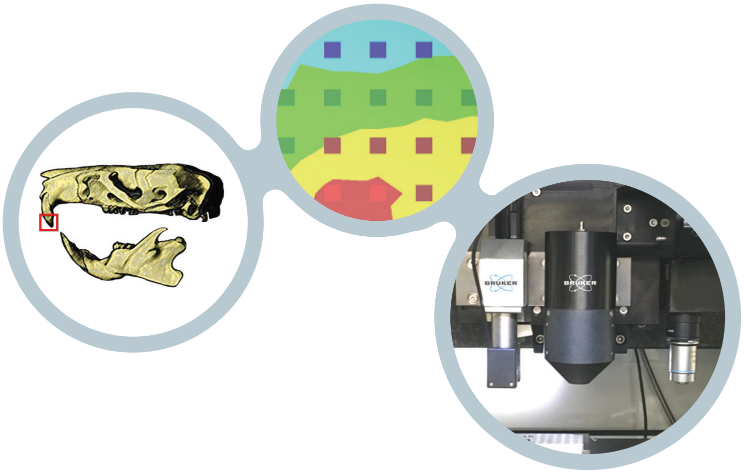

Application Note #1522

Raman and Indentation Mapping of a Rat Tooth

In general, a tooth is composed of stiffer, exterior enamel and softer, interior denten. Enamel is comprised primarily of a mineral phase (carbonated apatite) with less organic matter compared to dentin. Mineral phases are identified by their high PO_4 content (Figure 1), and amide Raman bands show organic phases [2]. Mineral peak intensities are linked to the content of hydroxyapatite, which influences local mechanical properties of tissue. Bruker's Hysitron® TI Series TriboIndenter® nanomechanical test instrument equipped with Raman spectroscopy is an ideal tool for biomaterials research (Figure 2). This combination of technologies provides the solution for small-scale mechanical characterization and its direct correlation to chemical composition. The vibrational (phonon) states of molecules detected using Raman spectroscopy provides a molecular fingerprint of the physical state of a material. At the same time, a nanoindentation curve serves as a fingerprint of a material's mechanical properties. Together, the two techniques produce a wealth of information about the relationships between composition, structure, and properties.

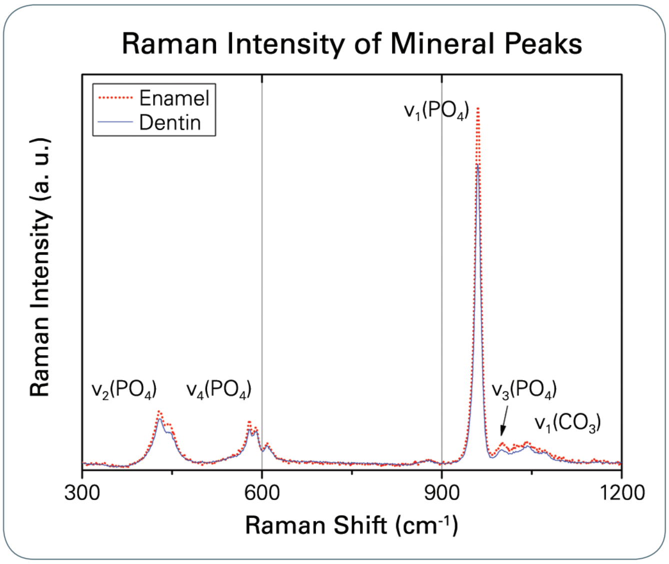

Figure 1. Raman spectra in the range of internal vibrations of the mineral part of dentin and enamel. Intensity of the band of hydroxyapatite at 962 cm^{-1} was used for mapping the spatial distribution of minerals and correlated with indentation mapping.

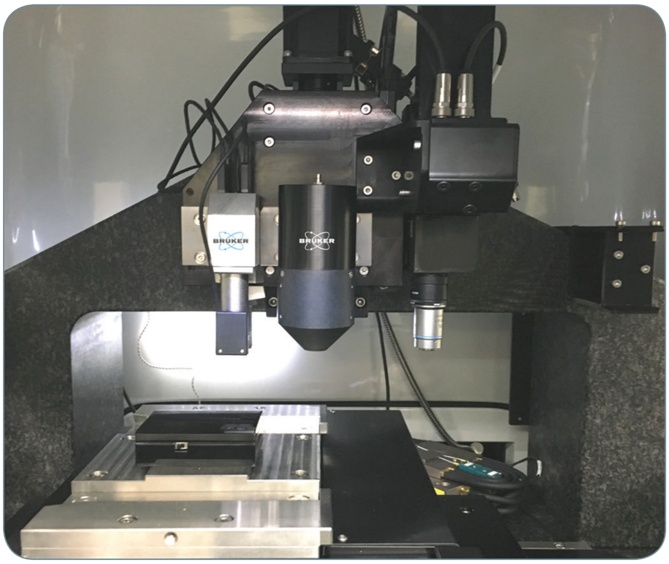

Figure 2. Direct integration of a micro-Raman probe on a TriboIndenter granite base. X and Y distance between tip and Raman optics is calibrated to enable acquisition of the microscopy image, Raman spectra, and nanoindentation.

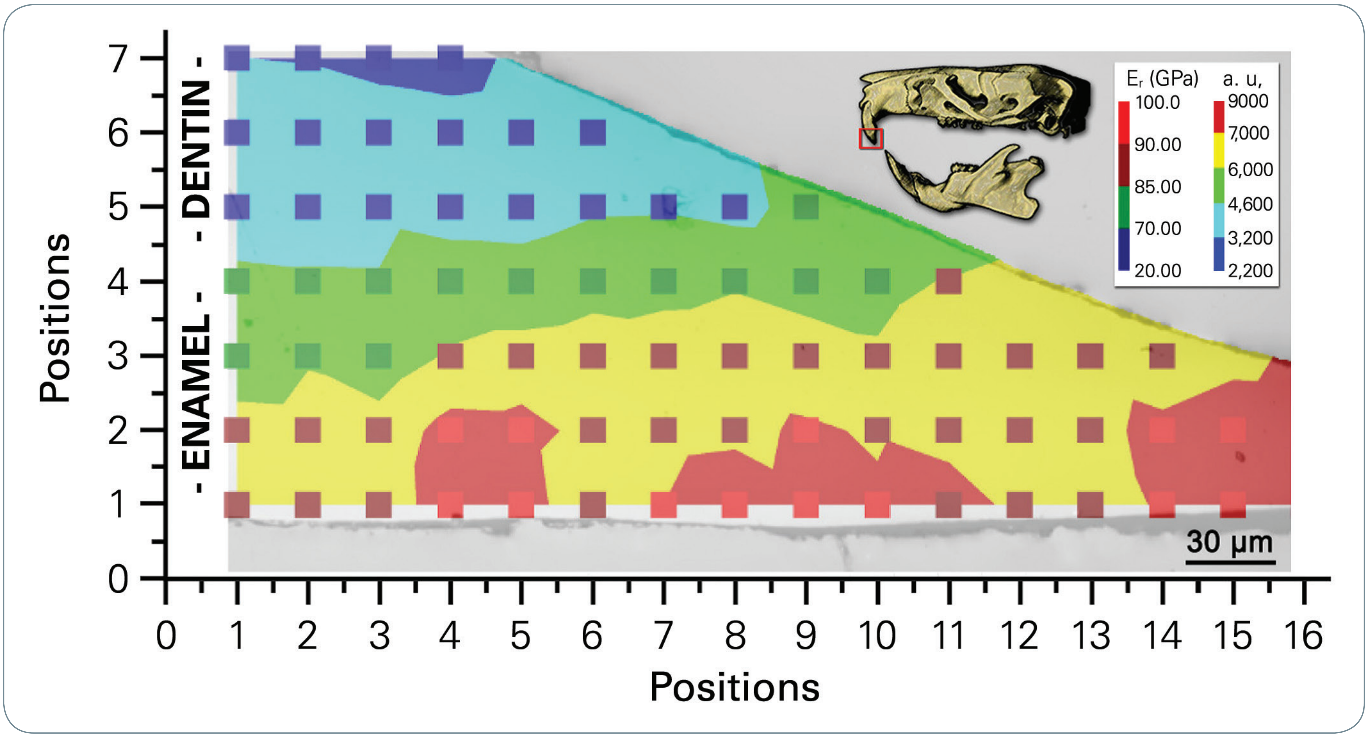

Figure 3. An overlay of a rat tooth micrograph with Raman and modulus maps. The Raman map visualizes intensity (a. u. = arbitrary unit) of $v_1(PO_4)$ bond at wavenumber 962 cm^{-1}. Intensity of internal vibration mode of PO_4 is associated with a volume of hydroxyapatite ($Ca_5(PO_4(OH))$ crystal which makes mineralized tissue stiffer. The higher intensity of $v_1(PO_4)$ peak represents higher mineral concentration. As expected, nanoindentation results (contoured squares) measured at exactly the same spots where Raman spectra were acquired show higher stiffness corresponding with higher mineral content. Enamel had higher mineral content and higher modulus than dentin.

Procedure

A dried rat incisor was embedded in epoxy resin, sectioned along the sagittal plane, and polished to a smooth surface. An automated array of Raman and nanoindentation measurements was performed across the enamel and dentin, with tests spaced at 30 µm intervals (Figure 3). Measurements were performed using a Hysitron TriboIndenter with a Berkovich indenter. Raman spectra were collected at 785 nm laser excitation wavelength within the range of 50 to 1800 cm^{-1}. The laser spot size of ~2 µm was focused with a 50x objective lens (NA 0.55) on the indentation positions defined by the automation routine.

Results

An overlay of an optical micrograph with correlated Raman and indentation maps shows a descending gradient in mineralization followed by decreasing elastic modulus from the enamel outer layer to the dentin. The highest mineral content and modulus were found especially close to the incisor apex.

Conclusions

In-situ correlation of chemical composition by Raman spectroscopy and modulus/hardness maps by nanoindentation is a fast and effective method to determine the influence of chemical composition on mechanical properties. In this case, the two complimentary methods demonstrate the relationship between mineral content and stiffness in mineralized tissue.

References

1. Nanoindentation mapping reveals gradients in the mechanical properties of dental enamel in rat incisors, B. Frydova, J. Sepitka, V. Stejskal, F. Fryda, J. Lukes, Comput. Methods Biomech. Biomed. Engin., Vol. 16, 290-291, 2013.

2. Molecular Spectroscopy Study of Human Tooth Tissues Affected by High Dose of External Ionizing Radiation (Caused by Nuclear Catastrophe of Chernobyl Plant), L. A. Darchuk, L. V. Zaverbna, A. Worobiec, R. Van Grieken, Current Topics in Ionizing Radiation Research, InTech, 2012.

Authors

Jaroslav Lukeš, Ph.D.
jaroslav.lukes@bruker.com

Bruker Nano Surfaces Division
Minneapolis, MN · USA
+1.952.835.6366
productinfo@bruker.com
www.bruker.com/nanomechanical-testing