MATERIALS SCIENCE

Probing the Nanoscale

Probes that can now measure a variety of nanomechanical properties provide opportunities for new science and devices.

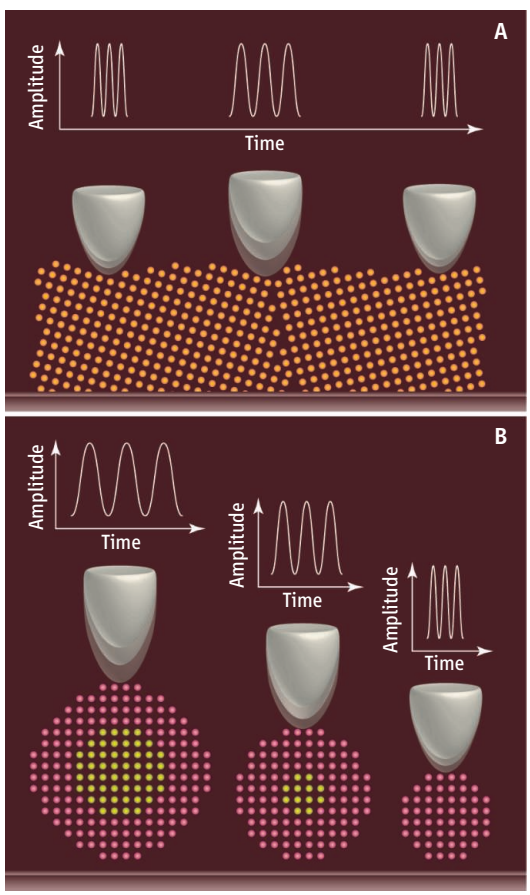
Robert F. Cook

Measurement probes to determine nanomechanical properties are confirming long-held beliefs, revealing new phenomena, and enabling commercialization of devices based on micro- and nano-electromechanical systems (MEMS and NEMS). The resistance of materials and structures to the complete range of deformation modes—reversible, irreversible, and time-dependent—can now be measured with great accuracy, precision, and spatial resolution.

As most devices are designed to perform mechanically in an elastic (reversible) manner, the mechanical property of greatest importance is the elastic modulus. The technique that provides the greatest spatial resolution is contact resonance atomic force microscopy (CR-AFM). Changes in the resonance frequencies of an AFM cantilever probe are measured as the probe tip is brought into contact with a surface; the contact stiffens the cantilever response, increasing the cantilever resonance frequencies. Knowledge of the cantilever dynamics and contact mechanics of the tip-surface interface allows the modulus of the contacted material to be determined. Contact radii of 2 to 3 nm are generated by means of probe tips of 20- to 40-nm radius and controlled contact loads in the 100 nN range, providing the ability to map the stiffness (and hence the modulus) with better than 10-nm spatial resolution. This system was used to map the elastic modulus of a nanocrystalline gold film (*1*) and confirmed directly the long-held belief that, as they are less dense, grain boundary regions are more compliant than grains, up to a factor of two smaller in modulus in some cases. Such measurements are critical in predicting the response of nanomaterials to stress, especially nanograined polycrystals, because they contain a much greater proportion of grain boundary material than their macrograined counterparts and are thus more compliant (see the figure, panel A).

CR-AFM was also used to measure the elastic moduli of zinc oxide (*2*) and tellurium nanowires (NWs) (*3*) and revealed new mechanical phenomena intrinsic to the nanoscale. NWs with radii greater than about 100 nm exhibited moduli comparable to bulk material. However, NWs with radii less than 100 nm exhibited dramatically enhanced moduli. The change in the NW properties is opposite to that for grain boundaries: Surface tension effects lead to a rearrangement of atomic positions and greater atomic densities at surfaces and thus lead to a stiffer shell of surface material surrounding a NW core of bulk-like material. As the NW radius decreases, the ratio of shell/core material increases, leading to stiffening. For NWs smaller than a critical radius, the bulk core disappears, the NW is all "shell," and the modulus is invariant (*3*) (see the figure, panel B).

Other mechanical properties define limits on the loading that a device can withstand and still perform. The technique that provides the greatest resolution for measurement of material resistance to (irreversible) plastic deformation is instrumented indentation testing, or nanoindentation. Here, the load and displacement of a hard, stiff probe, typically a pyramidal diamond with a 50-nm tip radius, are measured as the probe contacts a surface. For very small indentation contacts on metals, the deformation is elastic and yield—the onset of plastic deformation—is quantized, as irreversible deformation is associated with the nucleation or motion of individual dislocations. Such quantized yield is discernible as an abrupt displacement of the indenter into the surface of the material at a fixed load, a "pop-in" event. Nanoindentation measurements, combined with AFM of the real shape of the indenting probe and finite element analyses of the indentation stress field, were used to measure the single-crystal shear yield stress of tungsten (*4*). The loads required for such irreversible events were in the 100 μN range, generating contact radii of about 30 nm; both much greater than those for the purely elastic responses sensed in CR-AFM.



Material probes. The oscillation frequency of an AFM probe is altered as it encounters materials with different structure and stiffness. (**A**) The frequency decreases as the probe is scanned over disordered and less dense grain boundary regions, confirming directly that grain boundaries are more compliant than grains. (**B**) The frequency increases as the probe contacts nanowires of decreasing radii, indicating that surface effects lead to a stiffer, denser shell surrounding a bulk-like core. Nanowires smaller than a critical radius are all surface-affected shell.

Nanoindentation techniques have also been developed to measure viscoelastic (time-dependent) properties. The response of the indenter during fixed-load or fixed-displacement is measured as a function of time. Such methods have been used to determine the viscoelastic properties of polyurethane (*5*) using a spherical indenter and thence to predict the time-dependent responses over different time scales and indenter geometries. Flat-punch indentation measurements of the amplitude and phase of the probe displacement for an oscillating contact load were used to predict the time-dependent nanoindentation response of polyvinylchloride (*6*). Conical indentation models can describe and predict the time-dependent and irreversible viscous-elastic-plastic properties of glassy polymers (*7*) such as polymethylmethacrylate.

Although enormous progress has been made in the past 20 years in nanomechanical probing, two challenges remain, at either end of the

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materials spectrum. The first challenge is brittle materials and fracture. Although nanoindentation techniques with acute probes can generate cracks at small length scales (*8*), and the mechanics of acute indentation have been identified and analyzed (*9*), postindentation observation of cracks is required to determine toughness. The methodology has been demonstrated on nanoporous dielectric films with crack lengths as short as 300 nm in 500-nm thick films (*8*, *9*). A method that determined fracture properties in situ—for example derived from the indentation pop-in associated with crack nucleation—would be an obvious advantage in improving the reliability and thus commercialization of MEMS and NEMS devices.

The second challenge is biological and other gel-like materials that contain an appreciable fluid phase. In these systems, flow through a porous elastic or viscoelastic matrix determines the time-dependent deformation properties. Probe-based techniques, rooted in poroelastic mechanics, have been used to characterize the behavior of hydrogels (*10*). Such measurements are also able to determine the permeability of the matrix to the fluid and indicate reduced permeability at the nanoscale in bone (*11*). Development of nanomechanical probes for poroelastic systems would enable rapid development of artificial tissues and biomedical devices.

The primary focus of the Feynman lecture "There's Plenty of Room at the Bottom" (*12*) was fabrication of nanoscale devices, although the important differences between mechanical properties at the nanoscale and those of bulk materials were mentioned as critical in designing and operating such devices. This need is now being met as nanomechanical probes can determine elastic, plastic, viscous, and fracture properties of materials and structures with nanoscale spatial resolution. Such probes are enabling bounds to be placed on the length scales at which materials behave as the bulk and revealing exciting new phenomena when these bounds are exceeded.

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MEDICINE

Poisonous Contacts

The therapeutic effect of arsenic on promyelocytic leukemia depends on direct binding of the compound to the causative oncogenic protein.

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In the 1939 play *Arsenic and Old Lace*, the main character discovers many secrets about his family, including that his aunts are serial killers who murder lonely old men by serving them elderberry wine poisoned with arsenic. In addition to its use as a lethal poison, arsenic has been used since ancient times to treat human illnesses, including infectious diseases and malignancies (*1*). The remarkable story of arsenic as a modern medical treatment continues, and on page 240 of this issue (*2*), Zhang *et al*. add to our understanding of how its therapeutic effects have made acute promyelocytic leukemia "curable," with survival rates of ~90% (*3*).

Until recently, the use of arsenic in modern Western medicine was limited to treating parasitic infection. This began to change when in 1992 a group from Harbin, China, reported using a traditional Chinese medicine, "Ai-ling 1," to successfully treat patients with promyelocytic leukemia (*4*). The active ingredient in Ai-ling 1 was shown to be arsenic trioxide, which induced remissions in patients with promyelocytic leukemia, including those who had relapsed after other prior therapy (*5*, *6*).

Acute promyelocytic leukemia is one subtype of acute myeloid leukemia. It is characterized in nearly all cases by a recurrent reciprocal translocation between chromosomes 15 and 17, involving the gene encoding the retinoic acid receptor alpha (*RARA*) which is fused to the promyelocytic leukemia gene (*PML*) (see the figure). The leukemic cells of promyelocytic leukemia are morphologically distinctive (they exhibit numerous primary granules) because of their growth arrest at the promyelocyte stage of a differentiation process that produces mature neutrophils. The PML-RARα fusion protein represses transcription at multiple sites in the genome. In combination with other events, often including an activated FLT3 tyrosine kinase, PML-RARα blocks myeloid differentiation. However, retinoic acid induces differentiation of the leukemic promyelocytes, and both retinoic acid and arsenic trioxide together cause degradation of PML-RARα protein, a key event in their ability to eliminate the leukemia (*7*). Current treatment protocols for promyelocytic leukemia include combining both agents (*3*).

The discovery of arsenic therapy for promyelocytic leukemia was made empirically, but was quickly found to be a gene-targeted therapy. Arsenic causes the degradation of normal PML, a protein important for regulating growth and programmed cell death (apoptosis), as well as the destruction of the PML-RARα fusion protein. Arsenic also induces modification of PML-RARα with the molecules SUMO and ubiquitin, the transfer of PML-RARα to an insoluble nuclear matrix, and the degradation of both PML and PML-RARα (*8*–*12*). How arsenic exerts these effects has not been clear. Arsenic trioxide binds to thiol groups and thereby can inhibit protein phosphatases. This promoted the hypothesis that arsenic trioxide increases the phosphorylation of PML at a site that stimulates SUMOylation and subsequent degradation. Surprisingly, Zhang *et al*. show that arsenic works not through binding and inhibiting a phosphatase, but by direct binding to thiol groups within PML itself.

Zhang *et al*. used a combination of immunofluorescent localization within cells, cellular fractionation, mass spectrometry, nuclear magnetic resonance, near-ultraviolet and x-ray spectroscopy, and circular dichroism to examine the interactions of PML with arsenic. Their results show a direct interaction of arsenic with PML, including through cysteines located in PML zinc fingers (structural motifs that coordinate zinc) within the RING and B2 domains of the protein. The interaction of arsenic (instead of zinc) with zinc fingers induced subtle changes in the structure of the fingers, and these changes were associated with oligomerization of PML proteins. The exact molecular mechanisms by which arsenic triggers this oligomerization, as well as

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