SCANNING PROBE MICROSCOPY

Probing the Future

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Imagine a microscope that creates three-dimensional (3D) images down to the atomic scale, that works in air and in liquid as well as in vacuum, that uses a technique for which biological specimens need no staining, and that can map electronic, mechanical, and optical properties, and, moreover, that can manipulate a surface to the level of moving atoms one by one. These are the remarkable capabilities of scanning probe microscopy (SPM), which is being used to solve problems in fields from condensed-matter physics to biology. SPM can be used to study the structure and physical properties of the specimen surface. The exact nature of these problems depends on the field of research. In semiconductor physics, SPM techniques might be applied to investigate the arrangements of atoms at the surface or their electronic states. In biology, the questions relate to the structure and interaction of molecules adsorbed to inert or biological surfaces. In manufacturing, SPM provides quantitative topography for silicon wafers, lithography, compact-disc production, and so forth.

TECH VIEW

Scanning probe microscopes have no lenses. Instead, a "probe" tip is brought very close to the specimen surface, and the interaction of the tip with the region of the specimen immediately below it is measured (Fig. 1). The type of interaction measured defines the type of SPM: When the interaction measured is the force between atoms at the end of the tip and atoms in the specimen, the SPM technique is called atomic force microscopy (AFM) (*1*); when the quantum-mechanical tunneling current is measured, the technique is called scanning tunneling microscopy (STM) (*2*). AFM and STM are the parents of more than a dozen SPM techniques. Think of a physical property, and there is likely to be an SPM technique to measure it.

The interaction between tip and surface is converted to an electrical signal to be processed by the microscope's control electronics. For STM, the interaction is itself an electrical current, and so it is simply boosted with low-noise amplifiers. Other SPMs require a transducer for conversion. In AFM, the force transducer is a sensitive cantilever spring, about 100 μm long, with the probe mounted at its free end (Fig. 1, inset). Any force interaction between probe and specimen results in the cantilever bending, which can be measured optically. When the bending is small, it is proportional to the force. Commercial implementations have typical force sensitivities of ~100 piconewtons.

The 3D landscape of the specimen, its topography, is imaged by scanning the probe over the surface in a TV-like raster, while keeping the strength of the interaction constant. In each of the SPM techniques, the interaction depends very sensitively on the probe-specimen gap. This is the origin of the very high resolution. Think of the probe scanning over the specimen landscape: As it approaches a hill (perhaps a single atom) the distance between the probe and the specimen decreases, and so the interaction increases. However, if the cantilever is allowed to move so that the interaction is maintained at some preset constant value, then the probe will "fly" over the landscape at a fixed distance above the surface. The topography of the terrain is then imaged by recording the motion of the probe in the three dimensions. Because the image is a 3D surface, it may be viewed from different directions. In movies generated from these data, it is even possible to take flights over these atomic landscapes, fly around a human chromosome, or fall into a hole in a substrate digested by an enzyme. This 3D information can be used to measure the volumes of microscopic objects such as individual chromosomes (*3*). Such techniques can be used as an alternative method of karyotyping.

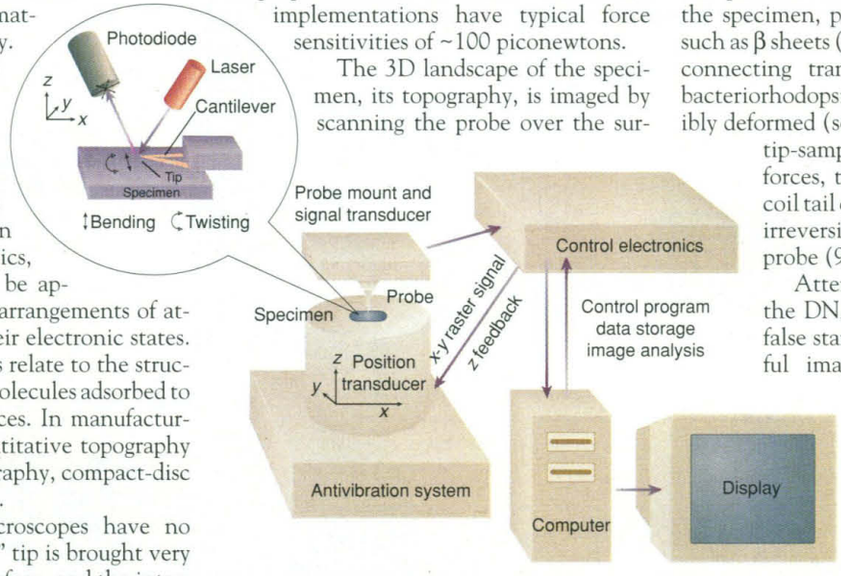

Fig. 1. A schematic diagram of the SPM technique. Inset shows a force transducer for AFM.

Besides this topographic imaging, many other more complex modes have been invented that can map physical properties such as relative (*4*) and absolute (*5*) mechanical stiffness, adhesion, friction, magnetic and electric fields (*4*), density of electronic states (*6*), and so forth.

The AFM is now the most widely used of the SPM techniques. It is also the most straightforward conceptually. For these reasons, I will focus mostly on AFM. Measuring the repulsive force between the probe and the specimen with the probe in contact with the specimen, protein secondary structures such as β sheets (*7*) have been imaged. Loops connecting transmembrane α helices in bacteriorhodopsin were found to be reversibly deformed (see Fig. 2) by changes in the tip-sample force (*8*). With higher forces, the α helices in the coiled-coil tail of the myosin molecule were irreversibly teased apart by the probe (*9*).

Attempts to use SPM to image the DNA double helix got off to a false start (*10*), but recently beautiful images revealing the helical structure were produced (*11*). An exciting demonstration of AFM's ability to image biomolecular processes is the direct imaging in real time of *Escherichia coli* RNA polymerase transcribing linear double-stranded DNA templates (*12*), in which the translocation of the DNA by the polymerase molecule is seen on the addition of ribonuclease 5′ triphosphates. The AFM also detected conformational changes in the protein during transcription. The DNA molecules must be only weakly bound to the surface for the enzyme interaction to occur, but weakly adsorbed molecules are swept away by contact-mode scanning. Instead, the AFM was operated in "tapping" mode (*4*), which considerably reduces the shear forces imposed by the probe on the molecules. In this mode, the probe and cantilever are oscillated in a vertical plane above the specimen, such that the probe makes only intermittent contact with the specimen. The probe "tapping" the surface decreases the oscillation amplitude, and so the specimen topography

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can be derived by scanning, advancing, or retracting the probe so as to maintain a constant oscillation amplitude in a manner analogous to maintaining a constant force in contact mode.

With the oscillating cantilever further from the surface so that it no longer taps the surface, noncontact imaging with essentially zero shear force applied to the specimen can be achieved. This dynamic mode makes use of the shift in resonant frequency resulting from the van der Waals force gradient close to the surface. In practice, this technique requires an ultrahigh vacuum environment. Atomic resolution has been achieved with this technique (*13*), and recently Güntherodt and colleagues

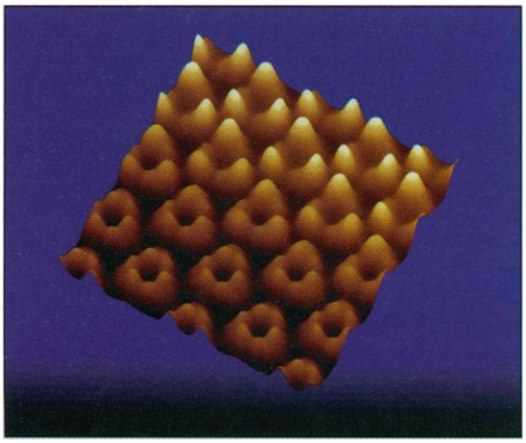

Fig. 2. A three-dimensional presentation of AFM data from an array of bacteriorhodopsin molecules showing that the E-F loops can undergo a conformational change as the force exerted by the probe is increased from the back to the front of the image.

(*14*) have imaged thermally activated movements of atomic defects at the surface of an inorganic crystal. Noncontact imaging has also been demonstrated for certain implementations of so-called shear-force microscopy (ShFM), which is more widely applicable because it operates in gaseous and aqueous environments. Here, a vertical cantilever is oscillated in a plane parallel to the specimen surface. The amplitude and resonant frequency depend on the distance of the probe from the surface for distances <10 nm. ShFM is also used to control the probe-specimen gap in near-field scanning optical microscopy, which gives resolution of more than an order of magnitude better than conventional optical microscopy.

SPM is essentially a surface microscopy, but its applications overlap those of both scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SPM's principal advantages are ultra-high resolution 3D imaging on a wide range of specimens and the ability to work in gas or liquid compared with limited gaseous environmental stages in electron microscopy. This is essential for studying biological specimens and biomolecular processes in native environments. Equally important for biology is that SPM contrast, unlike TEM, does not depend on the atomic number of the elements present, so that biological and organic materials do not need staining with heavy metals, nor are they subject to the high-energy radiation damage of an electron beam. The greatest disadvantage is that most SPM techniques image only the surface region of the specimen. Another disadvantage is the relatively low scan rate, typically about one image per minute. The cost of a versatile commercial SPM instrument falls between that of a relatively good conventional optical microscope and the lower end of TEM cost range. As with TEM, considerable experience and skill is required for anything more than routine imaging.

SPM has created a revolution in microscopy, and some unexpected twists in its development undoubtedly lie ahead. Some developments, such as increased scan rates, can easily be predicted. Low-mass cantilevers (*15*) are being developed to respond 10 times faster than those currently in use. An alternative approach is to use arrays of independently controlled probes (*16*) such as those being developed in Quate's group at Stanford University. The probes simultaneously scan neighboring regions of the specimen. The speed increase achieved by these arrays is not useful in following a high-speed molecular transformation, but rather, allows large regions of a specimen to be imaged very rapidly. A biological application might be an extension of the AFM gene-mapping strategy, demonstrated by Allison's group at Oak Ridge National Laboratories (*17*), to large chunks of a genome. Using a 1-cm linear array of 50 probes, the possibility of ultrahigh resolution lithography of a whole silicon chip may become a realistic route to future high-density circuits in silicon device technology. The ultimate example in surface modification must be the spectacular achievements of Eigler's group at IBM in positioning iron atoms one by one to form structures on a copper substrate (*18*). On a much larger scale, Heckl's group in Munich used an AFM probe as a nanoscalpel to dissect DNA from a particular region of a chromosome (*19*) and used polymerase chain reaction and fluorescence in situ hybridization techniques to demonstrate that they had succeeded in removing DNA from a specific site.

Other recent developments involved use of the AFM in a nonscanning mode to measure forces on individual molecules, such as the force-distance measurements of single strands of DNA by Lee and colleagues (*20*) at the Naval Research Laboratory in Washington, D.C., and the force denaturation of a molecule of the muscle protein titin by Gaub's group in Munich (*21*). The magic of the microcantilever continues into other nonscanning areas of biosensors, such as femtogram thermogravimetric analysis and femtojoule thermal analysis, among others (*22*).

The ultimate SPM technique would be capable of identifying individual atoms and specifying their 3D location to atomic resolution. This is the dream of magnetic resonance force microscopists (*23*). To achieve this goal, the force sensitivity must be sufficient to detect individual nuclear spins. This requires an improvement of a further two orders of magnitude on the astonishing attonewton sensitivity achieved by Rugar and colleagues (*24*). Having already scaled down eight orders of magnitude in their quest, this dream may yet become reality.

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