APPLIED PHYSICS: High-Speed Atomic Force Microscopy

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APPLIED PHYSICS

High-Speed Atomic Force Microscopy

Innovations in engineering, miniaturization, and control-system design have the potential to allow faster imaging by atomic force microscopes.

Paul K. Hansma, Georg Schitter, Georg E. Fantner, Craig Prater

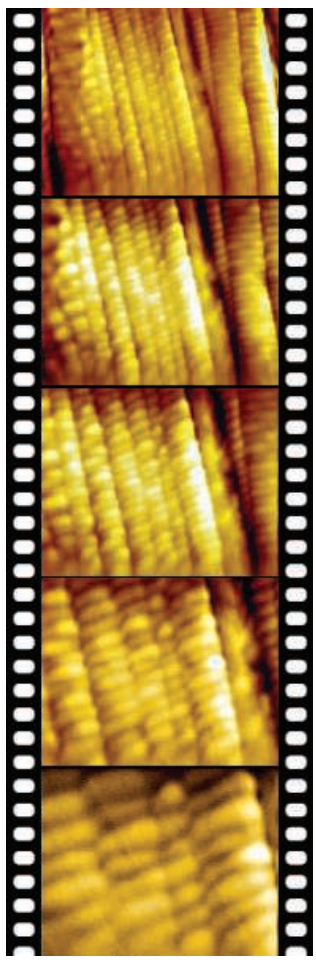
A graduate student was recently heard lamenting, "I feel like my life is passing me by!" as he waited for an atomic force microscope (AFM) image to form line-by-painstaking-line. In AFM, a sharp tip at the end of a tiny cantilever is scanned across a sample to image its topography and material properties. The images can be obtained for samples in air, water, or vacuum with typical resolution on the order of 10 nm. Despite the enormous success and widespread use of AFM, however, most users want higher speed imaging. Conventional AFMs typically take 1 to 100 min to obtain a high-quality image. The productivity and use of AFMs would increase dramatically if the speed could match the millisecond to minute image times of other scanning microscopes such as confocal and scanning electron microscopes. Moreover, there are many experiments, such as watching biological processes in liquids, that simply cannot be done without faster imaging.

The first paper on high-speed AFM was published 15 years ago (*1*). So why are faster AFMs generally not available? Just as a chain is only as strong as its weakest link, AFM speed is limited by the slowest component in its entire control loop. The achievement of high-speed scanning has required innovations in cantilevers, deflection measurement, scanners, and controllers. These innovations have pushed the state of the art in micromachining, electromechanical engineering, and control engineering.

About 10 years ago, small cantilevers (*2*) and heads for small cantilevers (*3*) were first reported. These small cantilevers can have much higher resonant frequencies at the same spring constant because their mass is much smaller. Typically the mass is smaller by a factor of 1000, making the resonant frequency higher by a factor of the square root of 1000, or about 30. The problem has been that, although individual research groups have made limited quantities (*4*–*7*), there has been no commercial source. And it has been a sort of "chicken or egg" problem: Major cantilever manufacturers have been reluctant to invest in small cantilevers because there were no commercial AFMs that could use them; AFM manufacturers have been reluctant to make small-cantilever AFMs because there were no commercially available small cantilevers. One of us (G.F.) has founded a start-up company, SCL-Sensor. Tech., to produce small cantilevers with integrated tips for this purpose.

Faster scanners are also required to take full advantage of the higher speed possible with small cantilevers. Here too, there has been substantial progress beginning with pioneering work by Ando *et al*. (*7*) and the work of Humphris *et al*. on resonant scanners (*8*). A recently reported scanner (*9*) based on finite element analysis of optimally constrained designs (*10*) achieves the necessary factor of ~30 improvement in scanner resonant frequencies in a scanner with a practical range of 13 μm in the *x* and *y* (horizontal) directions and 4.3 μm in the *z* (vertical) direction.

This leaves the control system. Here as well, substantial progress has been made both in electronics (*7*, *11*) and in control algorithms (*12*, *13*) and high-speed data acquisition (*14*, *15*). For fully functional high-speed AFM imaging, it is also necessary to increase the speed of the feedback loop that controls the height of the AFM tip by a factor of 30 to maintain minimal imaging force and high image accuracy. Fortunately, this appears within reach with emerging developments in high-speed digital electronics. For now, most of the detail in high-speed images is in the so-called error mode, such as those shown in the figure. The feedback is simply not fast enough to maintain constant cantilever deflection and accurately track the subtle details in sample topography. The information about these subtle details, such as the bands on the collagen fibrils, comes from measuring the subtle changes in cantilever deflection, which the feedback electronics are not fast enough to keep constant. The resulting images are called error mode images because they display the errors in maintaining constant cantilever deflection. As the speed of feedback increases, users increasingly will move from error mode images to quantitative topography images. Also, the imaging will be gentler because the force, which is proportional to cantilever deflection, will be kept constant.



Fast imaging. This series of images of rat tail collagen illustrates how high-speed AFM allows zooming in on areas of interest rapidly. This entire zoom series from an image width of 2 μm to a width of 470 nm was taken in 0.56 s and shows every fourth image in the series. Collagen's characteristic 67-nm banding pattern is clearly resolved in the raw data and enhanced with image processing for easy visibility. A conventional AFM would need about 15 min of imaging to obtain a comparable series of images.

In addition to relieving the tedium of waiting for images, commercial high-speed AFMs will also enable researchers to study fast processes such as protein motion (*7*, *16*) and crystal growth (*4*) and to do faster force spectroscopy (*5*) that has only been possible in a few labs with homebuilt equipment. High-speed AFM also offers enormous promise to increase the use of AFM for industrial measurements, where metrology is often monitored by the cost per measurement site. In the case where an AFM can

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operate faster by a factor of 30, this can translate into a substantially lower cost per measurement.

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BIOMEDICINE

Avoiding Collateral Damage in Alzheimer's Disease Treatment

Although β-secretase has been considered a relatively harmless target for drugs against Alzheimer's disease, such drugs may damage nearby nerves.

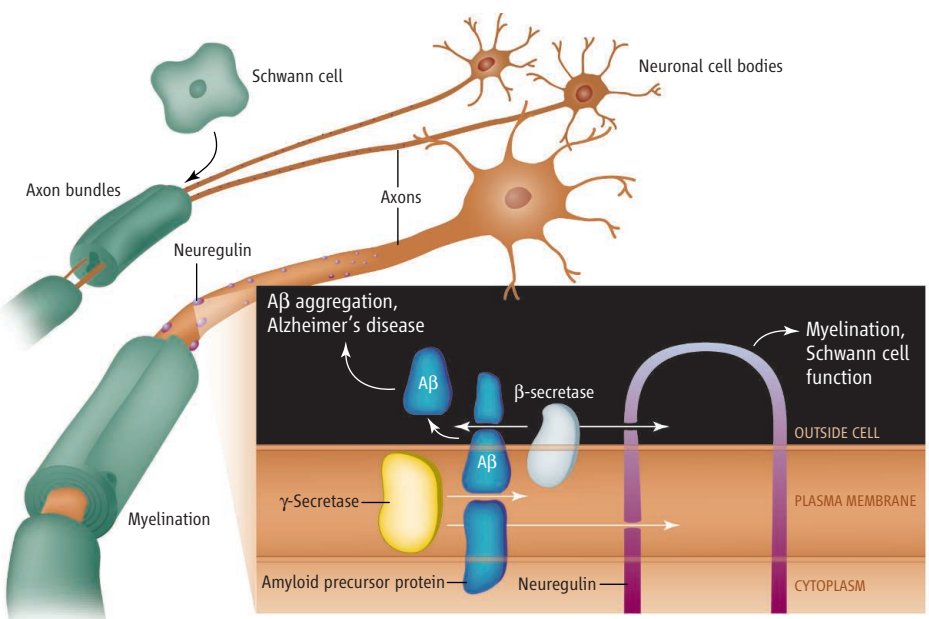
Charles Glabe

The abnormalities of Alzheimer's disease include the accumulation of a 40 to 42-residue peptide called amyloid beta (Aβ) in the brain, which eventually forms the characteristic plaques that are associated with the disease. Ever since the discovery that Aβ is generated through the proteolysis of a precursor protein, the cleaving enzymes have been considered potential targets for the development of drugs to treat the disease. In particular, BACE1 (beta-site amyloid precursor protein–cleaving enzyme 1; also called β-secretase) (*1*) has been viewed as a particularly "safe" drug target because deletion of β-secretase in transgenic mice shows no apparent detrimental side effects, although Aβ production is eliminated (*2*, *3*). But as Willem *et al.* report on page 664 in this issue, β-secretase targets another molecule that is crucial to heart development, peripheral nerve development, and neuroplasticity (*4*). This raises new concerns about the potential negative consequences of inhibiting β-secretase activity to treat Alzheimer's disease.

Aβ is genetically implicated as a causative agent in Alzheimer's disease. It is constitutively generated by proteolysis of amyloid precursor protein, a membrane protein. The proteolysis that generates Aβ occurs in two steps. First, β-secretase–mediated cleavage releases a soluble ectodomain and leaves a membrane-associated fragment. Then γ-secretase cleaves within the transmembrane domain of the latter, releasing soluble Aβ extracellularly and a soluble cytosolic fragment. As luck would have it, γ-secretase was discovered first. Mutations in presenilin, the catalytic component of γ-secretase, were associated with inherited forms of early-onset Alzheimer's disease. The mutations generally alter the cleavage site in the precursor protein, generating the longer 42-residue form of Aβ that aggregates faster (*5*). This discovery was one of the key pieces of evidence that Aβ plays a causal role in disease pathogenesis.

But there has been limited enthusiasm for γ-secretase as a drug target to decrease Aβ production. Other functions of the enzyme have proven broad and critical for normal biological processes, as transgenic mice lacking presenilin 1 and 2 have a severe developmental phenotype (*6*). Among the large number of γ-secretase substrates that have been identified are important morphogenic molecules, including Notch, Delta, and Jagged (*7*). So inhibiting γ-secretase may have a variety of unwanted side effects.

Once β-secretase was discovered, it appeared to be a more appealing drug target, as amyloid precursor protein seemed to be its only known substrate and transgenic mice lacking β-secretase displayed no obvious phenotypic aberrations. This suggested that



Myelination in jeopardy? Just like amyloid precursor protein, type III neuregulin 1 is also cleaved by β-secretase. Proteolytic cleavage of neuregulin 1 by β-secretase is critical for peripheral nerve myelination by Schwann cells. Drugs that target β-secretase could affect peripheral nerve development and function.

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