

Combined Raman spectroscopy and nanoindentation assessment of complex materials

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INTRODUCTION

The current rapid evolution of new materials for applications ranging from biomaterials and biomedical engineering to energy storage, necessitates facile methods to evaluate how a material's chemistry and structure contribute to mechanical behaviour. These combined measurements are necessary to inform design of advanced materials that achieve desired mechanical function and performance. Here, we present a new system that

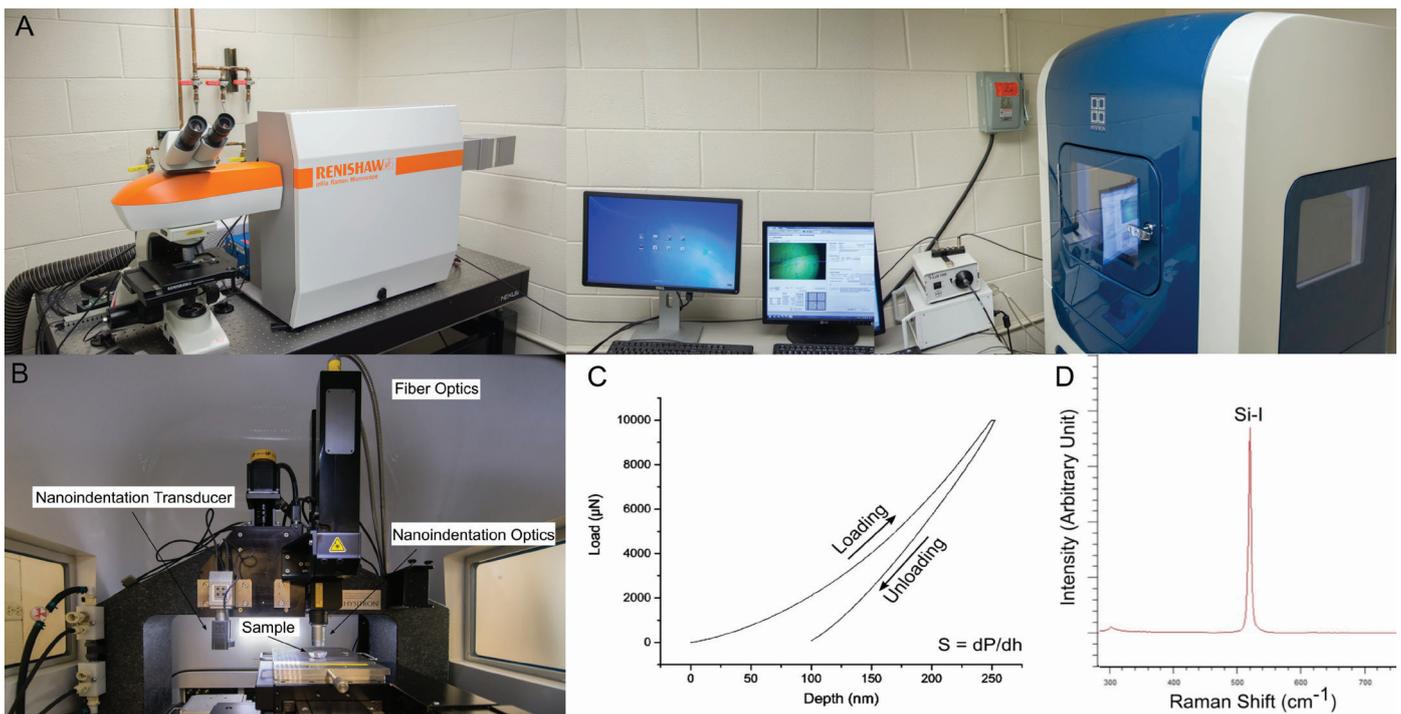
physically couples a Raman spectroscopy system with a nanoindenter on a shared stage that uniquely enables combined assessment of materials with sub-micrometre spatial resolution at single sites or spanning millimetre-sized regions (Figure 1).

In the current materials environment, researchers have traditionally assayed chemistry, structure and mechanical properties using different instruments where it is often difficult or impossible to locate the exact same region of

interest (ROI) on a single sample.

When matched ROIs are evaluated in separate assays, registering multiple data sets (e.g., images that differ in length scale, aspect ratio, and rotation angle) is expensive and time-consuming. One exciting solution lies in Raman spectroscopy - a versatile tool capable of evaluating chemistry and physical properties of a variety of organic and inorganic materials. When Raman spectroscopy is coupled with an atomic force microscope (AFM) equipped with

FIGURE 1 a) Combined Raman spectrometer (Left) and Nanoindenter (Right) System; b) View inside of Nanoindenter Enclosure showing the fibre optic cables and probes for both the 514 nm and 785 nm lasers.; c) Typical nanoindentation Load vs. Displacement curve; d) Typical Raman spectra for (100) Si



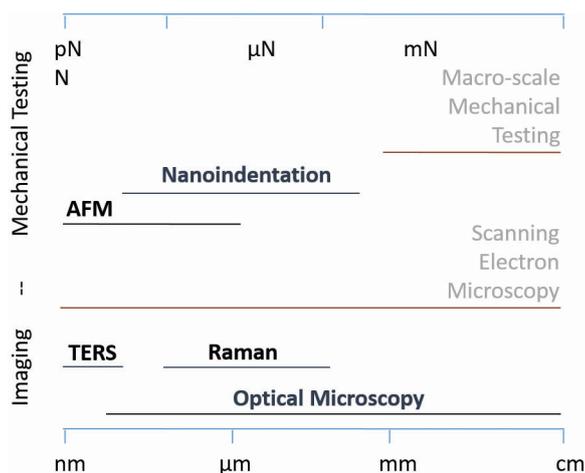
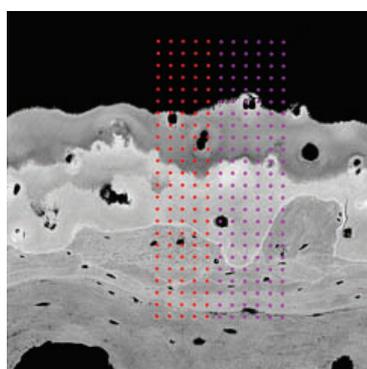


FIGURE 2 Length scale of nanoindentation overlaps that of Raman spectroscopy

FIGURE 3 Quantitative backscattered electron image of bone and calcified cartilage, where grayscale indicates variations in mineral density (white = 100%, black = 0%) but lacks chemical information. Nanoindentation sites are shown from testing with conico-spherical (red) and Berkovich (purple) probes
Image adapted from [6]



plasmonic tips, it is possible to collect Raman spectra and images with high resolution (SERS)¹. This novel approach was transformative as it enabled, for the first time, evaluation of chemistry and properties *in-situ*, at exactly matched nanometre-sized sites and spanning regions sized up to 100's micrometres.

Nanoindentation is a mechanical testing method in which a small probe is pressed into the surface of a material of interest, and the resulting load and displacement are monitored. Displacements into the material are typically on the order of nanometres, and contact areas often extend well into the micrometre range. Unlike AFM, commercial nanoindenter systems are readily programmed to collect data at single indent sites or to map property variations over large (millimetre to centimetre) 2D indent arrays⁶ – a feature that is particularly useful for assessment of heterogeneous samples. Indentation testing readily produces a measurement of stiffness, from which modulus and hardness are calculated. The most common tip geometries include three-sided pyramidal Berkovich and conico-spherical probes. Berkovich probes are sharp and can consistently probe depths < 50 nm, and are especially useful for testing at small length scales. The larger conico-spherical probes, possessing radii ranging from ~1-100 μm, require contact with a larger surface area yet

approximate an elastic contact with the test material to produce a more uniform strain field and are therefore used for soft material testing (e.g., hydrogels and tissues).

While originally developed to evaluate materials possessing predominantly elastic-plastic behaviour [2], refinements to these methods became necessary to appropriately evaluate polymers, hydrogels, biological tissues, and other materials possessing complex behaviours. Thus analytic methods have been developed to quantify time dependent material behaviour, such as creep and relaxation, using viscoelastic³ and poroelastic^{4,5} frameworks. These methods extend the scope of materials tested with nanoindentation to porous, hydrated materials such as biological tissues, hydrogels for tissue engineering, or oxygen-reactive materials submerged in fluid to enable testing without a specialized environmental chamber. Specialized nanoindentation systems can be located within a scanning electron microscope to visualize *in-situ* deformation. Alternately tips can be engineered to conduct heat or to evaluate secondary factors such as electrical contact resistance. While these advancements are useful, nanoindentation alone lacks the valuable inputs of chemical and structural information and how these factors influence mechanical behaviour.

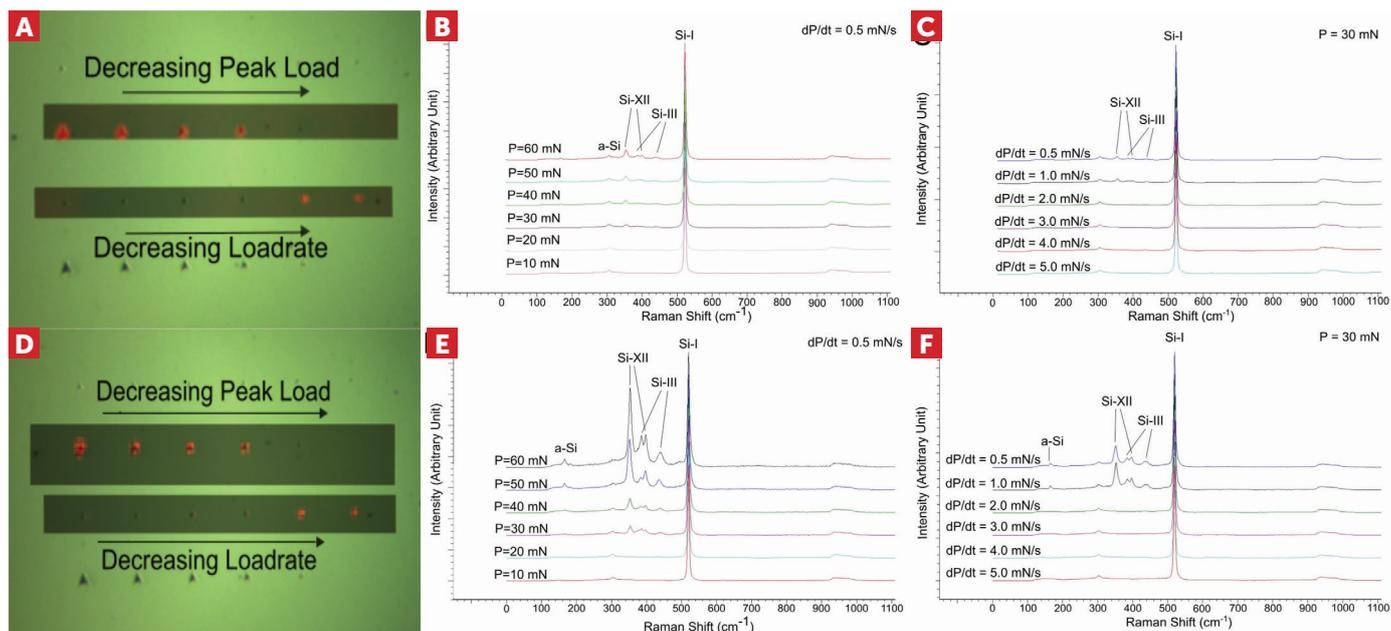
Nanoindentation is uniquely matched to conventional Raman spectroscopy, where both assays enable assessment of individual, micrometre-sized sites (both with lateral resolution ≤ 1 μm) (Figure 2). In a typical Raman test, monochromatic light from a laser source illuminates a specified area of a sample with a spot size given by $1.22 \times \text{wavelength} / \text{numerical aperture}$ (e.g., spot size = 0.84 micrometres for a 514 nm laser and 0.75 NA objective). A small fraction of the incident monochromatic light inelastically interacts with chemical bonds of specific energies, resulting in characteristic wavelength shifts that are measured by the detector. The intensity of light measured at the detector is plotted versus the shift in wavelength to create a Raman “signature” of a material (Figure 1D). Raman intensity is exquisitely sensitive to beam focus; thus intensity varies when Raman maps span even subtle topographic differences. Thus, for assessment of material chemistry, peaks of interest from a Raman spectrum are typically normalized, and these ratios compared between samples or groups of interest. Similar to nanoindentation, Raman spectra are readily collected at individual points or can be mapped over entire samples to evaluate material heterogeneity, functional gradients, or dissimilar material interfaces.

Nanoindentation's ability to survey large areas that span micrometres to millimetres (Figure 3) is critical for characterizing biological tissues as well as for a wide range of emerging materials for biological and other applications. Moreover, surface preparation for nanoindentation and Raman spectroscopy is similar, where flat surfaces are optimal; otherwise minimal sample preparation is required and samples can be tested when hydrated, embedded in plastic, or dry. Thus in combination with Raman spectroscopy, nanoindentation is a promising and exciting tool for non-destructive assessment of a broad range of materials where both techniques require little in the way of sample preparation.

Here we present a novel Raman Spectroscopy - Nanoindentation system, co-located on a shared stage (Figure 1B), which couples through fibre optic probes a Renishaw inVia confocal Raman microscope and spectrometer with a Hysitron TI-950 Triboindenter. This combined system achieves precise positioning of Raman and nanoindentation maps thus allowing investigation of how material chemistry and structure correlate with mechanical properties. Such an approach enables new directions in the study of heterogeneous, composite or functionally-graded materials as well as to elucidate root causes for complex material behaviours and phenomena (e.g., pressure-induced phase transitions). Here, we demonstrate the positional accuracy and resolution of our combined system with Raman maps of nanoindentation-induced phase transitions in silicon. We then display the utility of our approach by comparing chemistry and nanoindentation modulus maps of bone, a heterogeneous bio-composite material whose properties change with age. These two case studies demonstrate key capabilities of combining these two instruments into a single platform as well as the potential for future analysis of large numbers of test sites. A particularly exciting feature of these combined assays lies in the potential to develop statistical correlations over 1000's of test sites to elucidate mechanisms underlying a material's functional performance.

MATERIALS AND METHODS

All measurements were collected using a Renishaw inVia spectrometer that is optically coupled through fibre optic cables to the optical setup located within a Hysitron TI-950 Triboindenter. The Triboindenter uses a capacitive transducer system capable of measuring loads as small as 30 nN and displacements as small as 0.2 nm. Transducers are changeable, thus offering the possibility to perform indentation in both load


FIGURE 4

a) Ratio map of 353 cm^{-1} (Si-XII) to 520 cm^{-1} (Si-I) (Confocal);
 b) Raman spectra for peak loads of $P = 60\text{ mN}$ to 10 mN at $dP/dt = 0.5\text{ mN/s}$ (Confocal);
 c) Raman spectra for unloading rates of $dP/dt = 5.0\text{ mN/s}$ to 0.5 mN/s at $P = 30\text{ mN}$ (Confocal);
 d) Ratio map of 353 cm^{-1} (Si-XII) to 520 cm^{-1} (Si-I) (Nanoindentation Optics);
 e) Raman spectra for peak loads of $P = 60\text{ mN}$ to 10 mN at $dP/dt = 0.5\text{ mN/s}$ (Nanoindentation Optics);
 f) Raman spectra for unloading rates of $dP/dt = 5.0\text{ mN/s}$ to 0.5 mN/s at $P = 30\text{ mN}$

Nanoindentation Optics

and displacement control as well as quasistatic (12 mN max load), nanoDMA, nanoscratch, and high load (500 mN max) tests. A translatable stage with $250\text{ mm} \times 150\text{ mm}$ travel, to permit analysis of large specimens or multiple specimens within one test setting, and 500 nm encoder resolution enables precise sample movements. The Raman system is outfitted with a confocal microscope for independent Raman spectra collection and the spectrometer offers two lasers (green 514 nm and NIR 785 nm lasers). StreamLine acquisition is available for fast spectra recording on large sample volumes and WIRE software for analysis.

Engineers from Renishaw and Hysitron collaborated to develop a custom connection to the nanoindenter for combined assessment of Raman spectra and nanoindentation. This optical coupling is achieved by routing two fibre-optic cables from the Renishaw spectrometer, to separately communicate 514 nm or 785 nm laser light, to fibre optic probes that directly couple with the Leica (LWD 50x, 0.5NA) microscope objective mounted on the Triboindenter. This objective is also used to visualize the sample and locate sites for nanoindentation testing. The fibre-optic cables enable transmission of the monochromatic light from the laser source to the sample on the nanoindenter stage. The light interacts with the sample, and the signal is collected in backscatter mode and is then returned to the spectrometer via different fibre optic cables. This collected light is then routed into the spectrometer. Spectra are then analysed using the standard WIRE software. The Hysitron Triboscan software was modified to adjust and communicate stage coordinates to the Raman system

and trigger execution of Raman measurements; the Renishaw software required less modification because it has previously been configured to allow probes for AFM systems. The resulting combined system is capable of collecting Raman spectra on either the confocal side (without fibre optic transmission losses of signal), or using the fibre optic probes on the nanoindenter. For samples located on the Triboindenter translation stage, Raman spectra are readily collected at single sites or over programmable 2D arrays, where the two software tools communicate to move the stage and reliably collect and record data.

RESULTS AND DISCUSSION

APPLICATION 1. PRESSURE-INDUCED PHASE TRANSFORMATION IN Si

In order to demonstrate the positional accuracy and resolution of the combined system, we sought to create nanoindentation-induced small volume phase transformations in silicon, and then map the transformed and surrounding areas with Raman Spectroscopy using both the inVia confocal Raman microscope/spectrometer and the fibre optic Raman-indentation systems. Pressure-induced phase transitions are known to occur in (100) Si, where the Si-I structure transitions to Si-III/Si-XIII under nanoindentation load⁷. When (100) Si reaches a hydrostatic pressure of $11\text{--}13\text{ GPa}$, the diamond cubic structure (Si-I) transforms into the metallic β -tin structure (Si-II). If unloaded sufficiently slowly, Si-II reverts to Si-XII and then Si-III. The different silicon phases are known to produce separate Raman peaks; a peak at 520 cm^{-1} identifies Si-I, whereas Si-XII is identified by a peak at 353 cm^{-1} .

We performed two types of nanoindentation tests with a Berkovich indenter tip (Figure 4), following Jang *et al.*, 2005⁷. In the first, the peak load was varied from 60 mN to 10 mN over a row of six indents with a constant unloading rate of 0.5 mN/s . In the second test, the peak load was kept at a constant 30 mN and the unloading rate was varied from 5 mN/s to 0.5 mN/s over a second row of six indents. Phase transformations were identified by a “pop-in” on the load vs. displacement curve during unloading. For the tests with varying peak load, phase transformations were observed with peak loads of $60\text{ mN} - 30\text{ mN}$, while no transformation occurred for peak loads of 20 mN and under. While varying unloading-rate, phase transformations were observed at rates of 1 mN/s and 0.5 mN/s , and no transformation was observed for unloading rates greater than 1 mN/s .

For both types of tests, phase transformations were identified by a clear peak at 353 cm^{-1} from Raman Spectroscopy. As previously reported⁷, the intensity of the peak at 353 cm^{-1} scales with peak load, with the 60 mN load resulting in the largest peak. Phase transformations were also expected at lower peak loads so long as unloading rate was sufficiently slow. Indeed, Raman spectra from both the confocal microscope, with fixed and direct optical coupling to the spectrometer, and the fibre optic coupling to the Triboindenter reveal a peak at 353 cm^{-1} for the combination of low load (30 mN) and slow unloading rate (less than 1 mN/s). Our results reproduce expected pressure-induced transformation behaviour in silicon and demonstrate the similar capabilities of the traditional confocal Raman and novel integrated Raman-Nanoindenter system to capture

structural changes to small volumes of material.

APPLICATION 2: CHEMISTRY-PROPERTY CORRELATIONS IN AGING BONE

We next applied our combined system to the study of dense cortical bone tissue in a study of aging mice. Bone is a highly heterogeneous composite consisting primarily of carbonated hydroxyapatite nanocrystals within a collagen matrix. Bone tissue is dynamic; age and disease can change the nano- to micro-scale mineral chemistry and mechanical properties⁸, and produces a well-characterized Raman spectra⁹ (Figure 5). Creating matched Raman spectra + nanoindentation maps allows study of how mineral and matrix chemistry correlate with bone nanoindentation modulus, and how these correlations are affected by a variable such as age.

Raman-Nanoindentation maps were generated for early mature (6 months) and mid-aged (24 months) mouse cortical bone in the mid-section of the femur. Samples were embedded in polymethylmethacrylate (PMMA) and polished using a series of aluminium oxide pastes to a final 0.05 μm finish before testing¹⁰. Raman spectral maps (785 nm laser) were obtained for arrays spanning the cortical bone thickness (Figure 6). The background spectrum for PMMA was removed, and the baseline was subtracted. Peaks of interest were limited to the mineral: matrix ratio, given as ν_1 phosphate: amide I (961 cm^{-1} ; 1661 cm^{-1}). At matched sites, nanoindentation arrays were then generated. At each indent site, bone was indented to 500 nm with a 5 μm spherical tip (30 second load, 120 second hold, 30 second unload). The reduced modulus was measured at each site from the unloading curve and Oliver-Pharr analysis².

Our results demonstrate the changing relationship between chemistry and mechanical properties with tissue age. The reduced modulus from nanoindentation decreased with age from $28.9 \pm 3.4\text{ GPa}$ in young bone to $23.7 \pm 3.6\text{ GPa}$ in old bone. The mineral: matrix ratio assessed via Raman spectroscopy increased with age, from 14.8 ± 1.7 in young bone to 15.6 ± 1.8 in old bone. The correlation between modulus and mineral: matrix increased with age (statistical regression analyses produce a correlation coefficient of r^2 , where $r^2 = 1$ indicates a perfect relationship). A weak correlation was observed in young bone, measured by $r^2 = 0.12$, whereas a moderate correlation, $r^2 = 0.29$, was observed in old bone (Figure 6).

SUMMARY AND CONCLUSIONS

Combining Raman spectroscopy with nanoindentation uniquely enables

correlation of *in situ* measures of material chemistry, structure, and properties of a single site of analysis. While this combination of techniques has been performed using separate instruments¹¹, the process to orient and relocate identical sites is expensive and time consuming once a sample is moved. Instead, fibre optic integration of Raman spectroscopy into the optical microscope on a commercial nanoindenter system enables in-situ materials assessment. Due to the versatility of both testing methods for testing materials that have been subjected to minimal preparation methods, this combined platform holds promise for facile and rapid evaluation of a range of advanced material systems spanning heterogeneous biomineralized

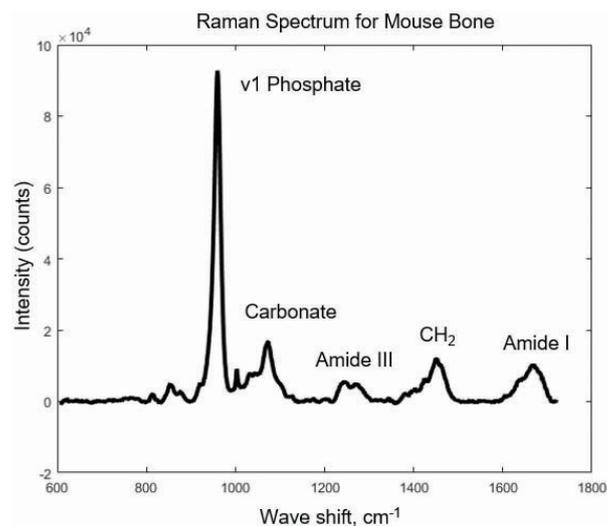


FIGURE 5 The Raman spectrum of bone. The Raman spectrum of bone includes peaks indicative of bone mineral (phosphate and carbonate) and the underlying collagen matrix (amide I, amide III, CH_2).

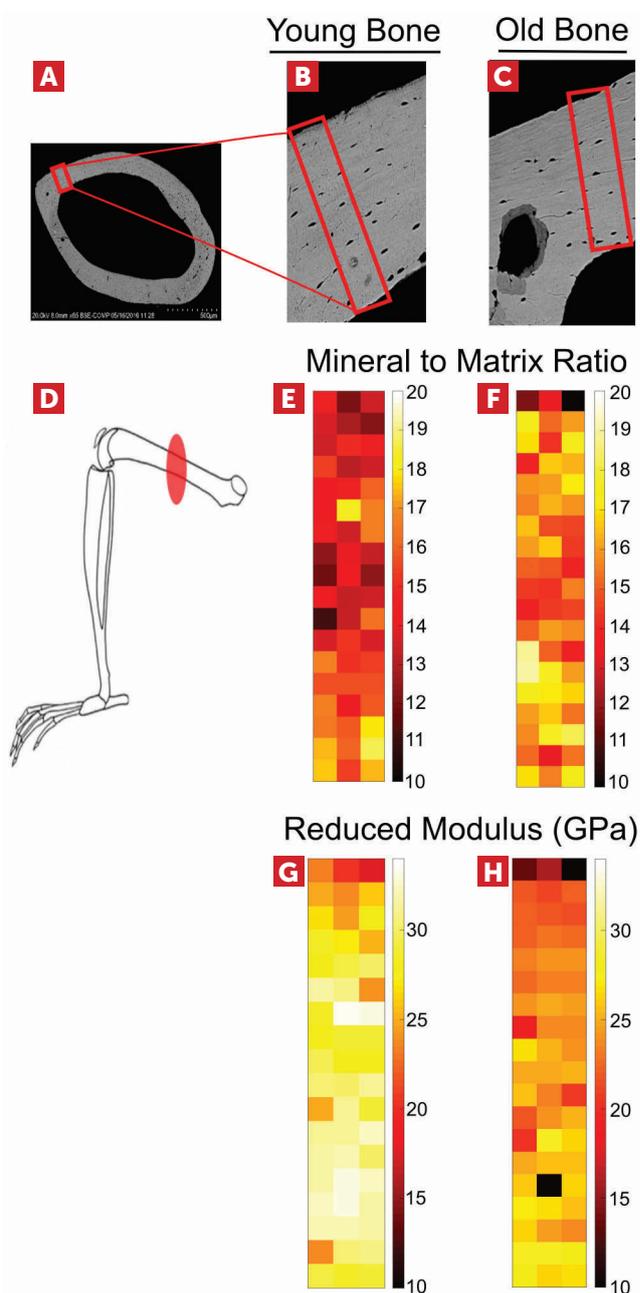


FIGURE 6 Mapping Raman chemistry and nanoindentation reduced modulus for bone. Raman chemistry and nanoindentation mechanical properties can be measured in site-matched arrays with the combined Renishaw inVia - Hysitron TI950 Triboindenter system. A transverse section of cortical bone (A) from a mouse femur (D) was studied in arrays spanning the cortical thickness (B, C) with Raman spectroscopy and nanoindentation. Microscale material properties (mineral: matrix ratio, reduced modulus) were mapped for young (E, G) and old (F, H) mouse bone. Compared with young bone, old bone has lower modulus and higher mineral: matrix ratio

composites to materials for solid-state batteries.

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BIOGRAPHY

Virginia L. Ferguson earned her PhD in Mechanical Engineering at the University of Colorado, Boulder and trained as a postdoctoral fellow in Materials Science and Biology in a joint position at Queen Mary, University of London and University College London. Her group studies problems rooted in mechanical behavior of materials with applications extending from understanding causes of functional tissue failure, developing solutions for tissue regeneration, novel biomaterials, and studying emerging and complex materials. With support of the U.S. National Science Foundation, she recently added correlated nanoindentation and Raman spectroscopy within her laboratory for use



by both internal and external researchers.

ABSTRACT

Material properties are influenced directly by underlying chemistry and structure, yet evaluating relationships between these factors across large surfaces with high spatial resolution is complex. We recently established a novel, combined system that couples a Raman spectrometer through fibre optic probes to the optical microscope within a commercially available nanoindenter. This novel system facilitates combined materials analyses via chemistry assessment and evaluation of nano-/micro-mechanical properties at sites matched for size and exact location. Here, we demonstrate the performance of this instrument by evaluating Raman spectra associated with nanoindentation pressure-

induced phase transformations in (100) Silicon. Using both the independent confocal Raman microscope and spectrometer and again through the fibre optic probes, we demonstrate that the combined system reliably collects accurate spectra. Additionally we use this combined instrument to evaluate aging bone, where we demonstrate correlative changes in both Raman spectra and nanoindentation modulus of 'young' and 'old' mouse bone. This unique combined system holds potential for rapid and facile evaluation of chemistry, structure, and properties of a wide range of heterogeneous advanced materials.

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