Scanned Probe Microscopy in Biology

Knute A. Fisher, University of California

History.

Numerous scanned probe microscopes (SPM) have been developed over the past decade.[1] Most are based on the precise positioning of sample and probe using piezoelectric transducers, and some have the capability of imaging flat surfaces with atomic resolution. The first atomic resolution SPM applied to biological samples was the scanning tunneling microscope (STM).[2] The atomic force microscope (AFM) was subsequently developed[3] and over the past few years has become the instrument of choice for biological applications.

Scanning Tunneling Microscopy.

Earlier investigators applied the STM to examinations of organic and biological systems ranging from small molecules to nucleic acids, globular and fibrillar proteins, and larger structures such as viruses, membranes, and even whole cells.[4] Much of this work was done during the mid-80s using electrically-conductive highly-oriented pyrolytic graphite (HOPG) as a substrate. Images were often difficult to obtain and control experiments were lacking. Unfortunately, when careful experiments were undertaken, they revealed the HOPG itself was capable of generating images previously thought to be biological. The HOPG artifact problem was exacerbated by the absence of convincing explanations of image formation by non-conducting samples, and by the lack of information that wasn't of the "me-too" variety. The unfulfilled expectations of easily achieved atomic resolution images of biological samples, plus the concerns just outlined established a somewhat shaky beginning for SPM in biology. At the same time it should be remembered that some exquisite and reproducible high-resolution STM imaging was accomplished with planar two-dimensional arrays such as liquid crystals and Langmuir-Blodgett films. In addition, several studies of metal-coated samples mounted on glass or mica, or freeze-fractured, demonstrated that the STM could be used to study features of biological interest. For example, the height of a phospholipid ridge in a freeze-fractured replica, and the thickness of a biological membrane and change in thickness after enzymatic cleavage, were examined.

Atomic Force Microscopy

The conductivity problem in STM was overcome by the development of AFM and subsequent development of an optical detector for reproducibly measuring the deflection of the AFM cantilever. Given suitable and commercially available instruments by the late-80s, the study of insulating as well as conductive samples could be initiated in earnest. For biological applications, several problems remain.[5] AFM resolution is limited by physical and chemical properties of the tip and by tip-sample interactions. Recently a variety of tips have been developed that are intended to improve resolution. With soft biological samples, however, simply reducing the size of the tip and/or producing more needle-like shapes has not significantly improved resolution, since the force is distributed over a larger area of the sample with a dull tip than with a sharp tip. Even with dull tips, the forces may still move or perturb soft samples. Nevertheless, exciting biological images are now being achieved at 2-10 nanometer resolution.

Sample Preparation

In the past few years attention has been given to preparing samples especially suited to AFM. Most of these approaches have employed methods to tether the sample to the substrate. For STM, coating with metal stabilizes the sample for subsequent scanning. for AFM, attachment of samples to mica iconically or covalently has led to reproducible imaging.[6] Because sample preparation and reproducible imaging has been a significant problem for SPM, obtaining images with relative ease has been a significant recent advancement for the field.

Applications

Although there continues to be STM studies of biological samples, SPM investigators have increasing turned to the AFM. For example, AFM is being used to examine single linear molecules such as nucleic acids and collagen monomers; model lipid monolayers, bilayers, and multilayers; native and

reconstituted biomembranes; and intact hydrated cells.

Future

Other SPM instruments such as the near-field scanning optical microscope will undoubtedly be developed bor biological applications. For AFM, as high-resolution tips continue to be developed, it will be necessary to focus increasingly on sample stabilization methods and/or to explore alternative imaging modes such as the non-contact modes of scanning. One approach we have taken to improve sample stability has been to develop a low-temperature AFM that can scan frozen samples.[7] We have imaged nucleic acids, ferritin molecules, colagen monomers, and purple membrane at 143 K and have found improved reproducibility, and instrument stability and sensitivity, but with resolution still apparently tip limited. Clearly the future of AFM lies in its unique capability of imaging hydrated samples in real time. Perhaps the most important advance for AFM in biology will be in asking appropriate questions! For example, rather than asking for the atomic structure of an active site of an enzyme, one might more reasonably ask for details of hydrated enzyme-substrate interactions (protein-protein or protein-nucleic acid). The future also will increasingly demand labels and approaches to identify structures unequivocally. In summary, for AFM to continue as a viable and creditable microscopic method will require increasingly thoughtful approaches, from experiment design to image interpretation. ■

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A WORD TO THE WISE

According to the personal secretary of Charles Steinmetz, the General Electric Wizzard, he framed a quotation which he particularity admired and hung on the wall behind his desk:

The man who once most wisely said,
Be sure you're right, then go ahead,
Might well have added this, to wit;
Be sure you're wrong before you quit

From the Daily Gazette of Schenectady, NY, May 29, 1995 and contributed by Sterling Newberry

A CORRECTION:

In the last issue of this newsletter, we advised that the FEI Company featured their LaB_4 cathode at the MSA Conference in Kansas City.

The FEI Company actually featured their LaB_6 cathode at this Conference.

Our apologizes to the FEI Company and to our readers!