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Infrared Chemical Biopsy for Non-Destructive Compositional Assessment of Intact Bone

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Abstract

Bone mechanical behavior reflects the integrated contributions of tissue composition and structure, yet compositional characterization generally requires tissue removal or specimen preparation. Here, we introduce Infrared Chemical Biopsy, a framework for non-destructive compositional interrogation of intact bone using fiber-optic near-infrared (NIR) spectroscopy. As a proof-of-concept, we acquired NIR spectra directly from the lateral surface of the human ilium at a clinically relevant biopsy site before retrieving a spatially matched trephine transiliac biopsy. Intact-surface spectra contained characteristic features associated with bone water, collagen, mineral, and fat. The corresponding biopsy was subsequently characterized by micro-computed tomography and compression testing, establishing a sequential workflow linking intact-site compositional assessment with three-dimensional bone structure and mechanical behavior. Serial trimming of the biopsy from the medial surface, while maintaining the original lateral NIR measurement surface, produced systematic spectral changes as tissue thickness decreased, demonstrating that the intact-surface NIR signal contains contributions from underlying bone. These findings establish the feasibility of Infrared Chemical Biopsy as a spatially matched composition–structure–mechanics framework and provide a foundation for investigating bone quality non-destructively at accessible skeletal sites.

Keywords: Near-infrared spectroscopy; Bone; Bone biopsy; Iliac crest; Bone composition; Fiber-optic spectroscopy

Introduction

Bone mechanical behavior is governed by the integrated contributions of tissue structure and composition. While bone density and morphology are key determinants of skeletal strength, tissue-level compositional properties also play important roles in determining stiffness, toughness, and failure behavior [1-4]. Bone composition is characterized by the relative content, organization, and interactions of mineral, collagen, water, and other matrix constituents, each of which contributes to tissue mechanical behavior through distinct yet interconnected mechanisms [5-10]. For example, mineral content largely determines tissue stiffness, collagen provides tensile resistance and toughness, and bone water contributes to viscoelastic behavior and energy dissipation. Accordingly, decades of biomechanics research have demonstrated that alterations in the amount, distribution, or properties of these constituents are associated with changes in bone mechanical behavior across aging, disease, and therapeutic intervention [11-16].

Characterization of bone composition is routinely performed using histological methods, biochemical assays, and vibrational spectroscopic techniques, such as Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy [16-20]. Collectively, these methods have substantially advanced our understanding of the relationships between bone composition and biomechanics. However, these approaches generally require tissue excision, sectioning, or extensive specimen preparation, limiting their ability to characterize composition directly at intact skeletal sites [16, 21, 22]. As a result, integrating localized compositional interrogation of intact bone into studies of bone structure and mechanical behavior at anatomically and biomechanically important skeletal sites remains challenging, thereby limiting fundamental understanding of composition–structure–mechanics relationships in bone.

Near-infrared (NIR) spectroscopy has emerged over the past decade as a complementary approach for compositional characterization of skeletal tissues [23-38]. In bone, NIR spectra provide information on major tissue constituents, including water, collagen, mineral-associated hydroxyl groups, and lipids. Quantitative NIR spectral parameters of bone, including peak intensities and band ratios [17], have been validated against established measures of bone mineral content [39, 40], collagen content [40-44], water compartments [40-42], and bone marrow fat [42], supporting their use for quantitative assessment of bone composition. Compared with FTIR and Raman spectroscopy, NIR interrogates a substantially larger tissue volume, enabling measurements from several millimeters beneath the tissue surface [37, 45, 46]. Additionally, coupled with portable spectrometers and fiber-optic probes [25, 47], NIR enables localized compositional assessment directly from intact skeletal surfaces.

Recent studies have demonstrated that NIR-derived compositional parameters provide meaningful information about bone structure and mechanical behavior. In osteochondral specimens [36, 37, 48] and human sternal bone [30], multivariate regression models based on NIR spectra have successfully predicted structural parameters, demonstrating that NIR measurements reflect underlying skeletal architecture. Subsequent studies extended these observations by demonstrating relationships between NIR-derived compositional parameters and bone mechanical behavior. In a mouse model of osteogenesis imperfecta [49], significant associations between NIR-derived protein and water parameters and bone stiffness, maximum load, and post-yield behavior were demonstrated. In human cadaveric tibiae [50], multivariate regression further showed that combined NIR-derived collagen and water measurements improved prediction of bone stiffness beyond structural measures alone. Most recently [51], multivariate regression models integrating NIR-derived compositional parameters with bone density and geometry substantially improved prediction of whole-bone proximal femur stiffness.

Despite these advances, studies relating NIR-derived compositional information to bone structure and mechanical behavior have largely relied on excised or sectioned specimens rather than measurements acquired directly from intact skeletal sites. The aim of this study was to demonstrate, as a proof-of-concept, an Infrared Chemical Biopsy workflow in iliac bone by integrating intact-surface fiber-optic NIR spectroscopy with spatially matched biopsy retrieval, structural imaging, and mechanical testing. Iliac crest biopsy remains the clinical reference standard when direct assessment of bone tissue composition and microstructure is required [52-55], making it an ideal model for establishing a non-destructive spectroscopic framework at an anatomically and clinically relevant skeletal site. To our knowledge, direct fiber-optic NIR interrogation of an intact iliac biopsy site has not been reported. By integrating intact-site compositional assessment with conventional imaging and biomechanical measurements, this framework provides a foundation for investigating composition–structure–mechanics relationships in bone while enabling compositional information to be obtained non-destructively before tissue retrieval.

Results and Discussion

Localized fiber-optic NIR spectroscopy enables non-destructive interrogation of intact bone

We used a portable NIR spectrometer coupled to a small-diameter fiber-optic probe to acquire spectra directly from localized regions of intact bone (Fig. 1a). The compact probe geometry allowed measurements to be obtained from anatomically defined sites while leaving the tissue intact and available for subsequent analysis (Fig. 1b). Fiber-optic NIR spectroscopy is particularly well suited to this type of interrogation because its comparatively large sampling volume enables measurement of tissue beneath the immediate surface [37, 45, 46], while the small-diameter probe permits localized measurements from geometrically complex skeletal sites. This combination provides the technical basis for the Infrared Chemical Biopsy concept: rather than removing tissue to determine its composition, spectroscopic information is first acquired directly from the intact skeletal surface. Importantly, the approach is not intended to replace conventional biopsy or established structural and mechanical measurements. Instead, it provides a non-destructive compositional measurement that can be integrated with subsequent characterization of the same anatomical site.

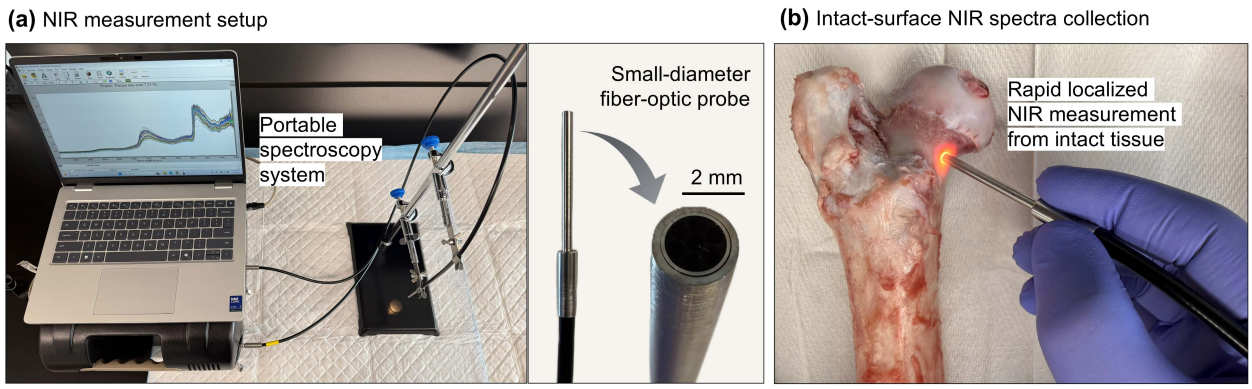


Figure 1. Fiber-optic NIR spectroscopy of intact bone. (a) Portable NIR spectroscopy system coupled to a small-diameter fiber-optic probe. (b) Localized intact-surface NIR spectral acquisition, illustrated here at a femoral neck region.

Infrared Chemical Biopsy establishes spatial correspondence between the intact-surface measurement and the biopsy specimen

We next implemented this approach at a human iliac biopsy site (Fig. 2a). Spectra were acquired from the lateral surface of the ilium near the anterior superior iliac spine (ASIS), corresponding to the anatomically accessible region conventionally used for transiliac bone biopsy [52-55]. Intact-surface NIR spectra were collected before tissue was removed (Fig. 2b), after which we obtained a trephine biopsy from the same location (Fig. 2c,d), establishing direct spatial correspondence between the surface-accessible infrared measurement and the retrieved tissue specimen. This one-to-one correspondence is central to the Infrared Chemical Biopsy framework. Conventional vibrational spectroscopic characterization of bone frequently relies on excised, sectioned, or otherwise prepared specimens [16, 21, 22]. Here, the order is reversed: compositional information is obtained first, from the intact site, and the corresponding tissue remains available afterward. The retrieved biopsy can therefore serve as the physical specimen corresponding to the spectroscopic measurement and subsequently undergo complementary structural, compositional, or mechanical analyses.

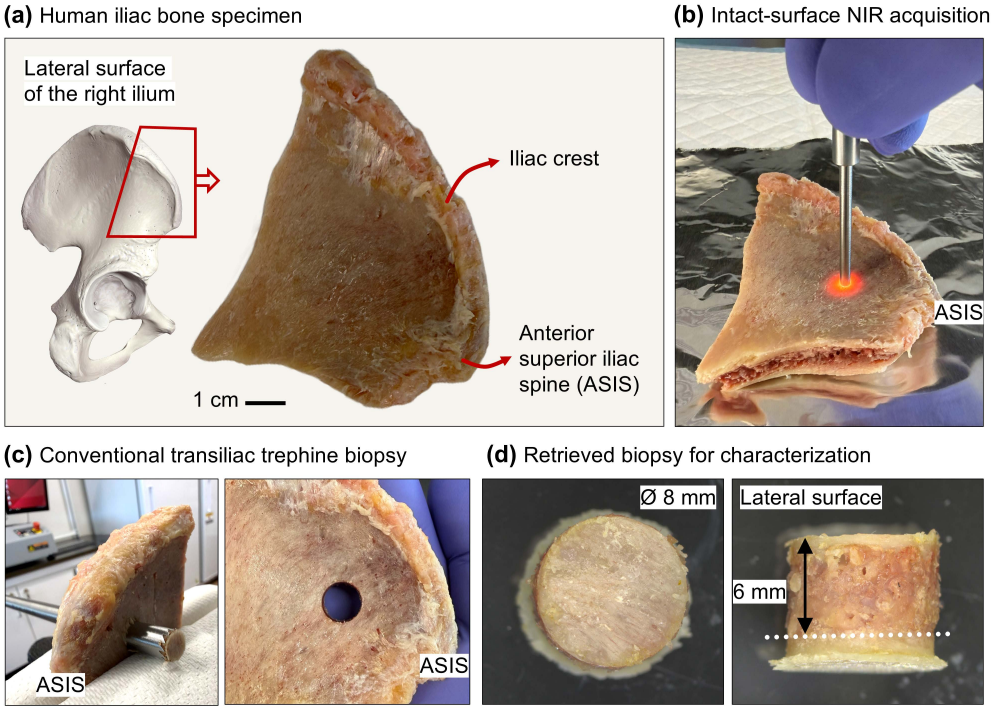


Figure 2. Intact-surface NIR acquisition and spatially matched transiliac biopsy. (a) Human iliac bone specimen showing the lateral surface and anatomical landmarks. (b) NIR spectra were acquired directly from the intact lateral surface at the selected biopsy site. (c) A trephine biopsy was subsequently retrieved from the same location. (d) The retrieved biopsy was trimmed to a final height of approximately 6 mm for subsequent structural and mechanical characterization.

Fiber-optic NIR spectroscopy has been applied extensively to investigate skeletal tissues, including articular cartilage and subchondral bone in intact joints [24, 34, 36, 56, 57] and excised specimens [23, 29, 30, 33, 37, 40, 51]. Importantly, studies of osteochondral tissues have demonstrated that NIR measurements acquired from intact or minimally prepared surfaces can reflect underlying structural and biomechanical properties, including cartilage mechanics and subchondral bone microarchitecture [30, 34, 36, 37, 48]. In situ fiber-optic NIR measurements have also been used to characterize regenerative cartilage in knee defects,

demonstrating the feasibility of localized spectroscopic assessment directly within anatomically relevant tissue environments [24, 57]. Clinical studies of the second metacarpal have further demonstrated that transcutaneous fiber-optic NIR measurements can capture information related to underlying bone structure [25]. To our knowledge, however, NIR has not been used to interrogate an intact iliac bone surface at a clinically relevant biopsy site in a manner that directly links the spectral measurement to tissue subsequently retrieved from the same location for spatially matched structural and mechanical characterization.

Intact-surface NIR spectra provide chemically interpretable information from iliac bone

Intact-surface measurements produced characteristic and consistent NIR spectra from the iliac bone surface across repeated acquisitions (Fig. 3a). The raw spectra contained the broad and overlapping absorption features typical of bone tissue, consistent with previously described overtone and combination vibrations associated with water, collagen, mineral-associated hydroxyl groups, and lipids [17, 40-42]. Second-derivative processing resolved individual spectral features more clearly and facilitated visualization of bands associated with these major tissue constituents (Fig. 3b). Such spectral features have previously provided the basis for quantification of peak intensities and spectral ratios associated with specific compositional properties of bone tissue [17, 40-42]. The presence of these features demonstrates that chemically interpretable information can be obtained directly from the intact biopsy site without sectioning, polishing, or other preparation of the bone surface. This makes NIR particularly attractive when the objective is non-destructive interrogation of a localized tissue volume rather than high-resolution chemical imaging. Together, these results demonstrate the feasibility of obtaining composition-sensitive information directly from a clinically relevant iliac bone site.

Matched-site structural and mechanical characterization of iliac bone biopsies

Following NIR acquisition and matched-site biopsy retrieval, we used micro-computed tomography (microCT) to characterize the three-dimensional structure of the corresponding specimen (Fig. 4a). Reconstruction of the complete biopsy revealed both the cortical shell and internal trabecular architecture, while segmentation allowed the cortical and trabecular compartments to be distinguished. This provides access to standard quantitative measures of bone microarchitecture, including bone volume fraction, cortical thickness and porosity, and trabecular thickness, number, and connectivity [58]. After imaging, the same biopsy remained available for unconfined compression using a standardized testing configuration (Fig. 4b), generating a representative stress-strain response from which apparent modulus can be derived (Fig. 4c). Compression testing of iliac and trabecular bone has previously been used to characterize apparent-level mechanical behavior and its relationship with tissue architecture [59, 60].

The significance of these measurements in the present study lies not in quantitative characterization of an individual specimen, but in demonstrating that the tissue corresponding to the intact-surface infrared measurement remains available for detailed three-dimensional structural analysis and subsequent mechanical testing. The workflow therefore preserves a continuous link between composition measured at the intact iliac bone site, the architecture of the spatially matched biopsy specimen, and the mechanical behavior of that same tissue.

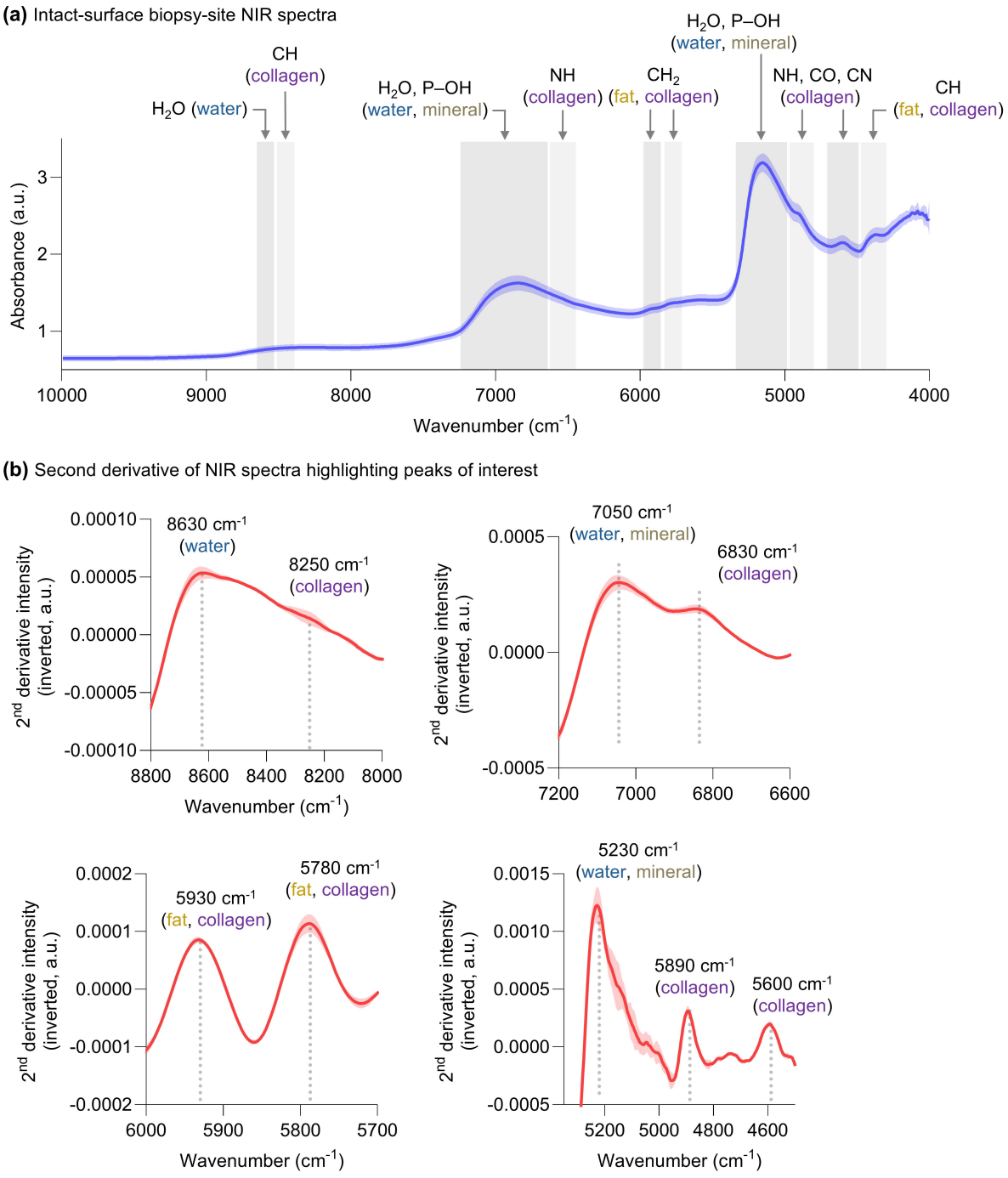


Figure 3. NIR spectra acquired directly from the intact iliac bone biopsy site. (a) Intact-surface NIR spectrum showing characteristic absorption features associated with major bone constituents. (b) Second-derivative spectra highlighting selected spectral peaks associated with water, mineral, collagen, and fat. Shaded regions represent the standard deviation across replicate spectra.

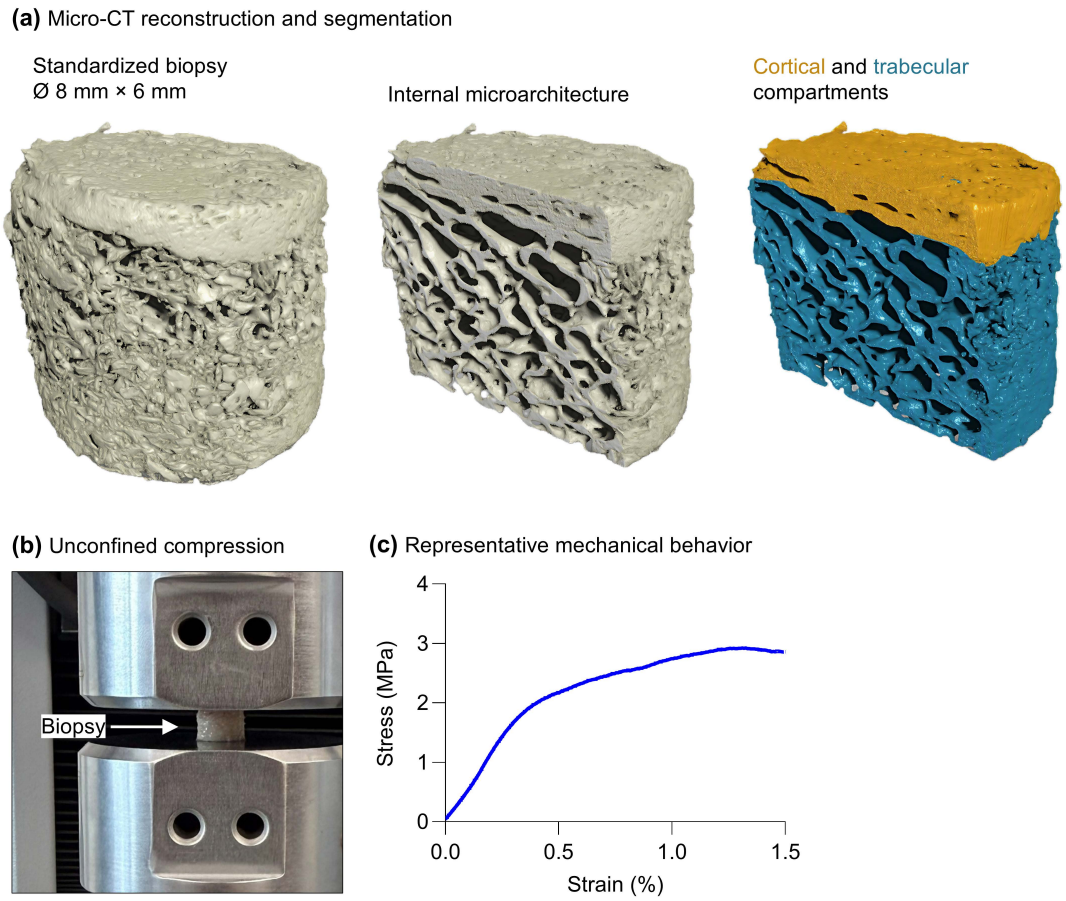


Figure 4. Structural and mechanical characterization of the spatially matched iliac bone biopsy. (a) MicroCT reconstruction of the biopsy showing internal microarchitecture and segmentation of cortical and trabecular compartments. (b) Unconfined compression testing of the same biopsy following microCT imaging. (c) Representative stress–strain response obtained during compression testing.

Intact-surface NIR measurements contain contributions from tissue beneath the cortical surface

A critical question for Infrared Chemical Biopsy is what tissue the intact-surface spectrum represents. If the measurement were dominated exclusively by the immediate outer surface, its relationship to the underlying biopsy would necessarily be limited. NIR radiation, however, can penetrate substantially deeper into biological tissue than mid-infrared radiation, and previous studies of bone and other tissues have demonstrated sensitivity to tissue several millimeters beneath the surface [37, 45, 46]. To directly investigate whether underlying bone contributed to our measurement, we performed a serial trimming experiment. We repeatedly acquired NIR spectra from the same lateral cortical surface while progressively removing tissue from the opposite, medial surface of the biopsy (Fig. 5a). Thus, the measurement surface and probe location remained unchanged while the amount of tissue beneath the measurement progressively decreased.

The spectra changed systematically as the remaining biopsy thickness was reduced (Fig. 5b). Because no material was removed from the measured cortical surface, these changes provide direct evidence that tissue beneath the immediate surface contributes to the intact-surface NIR signal. The measurement therefore interrogates a subsurface tissue volume rather than exclusively sampling the outer cortical boundary. This observation provides an important

physical connection between the surface-accessible spectrum and the spatially matched biopsy subsequently retrieved from the same site. It supports the premise that an intact-surface NIR measurement can contain information about the bone volume subsequently characterized by microCT and mechanical testing. At the same time, the present experiment should not be interpreted as defining a universal NIR penetration depth. Effective sampling depth depends on wavelength, optical properties, tissue composition, geometry, and acquisition conditions [45, 61, 62]. A larger systematic study is required to quantify the effective sampling volume and determine how it varies among specimens.

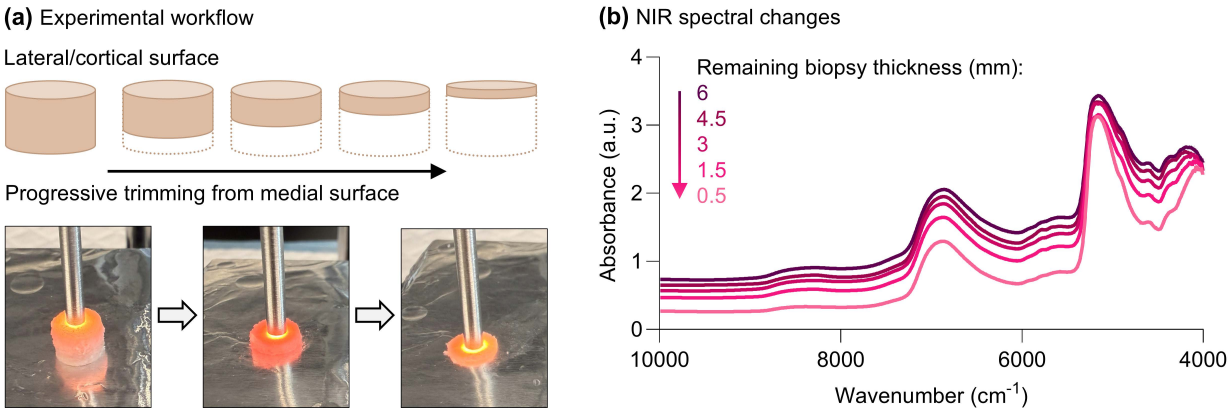


Figure 5. Influence of underlying tissue on intact-surface NIR spectra. (a) Serial trimming workflow in which tissue was progressively removed from the medial surface while NIR spectra were repeatedly acquired from the unchanged lateral cortical surface. (b) Intact-surface NIR spectra at decreasing remaining biopsy thicknesses, showing systematic spectral changes as underlying tissue was removed.

Infrared Chemical Biopsy as a framework for studying bone composition, structure, and mechanics

Taken together, these experiments establish Infrared Chemical Biopsy as a spatially matched workflow linking intact-site spectroscopy with subsequent structural and mechanical characterization of tissue retrieved from the same anatomical location. The serial trimming experiment further demonstrates that the intact-surface NIR signal contains contributions from tissue beneath the cortical surface, providing a physical basis for relating the surface-accessible spectrum to the underlying biopsy volume. To our knowledge, spectroscopic assessment of iliac bone has previously been performed predominantly on retrieved biopsy specimens, whereas direct fiber-optic interrogation of the intact iliac surface at a clinically relevant biopsy site prior to tissue removal has not been reported.

The present study builds on previous applications of NIR and other optical spectroscopic approaches to skeletal tissues, including transcutaneous bone measurements [25, 63] and arthroscopic spectroscopy of articular cartilage and subchondral bone [23, 24, 34, 36]. Notably, the concept of an “optical biopsy of bone” has previously been proposed using non-invasive time-resolved optical spectroscopy of the calcaneus, demonstrating the broader potential of optical measurements to interrogate bone tissue in vivo [64]. Together, these studies demonstrate the feasibility of obtaining compositional information from skeletal tissues non-destructively or with minimal tissue disruption. The key contribution of this study is extending localized fiber-optic NIR spectroscopy to a clinically relevant iliac bone biopsy site while preserving the corresponding tissue for subsequent structural and mechanical characterization.

The current study is intentionally limited to demonstrating the feasibility of this workflow and therefore does not establish population-level composition–structure–mechanics relationships. We do not attempt to quantitatively interpret individual compositional, structural, or mechanical metrics or establish statistical relationships among them. Biological variation in cortical thickness, trabecular architecture, tissue composition, and optical sampling volume will need to be addressed in larger cohorts. Nevertheless, establishing this experimental sequence is an important prerequisite for such studies. By obtaining compositional information before tissue removal while preserving the corresponding specimen for subsequent analyses, Infrared Chemical Biopsy provides a framework for investigating how surface-accessible spectral information relates to the structure and mechanics of intact bone. In the longer term, this framework may pave the way toward minimally invasive assessment of bone quality at accessible skeletal sites, potentially reducing reliance on physical biopsy retrieval.

Methods

Human iliac bone specimen

A fresh-frozen human iliac bone specimen obtained through the National Disease Research Interchange (NDRI, Philadelphia, USA) was used for this proof-of-concept study. The specimen was obtained from a 79-year-old male donor and stored frozen until use. The specimen was thawed and dissected to remove surrounding soft tissue prior to experimental measurements. The specimen was kept hydrated in phosphate-buffered saline (PBS) throughout the study.

Near-infrared spectroscopy

Intact-surface NIR spectra were acquired using an ASD LabSpec 4 spectrometer coupled to a small-diameter diffuse-reflectance fiber-optic probe with a 2-mm spot size (Malvern Panalytical). The measurement location on the lateral surface of the ilium was selected within Bordier's triangle, approximately 2 cm posterior to the anterior superior iliac spine (ASIS) and inferior to the iliac crest, corresponding to the anatomical region routinely used for clinical and translational transiliac bone biopsy [52-55]. A baseline spectrum was acquired from a Spectralon white diffuse-reflectance standard before spectral acquisition. The probe tip was positioned perpendicular to and directly against the intact lateral cortical surface at the selected biopsy site, and spectra were collected before any tissue was removed. Ten replicate spectra were acquired over the spectral range of 10,000–4,000 cm^{-1}, with 50 scans averaged per spectrum. Acquisition time was 6 s per spectrum. Spectra were processed using Orange Data Mining software. Second-derivative spectra were calculated using a Savitzky–Golay filter with a 21-point window to resolve overlapping absorption features and facilitate identification of spectral peaks associated with bone water, mineral, collagen, and fat, as previously described [17, 40-42].

Iliac bone biopsy

Following intact-surface NIR acquisition, a trephine biopsy was retrieved from the same anatomical location using an 8-mm trephine with a plunger (Life Instruments). This procedure established direct spatial correspondence between the intact-surface NIR measurement and the subsequently retrieved tissue specimen. The retrieved specimen was visually inspected using a Stemi 508 stereomicroscope equipped with an Axiocam 208 camera (ZEISS). The biopsy was trimmed at the medial surface to produce a flat surface approximately parallel to the intact lateral cortical surface and a final specimen height of 6 mm using an EXAKT 302 diamond band saw. The resulting specimen contained the lateral cortical surface together with the underlying trabecular bone and was retained for subsequent structural and mechanical characterization.

Micro-computed tomography

The retrieved iliac bone biopsy was imaged using a SkyScan 1272 high-resolution microCT system (Bruker). The specimen was maintained hydrated during imaging by wrapping it in PBS-soaked gauze. Scans were acquired at an isotropic voxel size of 7.5 µm using a source voltage of 80 kV and current of 100 µA with a 1-mm aluminum filter. Images were acquired over 360° with a rotation step of 0.2°, an exposure time of 1100 ms, and three-frame averaging. Projection images were reconstructed using NRecon software (Bruker) with a ring artifact correction of 5 and beam-hardening correction of 60%. The reconstructed image volume was processed using Dragonfly 3D World software (Object Research Systems) to segment and visualize the cortical shell and underlying trabecular architecture.

Mechanical testing

Following microCT imaging, the biopsy was subjected to unconfined compression testing using a Model 34SC-05 single-column universal testing machine (Instron) equipped with a 500-N load cell, following established approaches for compression testing of human bone [59]. The specimen was positioned between parallel compression platens with the lateral cortical surface facing upward and the loading axis oriented through the thickness of the biopsy. The specimen was maintained hydrated with PBS until immediately prior to testing. A preload of 2 N was applied, followed by compression at a displacement rate of 1 mm/min. Testing was terminated before macroscopic failure, preserving the specimen for subsequent analysis. Initial specimen height and cross-sectional dimensions were measured using a digital caliper immediately before testing and used to convert the continuously recorded force and displacement data to engineering stress and strain.

Serial trimming experiment

To investigate the contribution of underlying tissue to the intact-surface NIR measurement, the biopsy was progressively trimmed from the medial surface in increments of approximately 1.5 mm using an EXAKT 302 diamond band saw, while the lateral cortical measurement surface remained unchanged. The remaining specimen thickness was measured using a caliper after each step. Ten NIR spectra were reacquired from the same lateral cortical surface after each trimming step using the acquisition protocol described above.

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Author Contributions

W.Q. conceived and designed the study, performed experiments, supervised the research, and wrote the manuscript. A.P. performed experiments, processed the data, and contributed to manuscript review and editing.

Competing Interests

The authors declare no competing interests.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Declarations

The use of human cadaveric tissue in this study was reviewed and approved by the Stevens Institute of Technology Institutional Review Board (IRB) under protocol 2026-026 and was classified as Non-Human Subjects Research. The tissue was obtained through the National Disease Research Interchange (NDRI, Philadelphia, USA). In accordance with NDRI tissue procurement policies, informed consent for the use of donated tissue for research was obtained in writing from the donor or the donor’s next-of-kin and is maintained by the tissue acquisition organization. All methods were performed in accordance with relevant institutional guidelines and regulations.

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