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Imaging soft materials in fluids by nanowire detection

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Sub-Ångstrom-resolution sensing of tiny cantilevers is leading the way to a gentle physical probe for surfaces of materials such as living cells.

Atomic force microscopy (AFM) is an imaging technique used to study nanosized features on surfaces. It consists of a sharp tip at the end of a cantilever, usually suspended over a sample. The sample itself moves in a rasterlike scan beneath the tip, driven by piezoelectric materials, which expand in proportion to voltage. As the tip is lowered, it interacts with the sample. The cantilever's deflection can then be used as an indicator of the local characteristics of the surface with subnanometer resolution. AFM can image significant characteristics such as height, friction coefficients, chemical signatures, and magnetic properties. Because it offers force measurement, surface manipulation, and imaging with a resolution that far exceeds the optical diffraction limit, it serves as a fundamental research tool for nanoscale research. Indeed, researchers have used AFM to identify and manipulate individual atoms on surfaces.[1,2] A broad range of open questions in biology would benefit from this technology, particularly those dealing with protein and lipid dynamics in the outer membrane of the cell. However, performing AFM on soft materials in fluids (e.g., living cells) is difficult to do gently without damaging or destroying the sample.

Imaging in fluids in a nondestructive manner requires reducing the random fluctuations of the cantilever. These thermal motions are a fundamental property of the cantilever's interaction with surrounding fluid, and depend strongly on the device's size and shape.[3] In general, shrinking a cantilever's cross-section will reduce noise significantly. However, the minimum possible size is still limited by the manner in which the position of the device is detected. Recently, smaller cantilevers have demonstrated improvements in reducing these fluctuations,[4] allowing for gentler imaging. Going to diameters smaller than the diffraction limit of light could reduce thermal noise by another order of magnitude, but this would necessitate a new scheme to detect the cantilever's position. At the Lawrence Berkeley National Laboratory's Molecular Foundry, a nanoscience user facility, we have developed a means of optically discerning the position of tiny nanowire cantilevers with sub-Ångstrom resolution.

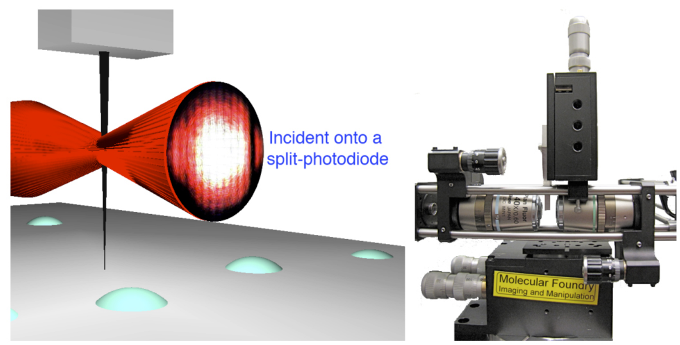

Figure 1. *(left) A conceptual drawing of the nanowire detection scheme. (right) Photo of a portion of the optics.*

A nanowire cantilever images a sample by operating in a 'shear mode,' where the oscillating nanowire is oriented perpendicular to the sample. As the sample is raised through piezoelectrics toward the nanowire, it interacts at the tip, and the amplitude of the nanowire's oscillation changes. We are able to detect this signal and use it in a feedback loop that lowers the sample accordingly, ensuring gentle contact during scanning.

Typically, the deflection of a cantilever is measured by an angle change of a reflecting laser. This requires a flat surface on the cantilever large enough to produce a specular reflection, which limits the minimum size of the device. In our scheme, we discern the position of the cantilever (a nanowire, whose diameter is significantly smaller than the wavelength of light) by placing it in the focus of a laser beam and observing the resulting light pattern (see Figure 1). The approach is reminiscent of a technique used to measure the location of beads in an optical trap.[5] When the pattern is incident on a split photodiode, we find that the diode's difference signal serves as an excellent indicator of the nanowire's position—see Figure 2(A)—in agreement with our

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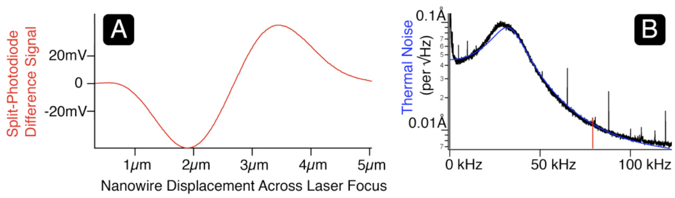

Figure 2. *(A) Trace of the difference signal from the split photodiode as a function of the nanowire's position relative to the laser focus. (B) The measured thermal spectrum of a nanowire in air.*

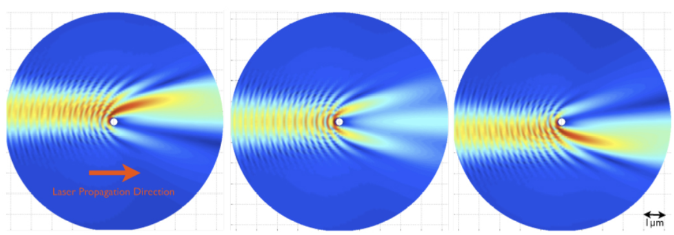

Figure 3. *Light-scattering simulations of the nanowire in a focused beam, as the nanowire traverses the beam's focus. The long axis of the nanowire is directed out of the page, and the beam crosses it from left to right.*

computational models. Our simulation of light scattering from a cylindrical nanowire (see Figure 3) in a focused laser beam indicates that, for small cantilevers, our scheme is more sensitive when the focused spot is tighter.

With this engineering guidance, we have designed and built a system that allows us to independently piezo-control the position of the nanowire with respect to the laser beam, as well as the position of the sample with respect to the nanowire. The nanowires are readily generated by a variety of well-developed techniques.[6,7] We have detected the position of commercially available nanowires in air, with noise that is sufficiently low for measurements to be limited by the fundamental thermal noise, instead of detector design: see Figure 2(B).

Having shown that the nanowire sensing scheme is viable, we are currently performing the first experiments with the wire immersed in solution to image 'soft' samples. Ultimately, we intend to image the surface of living cells to discern protein cooperativity and membrane structure.

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