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IMAGING BIOLOGICAL SAMPLES WITH THE ATOMIC-FORCE MICROSCOPE

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The application of atomic force microscopy (AFM) to biological investigation is attractive for a number of reasons. Foremost among these is the ability of the AFM to image samples, even living cells, under near native conditions and at resolution equal to, or exceeding, that possible by the best light microscopes. Moreover, the ability of the AFM to manipulate samples it images provides a novel and far reaching application of this technology.

We have been studying a number of biological samples by AFM. These include conventional and non-conventional nucleic acid structures, ribosomes, neural cells and synapses, cellular organelles (chloroplasts and nuclei), among others. Each of these projects has its own set of associated difficulties and each reveals information about the uses and limits of the AFM in biology. Fig. 1 shows AFM images of various biological samples. In the case of nucleic acids, which have been extensively studied in a number of labs by AFM the problems of signal/noise sample deposition have been overcome in air and organic solvents. Recently imaging of DNA in aqueous environments has been accomplished. Small biomolecules like ribosomes present the problem of deconvolving the tip architecture from the image. Mathematical algorithms to do this have been developed and methods for tip shape characterization and new tip construction are ongoing. Successful imaging of living cells is very dependent upon the type of cell, the type of tip, the growth substrate and imaging parameters such as applied vertical force and scan rate. Cellular organelles, lacking the extensive supportive skeletal structures found in some cells, have been extremely difficult to image in their native state, although images of fixed samples are routinely obtained.

One of the obvious future developments in AFM is the integration of this method with other imaging methods, for example, fluorescence and light microscopy. We have constructed a prototype coaxial optical/atomic force microscope. Similar instruments have been constructed by other laboratories. With these instruments one can visualize a biological (or other) sample with bright field, dark field or fluorescence microscopy and position the scanning tip over a region of interest. Subsequent scans of this area reveal surface topography and other information about the sample. In our laboratory this approach has been applied to the study of cellular cytoskeleton and chromatin structure. Fig. 1 shows fluorescence and AFM micrographs of *Xenopus* retinal glial cells (panels g and h) and a *Drosophila* polytene chromosome (panel i). Further development in this area is ongoing.

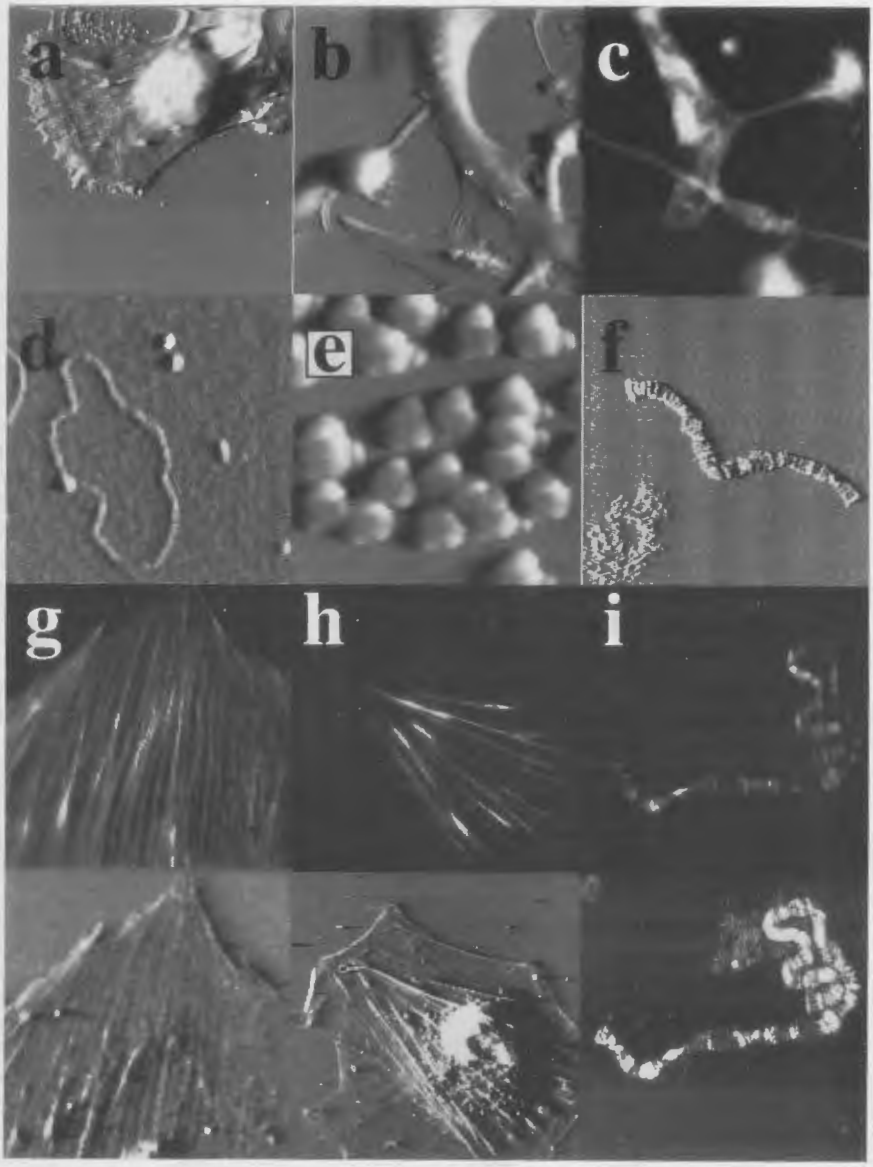

Fig. 1. AFM of biological samples. a, living *Xenopus* retinal glial cell, b, living rat hippocampal neuron and glial cells, c, specialized nerve terminal (calyx) from chicken, d, plasmid DNA bound by the restriction endonuclease EcoRI, d, bacterial large ribosomal subunits, f, fragment of a *Drosophila* polytene chromosome, g and h, fluorescence (top) and AFM micrographs of fixed *Xenopus* retinal glial cells, i, fluorescence (top) and AFM micrographs of *Drosophila* polytene chromosome. Approximate field sizes are, a, b, g and h, 80 μ^2; c, f and i, 20 μ^2; d, 1 μ^2; and e, 0.5 μ^2.