cortex displayed a relatively homogeneous but mechanically compliant profile, whereas the hippocampus showed characteristic laminar variations in strain amplitude consistent with its layered cytoarchitecture. In contrast, white-matter tracts such as the corpus callosum demonstrated markedly lower strain values, indicating stiffer mechanical properties associated with dense axonal packing and high myelin content. The caudoputamen exhibited intermediate stiffness with spatial variability, reflecting its mixture of projection fibers and neuronal populations. Likewise, the cerebellar cortex presented layered mechanical patterns that paralleled the molecular, Purkinje, and granular layers observed in the corresponding histological sections.

Optical clearing contributed additional improvements to image uniformity and depth sensitivity. After clearing, structural boundaries—particularly those between gray and white matter—appeared sharper, and deeper regions displayed enhanced phase stability. Fiber-rich regions, such as the corpus callosum and cerebellar arbor vitae, benefited most from this enhancement, enabling clearer delineation of substructure. Importantly, however, the spatial distribution of relative stiffness remained unchanged after clearing, demonstrating that the clearing process improved optical penetration without altering underlying biomechanical properties.

The close correspondence between mechanical contrast and histological architecture across all imaged coronal positions underscores the reliability and biological relevance of compressional OCE for characterizing neural tissue. The ability of OCE to delineate fine

anatomical features based on intrinsic biomechanical differences highlights its potential as a label-free tool for studying brain microstructure. Moreover, the consistency of these findings across multiple tissue depths and anatomical regions suggests that wide-field OCE can provide a comprehensive, quantitative view of brain biomechanics that complements conventional histological analysis.

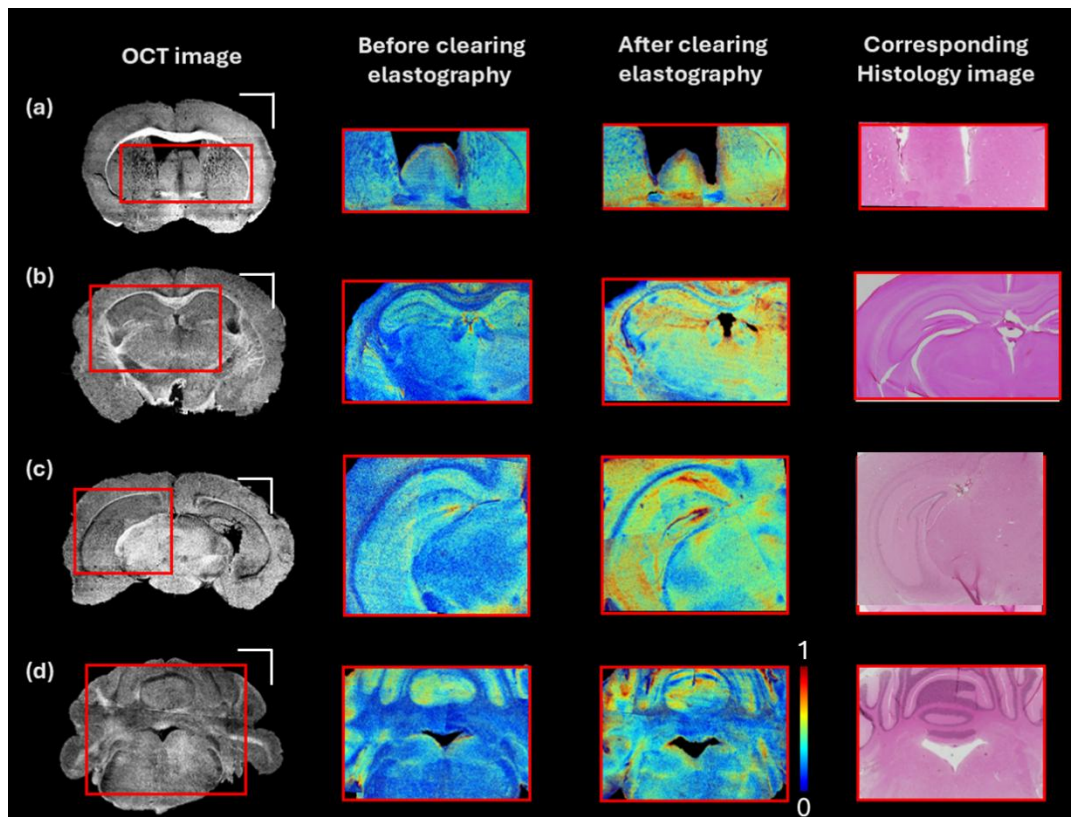


Figure 5.6. Wide-field OCE imaging across multiple coronal brain regions. Each row shows (a) OCT structural image, (b) OCE elastogram before clearing, (c) OCE elastogram after clearing, and (d) corresponding H&E histology. Mechanical contrast closely parallels histological architecture, with optical clearing providing improved structural visibility while preserving intrinsic stiffness patterns.

5.4 Discussion

The results of this chapter demonstrate that compressional OCE provides a reliable and high-resolution method for visualizing the mechanical heterogeneity of mouse-brain tissue. The system consistently distinguished gray and white matter based on their intrinsic stiffness, with gray matter showing higher strain (softer) and white-matter tracts exhibiting lower strain (stiffer). These mechanical patterns closely matched corresponding histological structures,

including laminar organization in the hippocampus and cerebellar cortex, highlighting the biological relevance of the OCE-derived contrast.

Optical clearing enhanced imaging penetration and boundary definition without altering the underlying stiffness distribution. This confirms that clearing functions as an optical improvement rather than a biomechanical modifier, making it a valuable tool for ex-vivo OCE of thick or scattering tissues.

Despite these strengths, several limitations must be acknowledged. The current system is restricted to ex-vivo imaging due to its reliance on stable surface contact, controlled loading, and limited OCT penetration depth. Uniform compression across the imaging field remains critical for accurate elasticity reconstruction, and minor variations in contact pressure can introduce artifacts. Motion sensitivity—particularly lateral drift between sequential B-scans—also poses challenges for phase-resolved strain estimation.

Depth-dependent sensitivity remains another limitation, even with clearing, as OCT signal and phase stability degrade with depth. Future efforts may include improved clearing strategies, longer wavelengths, adaptive optics, or faster acquisition to enhance depth fidelity and reduce motion-related artifacts.

Finally, this study focused on quasi-static elasticity. Since brain tissue is viscoelastic, dynamic excitation or frequency-dependent analysis may provide additional insight into microstructural and pathological changes.

Overall, compressional OCE shows strong potential as a label-free tool for studying neural biomechanics. Continued development toward improved depth penetration, more stable loading control, and potential in-vivo adaptation will further expand its utility for neuroscience research and disease characterization.

5.5 Summary

This chapter presented a wide-field compressional OCE framework for quantitative biomechanical mapping of ex-vivo mouse-brain tissues. By integrating high-sensitivity phase-resolved OCT detection with controlled axial loading and calibrated stress–strain analysis, the system produced strain and elasticity maps that delineated gray- and white-matter structures with high spatial fidelity. Validation with tissue-mimicking phantoms demonstrated the accuracy and stability of the strain-estimation workflow, while experiments on mouse-brain slices revealed robust mechanical contrast reflecting intrinsic microstructural organization.

Across multiple coronal levels, OCE consistently captured regional biomechanical heterogeneity in close agreement with corresponding histological images. Gray matter exhibited higher strain and softer behavior, whereas white-matter tracts showed lower strain and greater stiffness, aligning with known differences in cellular composition, axonal density, and myelination. Laminar organization within the hippocampus and cerebellar cortex was also visible, demonstrating the capacity of compressional OCE to resolve fine-scale mechanical features that parallel complex cytoarchitecture.

Optical clearing served as an effective auxiliary enhancement, improving depth penetration and boundary definition without altering the intrinsic stiffness relationships across brain regions. This reinforces the interpretation that the mechanical contrast measured by OCE arises from true biological differences rather than optical artifacts. The combination of clearing and compressional OCE therefore enables more comprehensive biomechanical assessment, especially in densely scattering or thick neural tissues.

Collectively, the results presented here highlight the strength of compressional OCE as a label-free, high-resolution modality for studying brain biomechanics. Its ability to generate quantitative, spatially resolved elasticity maps offers a valuable complement to histological analysis and has potential relevance for investigating neurological diseases characterized by subtle mechanical alterations. These findings lay the groundwork for future development toward in-vivo or minimally invasive neural elastography, integration with multimodal imaging platforms, and deeper exploration of biomechanical biomarkers in health and disease.

CHAPTER 6: Summary and Future direction

6.1 Summary of findings

This dissertation developed and validated a comprehensive quantitative optical coherence elastography (OCE) framework that spans system innovation, methodological advancement, and biological discovery. Across multiple chapters, the work integrated high-sensitivity OCT detection with carefully engineered excitation schemes to achieve reliable measurement of soft-tissue biomechanics at micrometer spatial scales. A core technical contribution was the establishment of multiple actuation modalities—including air-coupled ultrasound generation, controlled compressional loading, and synchronized mechanical perturbation—that enabled noncontact or minimally invasive stimulation of tissues across a wide range of geometries. These excitation mechanisms were paired with phase-resolved OCT processing pipelines optimized for nanometer-level displacement sensitivity, high repeatability, and robustness against environmental disturbance. Collectively, these system innovations laid the foundation for precise quantification of elastic wave propagation, strain distribution, and material stiffness.

Building on this technical groundwork, the dissertation applied OCE to several anatomically and clinically significant tissues within the eye. In the optic nerve head (ONH), a region central to glaucoma pathophysiology, OCE reliably detected intraocular-pressure–dependent increases in mechanical stiffness. The experiments demonstrated a monotonic relationship between wave speed and IOP, validating OCE’s ability to capture load-induced biomechanical changes in vivo. These findings underscored the sensitivity of the ONH to pressure modulation and highlighted the importance of mechanical characterization in glaucoma research. In the sclera, detailed mapping of wave propagation in orthogonal directions revealed clear regional anisotropy, consistent with collagen fiber architecture. By quantifying direction-dependent elastic moduli, the work provided new experimental evidence supporting fiber-guided mechanical reinforcement in the scleral shell, which plays a critical role in ocular biomechanics and IOP-related deformation. In the crystalline lens, in vivo OCE measurements demonstrated age-dependent stiffening and relative insensitivity to acute pressure changes, offering valuable insight into lenticular biomechanics relevant to presbyopia and cataract progression.

The dissertation further extended OCE beyond ophthalmology to neural tissue characterization. A wide-field compressional OCE platform was developed to image the mouse brain, incorporating an optimized preload control module and high-resolution phase-sensitive

strain mapping. Importantly, optical clearing techniques were introduced to partially improve signal penetration and delineation of microstructural boundaries. The resulting mechanical maps showed pronounced contrast between gray matter and white matter, reflecting their distinct cytoarchitectural organization. Correlative histological analysis strengthened the link between tissue structure and elasticity, demonstrating OCE's ability to capture biomechanical signatures that complement conventional imaging contrasts. This neural tissue work broadened the scope of OCE and demonstrated its capability for mapping heterogeneous, layered, and highly scattering biological systems.

Collectively, the results of this dissertation advance OCE as a unified, quantitative tool for multiscale biomechanical imaging across ocular and neural applications. The technical developments provide enhanced sensitivity, flexibility, and imaging depth, while the biological studies illustrate OCE's ability to identify clinically relevant mechanical biomarkers associated with pressure loading, fiber orientation, age-related changes, and neural microstructure. The findings establish a clear pathway toward translational OCE technologies that can support disease detection, therapeutic monitoring, and mechanistic insight in both ophthalmology and neuroscience.

Taken together, these findings demonstrate the feasibility and accuracy of the proposed OCE methodologies and highlight their value for quantitative assessment of soft tissue biomechanics across a range of ocular and neural structures. By integrating phase-resolved detection, optimized excitation strategies, and robust wave-propagation analysis, this work establishes a coherent framework for high-resolution mechanical imaging and provides new insight into the structure–function relationships that govern tissue behavior. Despite these advances, several limitations and technical challenges remain, particularly in the areas of imaging speed, excitation safety, and volumetric coverage. Addressing these challenges will be essential for translating OCE from controlled laboratory environments to real-time, clinically deployable systems. The following section outlines key future directions aimed at overcoming these limitations and enabling the next generation of ultrafast, low-exposure, and clinically practical OCE technologies.

6.2 Future Direction: MHZ OCT/OCE

Advancing OCE into the MHz regime represents one of the most impactful technological trajectories for the field. Current swept-source and spectrometer-based OCT systems, including those used throughout this dissertation, typically operate at tens to hundreds of kilohertz in A-scan rate. While sufficient for quasi-static and moderate-speed wave measurements, these acquisition speeds fundamentally limit the temporal resolution, volumetric coverage, and motion robustness needed for real-time elastography. As tissue mechanics are increasingly understood as dynamic and spatially heterogeneous, the ability to capture high-speed elastic wave propagation, transient responses, and large-scale fields of view becomes essential. Transitioning to MHz A-scan rates—enabled by recent advances in vertical-cavity surface-emitting lasers (VCSELs), Fourier-domain mode-locked (FDML) swept sources, and high-bandwidth digitizers—offers a compelling path toward the next generation of OCE performance.

The current confocal shear-wave OCE method already reduces tissue exposure compared to compressional-wave and modulation-based approaches. However, for delicate layers of the retina, even brief acoustic radiation force (ARF) excitation raises safety concerns. A promising future direction is therefore to further minimize excitation dose by using a **single-shot shear-wave pulse**, in which one ultrashort ARF impulse creates a broadband shear wave that propagates through the tissue. Implementing such a strategy requires extremely high imaging speed, as the entire displacement field must be captured before the wave dissipates or becomes confounded by physiological motion. This requirement is now achievable with modern **MHz swept-source lasers**, whose ultrafast A-line rates enable dense temporal sampling within a single excitation cycle. Importantly, our phase stability studies demonstrated that the phase fidelity of these swept-source systems is comparable to that of broadband sources, thereby removing a longstanding barrier to using swept-source platforms for phase-resolved Doppler imaging and nanometer-scale elasticity detection.

With MHz imaging, a single ARF beam can be applied to generate a shear-wave front that propagates along both the x- and y-axes of the sample. The ultrafast A-line rate enables rapid fast-axis scanning across the entire OCT field of view, allowing reconstruction of the wave's arrival time at each spatial location. From these measurements, local shear-wave velocity can be computed throughout the tissue, enabling generation of a **full elasticity map from a single excitation pulse**. This approach dramatically reduces mechanical exposure while

simultaneously providing high-resolution spatially resolved elasticity measurements—an essential step toward safe, clinically deployable retinal OCE.

At MHz acquisition speeds, the benefits extend beyond exposure reduction. The ability to acquire volumetric datasets within fractions of a second enables near-instantaneous 3D mechanical imaging and direct visualization of shear waves traveling at several meters per second without the need for repeated stimulation or phase-locked excitation. The reduction in temporal sampling gaps greatly improves wave-tracking fidelity, mitigating aliasing and interpolation artifacts that arise from undersampled propagation. Moreover, the dense temporal sampling provides inherent robustness against motion artifacts from breathing, heartbeat, and microsaccades, enabling reliable displacement extraction even in challenging in vivo scenarios. This positions MHz-OCE to approach real-time elasticity estimation comparable to ultrasound elastography but with substantially higher spatial resolution.

Realizing the full potential of MHz-OCE will require parallel advances in signal processing and excitation strategies. High-throughput pipelines incorporating GPU-accelerated reconstruction, real-time Fourier-domain resampling, and low-latency phase unwrapping will be essential to manage the enormous data rates. FPGA-based architectures may allow on-the-fly implementation of dispersion compensation, k-clock resampling, and Doppler demodulation, reducing computational bottlenecks. Novel excitation methods—including high-frequency air-coupled transducers, optical radiation-pressure stimulation, and broadband photonic excitation—may also be needed to fully exploit ultrafast sampling.

Beyond hardware, MHz OCE opens the door to more sophisticated mechanical modeling and reconstruction algorithms. Dense 4D sampling will enable estimation of full vector displacement fields, supporting simultaneous analysis of shear, compressional, and surface-wave modes and enabling inversion of viscoelastic, poroelastic, and anisotropic models that are underdetermined by current sampling rates. The ability to track transient events—pulse-induced deformation, accommodation dynamics, or rapid neural tissue responses—will extend OCE into the realm of functional elastography and dynamic biomechanical assessment.

From a translational standpoint, single-pulse MHz OCE represents a transformative path toward **safe, high-speed, clinically viable elastography**, particularly for the retina and optic nerve head. By delivering only one low-dose ARF excitation while capturing the full mechanical response at MHz rates, the method combines unprecedented safety with ultrafast 3D elasticity

mapping. This single-shot framework could support real-time monitoring in ophthalmic surgery, IOP modulation, retinal disease assessment, and neuro-ophthalmic diagnostics—areas where both speed and safety are paramount.

As MHz light sources, high-speed detectors, and efficient computational architectures mature, their convergence will establish OCE as a uniquely powerful modality for rapid, quantitative, and high-resolution biomechanical imaging. The transition into the MHz regime represents not merely an incremental technical improvement but a fundamental transformation that will redefine what is possible in both research and clinical elastography.

6.3 Future Direction: Real time ophthalmology OCE

The integration of optical coherence elastography into clinical ophthalmic imaging represents a major translational opportunity for the technology. Ophthalmology is uniquely positioned to benefit from real-time biomechanical assessment due to the strong mechanical dependence of diseases such as glaucoma, keratoconus, and presbyopia. Despite this need, current clinical instruments provide little or no direct mechanical contrast. The results of this dissertation—quantifying ONH pressure sensitivity, mapping scleral anisotropy, and characterizing lens stiffness—highlight the substantial clinical insight made possible by OCE. The next step is to transition these capabilities into real-time systems that can operate in vivo, at the point of care, and within the constraints of routine clinical workflows.

Achieving real-time ophthalmic OCE requires advances across multiple dimensions of system design. First, phase stability must be improved to withstand natural patient motion, including microsaccades, blinking, and head drift. This will involve enhanced common-path or active phase-stabilization designs, ultra-low-noise swept sources, environmental vibration isolation, and robust reference-arm management. Eye-tracking feedback and adaptive optics may further suppress motion artifacts to preserve nanometer-scale phase sensitivity in clinical environments.

Second, mechanical excitation must be safe, comfortable, and compatible with rapid, repeatable clinical use. In addition to air-puff actuation, acoustic radiation force, and optical perturbations, **air-coupled ultrasonic excitation** represents a highly promising strategy for clinical translation. Unlike contact transducers, air-coupled excitation delivers mechanical energy through the air interface, eliminating the need for gels, contact plates, or mechanical

coupling to the ocular surface. This avoids contamination risk, reduces patient discomfort, and enables rapid measurements within a routine OCT exam. Furthermore, the noncontact nature of air-coupled excitation inherently complies with corneal and retinal safety thresholds, as the transmitted acoustic intensity can be tightly controlled and spread over a broad beam profile. With continued improvements in bandwidth, focusing efficiency, and transducer miniaturization, air-coupled devices may become the preferred excitation modality for ophthalmic OCE in clinical settings.

On the computational side, real-time operation will require low-latency, high-throughput processing pipelines capable of handling millions of A-scans per second. GPU-based parallelization will be essential for phase demodulation, motion correction, wave tracking, and elasticity reconstruction. Machine-learning-assisted inversion algorithms may further accelerate modulus estimation by replacing iterative solvers with neural networks trained to infer mechanical properties directly from displacement fields. Hardware–software co-design—especially when paired with emerging MHz-rate swept-source OCT—will make it possible to visualize wave propagation and mechanical metrics in real time during patient examination.

Beyond the engineering challenges, successful integration into clinical workflows will require systems that are compact, automated, and compatible with existing OCT platforms. Air-coupled excitation is particularly attractive in this regard, as its noncontact nature lends itself to simple, hygienic, and automated positioning within slit-lamp–style or tabletop OCT devices. Combined with automated alignment, quality metrics, and multimodal co-registration with structural OCT and OCTA, OCE could be incorporated seamlessly into standard ophthalmic examinations. Regulatory pathways may also be streamlined with air-coupled excitation, as it avoids direct physical contact and can be engineered to operate well below ocular acoustic safety limits.

Clinically, real-time ophthalmic OCE could transform tissue assessment across multiple applications. For glaucoma, live ONH stiffness measurements could provide a biomechanical risk index that complements IOP, structural OCT, and functional testing. For keratoconus and refractive surgery planning, rapid corneal elasticity mapping could detect focal mechanical weakening before topographic changes emerge. For presbyopia and cataract management, lens stiffness measurements may enable personalized IOL selection and early detection of age-related stiffening. Importantly, air-coupled excitation would allow these measurements to be performed

quickly and comfortably without compromising corneal integrity, making routine mechanical screening feasible for a broad patient population.

Looking forward, real-time ophthalmic OCE has the potential to become as integral to clinical care as angiographic OCT. The convergence of noninvasive excitation—particularly air-coupled ultrasound—motion-tolerant phase detection, ultrafast imaging, and intelligent computational reconstruction will enable robust in vivo biomechanical measurements. As these technologies mature and are integrated into patient-friendly diagnostic platforms, OCE may evolve into a standard clinical modality that offers immediate mechanical insight, improves diagnostic precision, and supports personalized ophthalmic care.

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