A New Understanding of Soft Materials

The hardness and stiffness of soft materials—certain biological tissues, soils, and polymers—can be measured for the first time with a Livermore invention.

RESEARCH in nuclear physics at Lawrence Livermore is performed to serve the Laboratory's national security mission. But along the way, the research has spun off technologies beneficial to human health. One example is PEREGRINE, a tool for calculating the dose that patients receive during radiation therapy for cancer. PEREGRINE combines Livermore's storehouse of data on radiation transport with Monte Carlo statistical techniques. Modeling of radiation transport also came into play in developing optical coherence tomography, a technique that uses infrared light to image through highly scattering media such as blood and the walls of arteries.

Now, in the Chemistry and Materials Science Directorate, physicist Mehdi Balooch is using a new technique he pioneered for examining uranium and other materials inside aging nuclear weapons and applying it to soft materials in the human body. Working with researchers at the University of California at San Francisco (UCSF), he is using this method to learn more about the strength of human teeth. He has also used it to study both healthy and damaged human arteries.

Balooch's new applications make use of a modified atomic force microscope (AFM). Atomic force microscopy has been in use since the 1990s to produce topographic maps of nanostructures. In atomic force microscopy, an extremely sharp tip mounted to a cantilever arm senses the atomic shape of a sample while a computer records the path of the tip and slowly builds up a three-dimensional image.

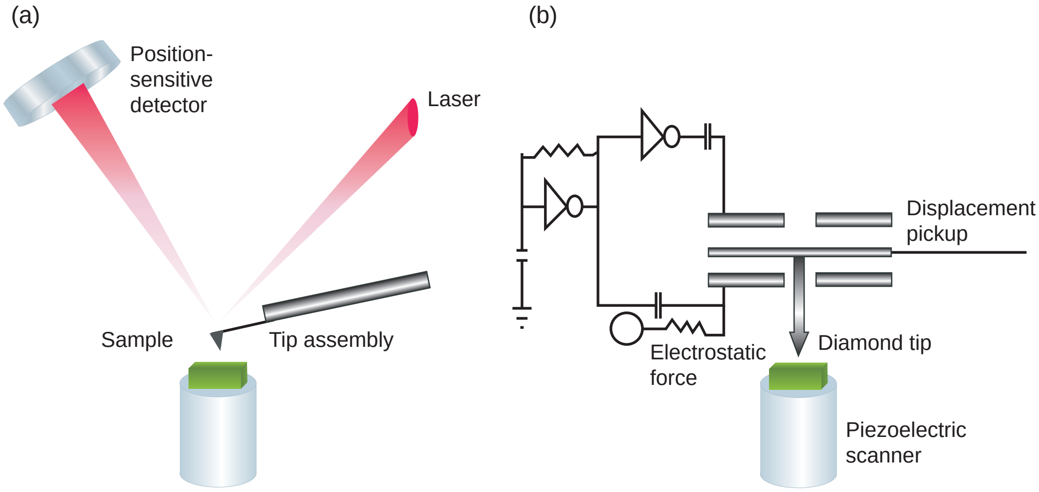
Balooch's modified AFM makes indentations just 20 trillionths of a meter deep on the surface of a sample material. Even such a tiny hole—100,000 times shallower than the width of a human hair—provides extraordinarily useful measurements about the sample. When force and displacement data are combined in various algorithms, the resulting calculations reveal information on mechanical properties—hardness, stiffness, or any other reaction to an applied force.

The modified AFM is unique in its ability to measure the mechanical properties of both hard and soft materials. Hard materials are usually easy to study, but measuring the mechanical properties of soft materials has been more difficult. Part solid and part fluid, soft materials include many biological tissues, polymers, and hydrated clays, an important component of soils. Traditionally, mechanical properties have been obtained using dried samples. But the modified AFM's ability to take measurements in liquid allows accurate measurements of the mechanical properties of soft materials for the first time.

Examining a hard material in liquid has been equally difficult. Teeth, for example, present a challenge because their normal environment is in saliva. The modified AFM allows for measurements of fully hydrated teeth. Two years ago, Balooch's team took some of the first nanoscale measurements of hardness and elasticity at the junction between tooth enamel, a hard mineral material, and the dentin just beneath it. Dentin is a soft material—part mineral, part protein, and part fluid.

Balooch has been working with dental researchers Bill and Sally Marshall at UCSF for 11 years, operating under a grant from the National Institutes of Health. A 5-year extension of the grant for continued research on the hardness and stiffness of dental material begins this month.

The modified AFM is also finding applications beyond weapons and health. Balooch recently began collaborating with colleagues in the Energy and Environment Directorate to study the effect of water on the mechanical properties of clay materials, which cannot be determined by conventional testing methods. The researchers are making first-ever measurements of the mechanical properties of single crystals of clay with water intercalated in the crystal



(a) A traditional atomic force microscope (AFM) traces the atomic shape of a sample with a tip on a cantilevered arm. The path of the tip is recorded on a computer and used to build a three-dimensional image. (b) The modified AFM incorporates the features of a nanoindenter to make tiny holes on the surface of a sample material. Instead of a cantilevered tip assemply, this AFM has a transducer-driven head and tip that can measure the mechanical properties of a fully hydrated sample.

structure. Here, as in teeth, a hard mineral material coexists with liquid matter. Until now, it has been impossible to understand how hard mineral and liquid matter work together on this very small scale.

Problems and a Solution

In Balooch's early work with UCSF on teeth, he used a commercially available nanoindenter, which made indentations 100 to 200 nanometers deep on the surface of samples. It was useful but not quite what was needed. For one thing, it could not operate under water or on hydrated samples. The nanoindenter also could not position an indentation within less than a few micrometers, which was not precise enough. Nor was the nanoindenter an imaging device, so it was impossible to know with certainty where indentations were being made.

To solve these problems, Balooch, in collaboration with a startup company named Hysitron, modified an atomic force microscope to incorporate the features of a nanoindenter. To obtain not only a topographic image but also mechanical-property measurements, the team replaced the AFM tip with a transducer-driven head and tip that can operate on a fully hydrated sample.

This modified AFM can operate in either an imaging mode or an indentation mode. In the imaging mode, the force applied to the cantilever is about 1 micronewton, a force so small that it does not dislodge a single atom in the sample. When used in the indentation mode, the force on the arm is up to 30,000 times as much, though it is still extremely small.

The modified AFM permits positioning the indentation with an accuracy of a few tens of nanometers for site-specific studies, which is a thousand times more accurate than that of a standard nanoindenter with an optical microscope attachment. It allows measurement of hardness values with indentation depths of less than 20 nanometers.

"Now," says Balooch, "with a coat-on-coat dynamic testing capability, we can get pixel-by-pixel information on stiffness or elasticity. Plus, we get topography so we know where we've been."

Getting a Grip on Teeth

Dentin is about 50 percent mineral, 30 percent organic material (mostly collagen), and about 20 percent fluid. Microscopic tubes, called tubules, run through dentin, from the pulp beneath to the junction with the enamel above. Dentin's structure is not precisely the same everywhere in a tooth, and both age and disease affect structure.

Collagen, a major component of dentin, is the most abundant animal protein in mammals, accounting for about 30 percent of all proteins. It is responsible for the tissues that hold us together, such as bone, cartilage, tendons, and skin.

For the UCSF researchers, knowing how hard, soft, brittle, or elastic dentin is will help improve restorative dentistry. Fillings, bridges, crowns, and other dental repairs must bond to the dentin, or they will fail.

Balooch is assisting the project by finding or creating the best tools to supply necessary measurements. His modified AFM may well be the best tool thus far for determining the mechanical properties of dentin. Because of the problems inherent in studying soft materials, especially very small samples of soft materials, there have been large discrepancies among measurements of the hardness and stiffness of dentin. This wide variation has made it impossible to establish the baseline mechanical behavior of dentin or to explore the effects of age, gender, or disease on tooth strength. The accuracy of the modified AFM allows evaluations of nanomechanical properties on a highly site-specific level, for the first time.

With the modified AFM, the team was the first to reveal major differences in the hardness of dentin in and around the tubes that traverse it. Hardness is evaluated using the pressure exerted by the indenter on the contact area. Using a different tip on the modified AFM, they measured fracture toughness by inducing cracks, a standard practice in examining the effects of stress on a material.

At the dentin–enamel junction, hard, brittle tooth enamel overlays soft, ductile dentin. Macroscopic tensile, compression, or shear tests are difficult at the junction, an area that represents a small percentage of the already small human tooth.

"Here, things got very interesting," says Balooch. As shown in the bottom figure on p. 14, the modified AFM exposed in detail the stiffness and hardness differences between dentin and enamel. The team's results agreed well with earlier macro- and microscale experiments by others. The smooth transition across the junction suggests that mineral content there must gradually change because the mineral component of calcified tissue is closely related to its mechanical properties.

While creating cracks in enamel is relatively easy, Balooch's team found that inducing cracks in the dentin–enamel junction is much more difficult. The strength of the junction suggests that this area is critical for preserving the physical integrity of the tooth. Because of these characteristics, the dentin–enamel junction may serve as a model for the linkage of other pairs of highly dissimilar materials, such as those in artificial hip replacements.

Estimates of the precise width of the dentin–enamel junction have ranged from 12 to 200 micrometers using various micro- and nanoscale methods. Balooch, ever the tinkerer, tackled this measurement problem by again modifying an atomic force microscope. He changed it to create nanoscratches across the junction while simultaneously measuring lateral force. By measuring changes in friction, his team estimated a width for the junction of from 1 to 3 micrometers, about 10 times smaller than the smallest width previously estimated.

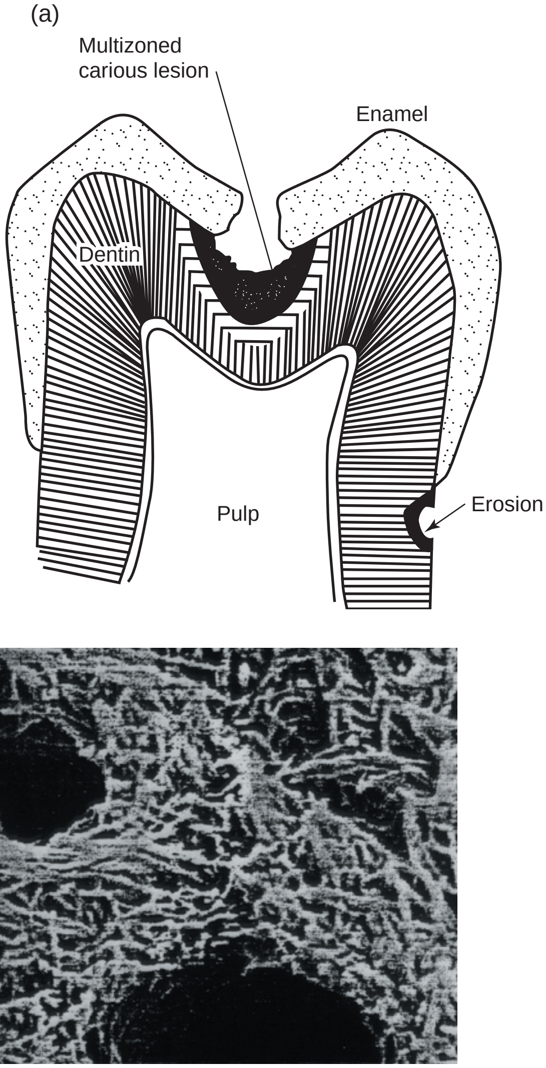
Fighting Vascular Disease

To investigate healthy and diseased human arteries, Balooch joined forces with Livermore physicist John Kinney, whose specialty is x-ray tomography.

Heart disease is the leading cause of death in the U.S. and is most commonly treated by catheter-based balloon angioplasty to dilate obstructed coronary arteries. While this procedure results in a 95-percent immediate success rate, 40 percent of all treated sites renarrow within 6 months. This lack of long-term success has led to considerable interest in how normal and diseased arteries become deformed. In particular, research focused on how the artery wall changes from stretching or from changes to fatty plaque deposits (calcifications).

Using his modified AFM, Balooch first measured local, in vitro mechanical properties of femoral artery tissue in saline solution. Then he moved to diseased calcified arteries. For this work, ultrasound images of arteries were recorded, and the healthy and calcified regions were marked. The healthy regions were extracted, and solid calcified deposits were dissected from the artery wall. More than 40 samples of both types of tissue were then mechanically tested with the modified AFM. Calcified deposits were found to be many orders of magnitude stiffer than the healthy artery wall, even as deposits varied from sample to sample and in their position on the wall.

Kinney took x-ray tomographs of dissected femoral arteries to make dynamic measurements of the deformation of plaque and vessel walls during graded stages of balloon



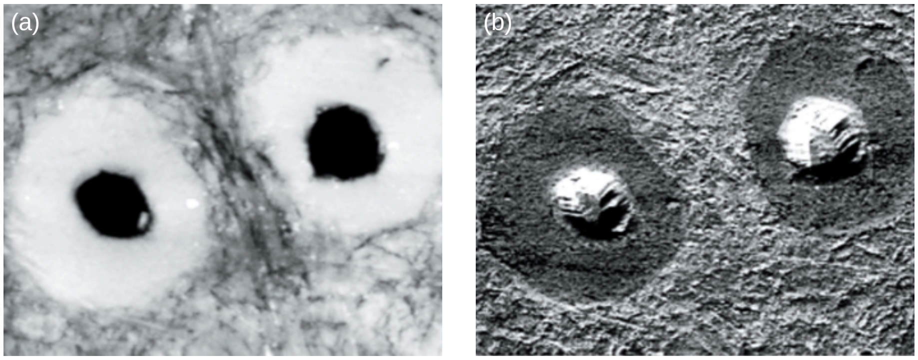
(a) Schematic diagram of a human tooth, showing enamel, dentin, and pulp. (b) A scanning electron microscope image of dentin showing the collagen fibers and tubules that run through dentin.

inflation and deflation. He found that fatty plaque deposits were less elastic than commonly assumed, which could affect the long-term success of balloon angioplasty.

Solving Seismic Problems

Geophysicists Brian Bonner and Dan Farber and geochemist Brian Viani are using the modified AFM to address seismological issues. Most of us equate seismology with earthquakes. But seismology in its broadest sense is simply the study of Earth's dynamic response to any mechanical stimuli. Seismic research thus encounters not just earthquakes but also problems related to slope stability, mitigating earthquake hazards, tracking the movement of pollutants underground, and exploring for oil, gas, and other hydrocarbons. With its Ground-Based Nuclear Explosion Monitoring program, Livermore also focuses on forensic seismology, which is the detection of clandestine explosions.

The project using the modified AFM addresses a fundamental problem in remote seismic sensing: the role fluids play in the transmission, modulation, and dissipation of seismic energy. "The importance of water, even at very low concentrations, was made clear during analysis of seismic events on our Moon," says Bonner.



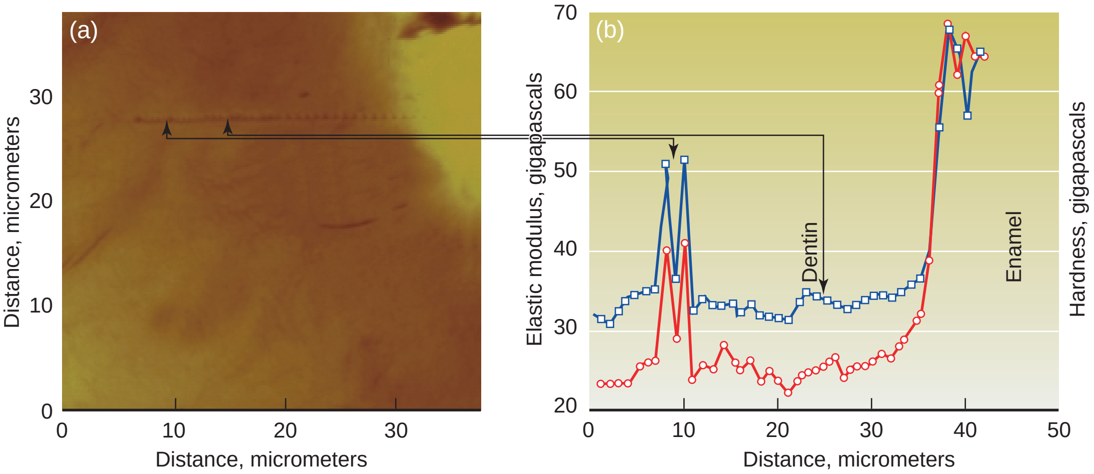
(a) A standard atomic force microscope (AFM) topographical image of a polished dentin specimen. The dark areas are the tubules that run through dentin. (b) An image with the modified AFM measures the stiffness of the same site. The peritubular dentin, the area immediately around the tubules, is stiffer than the rest of the surface.

When the Apollo astronauts blasted off, they took along instruments, including seismometers, to study the Moon's properties. The seismograms from the Moon proved to be totally different from seismograms here on Earth. "They showed that the Moon rang like a bell," says Bonner.

But later, when rock samples were brought back by the Apollo astronauts, their seismic response became Earth-like. Conversely, scientists found that when Earth rocks were baked in high-temperature vacuum ovens, they took on Moon-like attenuation characteristics. The scientists assumed that the change in the Moon rocks must have been caused by tiny amounts of water acquired in Earth-bound laboratories. Even a small amount of water made a big difference. Now, after more than 30 years, Balooch's apparatus finally is providing the means to study this effect directly.

Bonner, Farber, and Viani's experiments are similar to the Moon-rock scenario in that they are studying the effects of an extremely small amount of water in a mineral. They use a type of clay, montmorillonite, which can confine very thin layers of water (0.25 nanometer per layer) between the sheets of silicate that make up the clay.

(a) An atomic force microscope (AFM) image of indentations across the dentin–enamel junction. (b) Curves show the corresponding hardness and stiffness (elastic modulus) determined from indentations with the modified AFM. The smooth increase in stiffness across the junction suggests that mineral content must gradually change there because the mineral component of calcified tissue is closely related to its mechanical properties.



An image of the experimental sample is shown in the figure at bottom right. Samples were tested using the modified AFM in dry nitrogen and in air with about 30-percent relative humidity. Stiffness decreased dramatically when the sample was hydrated, as indicated by force modulation tests. Attenuation—the dissipation of mechanical energy—increases greatly in the hydrated sample. The measurements show the samples behaving like a classical viscoelastic material, that is, a viscous material that has some elastic properties. "Now we can say with confidence that a viscoelastic model is an appropriate one for seismic response in clay-dominated geologies, even for seismic displacements," says Bonner.

These results make perfect sense on an intuitive level because we know what water does to soil. But the experiments make this observation quantitative for the first time. Collecting data on the nanoscale removes the effect of other interfering phenomena, such as rock porosity. Nanoscale effects—which had never before been observed—reveal the important mechanisms of seismic deformation and are consistent with observations on a large scale.

The team is now studying other sheet silicates to observe the effects of additional layers of water. They are also beginning to use molecular dynamics and effective-medium modeling to make predictions on a scale that is useful in the field.

"These measurements of mechanical properties of clay are important for several other energy and environmental uses," says geophysicist Pat Berge, acting division leader for Geophysics and Global Security, who applies these results to field-scale issues. " With them, we can model larger-scale behavior of clay-bearing soils and rocks."

Geophysicists estimate the unknown composition of rock and soil underground by making seismic measurements at environmental cleanup sites, oil fields, or other regions of interest. Then they use rock-physics theories to interpret the seismic data and figure out how much fluid and what types and amounts of minerals are present. The success of these calculations depends on having good estimates of the seismic velocities of the pure fluids and minerals that make up the fluid-bearing soil or rock. Properties of water, quartz, calcite, and other minerals found in sandstones and sandy soils are readily available. Until Balooch's new technique came along, geophysicists did not have good estimates of the properties of clay, particularly at the smallest scales of individual clay platelets. This made modeling silty sands and shales difficult, leading to problems in underground imaging for oil reservoirs and environmental sites. Livermore researchers such as Berge suspected that the seismic velocity estimates commonly used for clay were too large by a factor of at least two and possibly a factor of five. But without laboratory corroboration, it was not possible to change the minds of the geophysical community. The new measurements by Farber, Bonner, and Viani show that clay is indeed a very soft material.

Most recently, Balooch has been working with chemist Wigbert Siekhaus to modify the AFM further so that stiffness can be imaged directly. This process, presently being patented, only adds to Livermore's unique ability for nanoscale examination of soft materials, an intermediate regime that until now has eluded researchers.

—Katie Walter

Key Words: atomic force microscope (AFM), dentin, mechanical properties, nanoindenter, plaque, seismic response, vascular disease.

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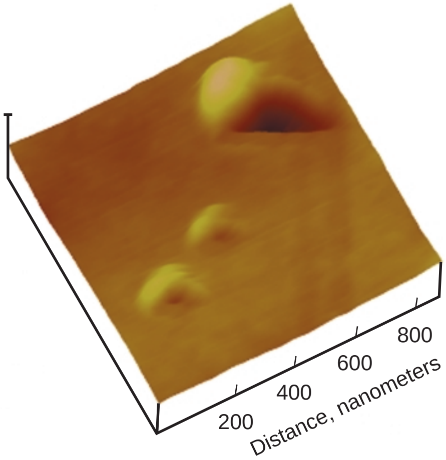
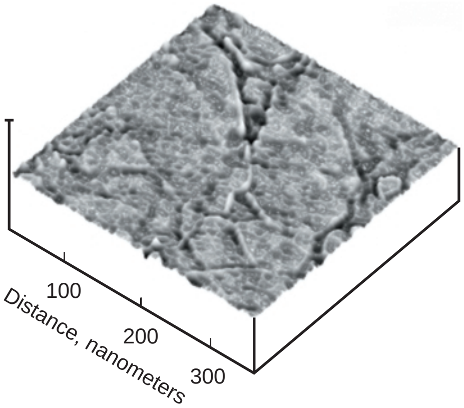


Nanoindentation on a calcified artery. The deposits can be mechanically tested with the modified atomic force microscope to determine their stiffness and thus reveal how the artery wall has been changed by calcification.



A prepared sample of a clay before it is measured with the modified atomic force microscope. The clay is montmorillonite, which can confine very thin water layers (0.25 nanometer per layer) between the sheets of silicate.