Sandor Kasas
M.R. Celio

The Applications of the AFM in Histology

The atomic force microscope (AFM) is a new kind of instrument allowing an atomic resolution on crystals and molecular resolution on some biological materials. Its ability to image samples immersed in a fluid medium makes it highly interesting for biologists. We demonstrate here images obtained using this microscope on slices of rat striated muscle, erythrocytes immersed in a physiological buffer and molecules of laminin.

Introduction

The atomic force microscope is a new kind of microscope reaching an atomic resolution on crystals and a molecular resolution on biological samples. An increasing number of scientific groups are utilizing it in biology and more and more AFM images of biological samples are published in scientific papers. We are presenting here our one year results of the AFM in histology.

We have been interested in the visualization of animal tissues prepared with classic histological techniques for light and electron microscopy. *Figure 1* depicts a rat skeletal muscle embedded in epon, cut in 1 µm slices with an ultramicrotome, mounted on glass, stained by toluidine-blue and observed in air using the AFM. The characteristic periodic striation of muscle tissue clearly appears on this image.

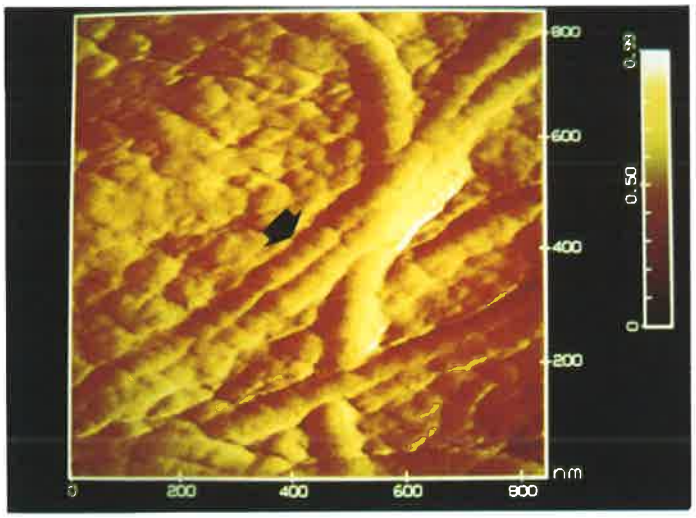

FIGURE 1. - *Section of rat skeletal muscle embedded in epon and imaged with AFM.*

Figure 2 represents the surface of a section of the mouse tongue. The fixed mouse tongue was frozen at − 21 °C, cut in 16 µm thick slices with a cryomicrotome, mounted on a slide and dehydrated in vacuum. The AFM image in air shows some collagen fibres running on the surface of the section. Their typical 64 nm periodic pattern is well visible.

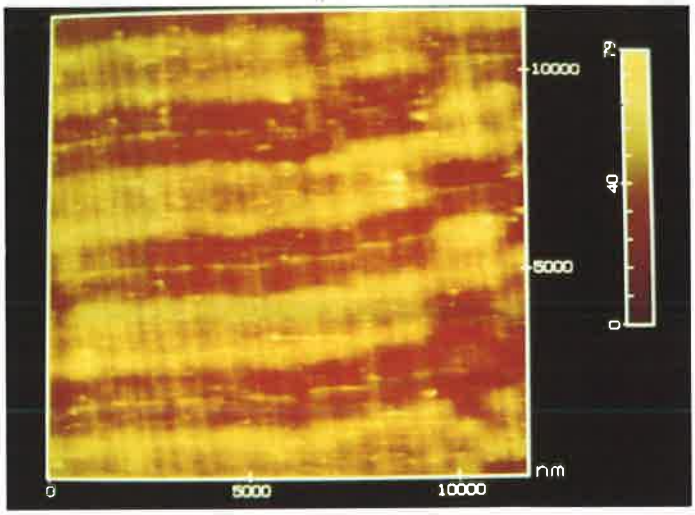

FIGURE 2. - *Collagen fibres (arrow) on the surface of a section of the tongue. Note the characteristic 64 nm periodic pattern.*

For the biologist, the main advantage of the AFM is that it allows to visualize samples immersed in a fluid medium. *Figure 3* shows a rabbit erythrocyte fixed with glutaraldehyde and imaged in phosphate buffer.

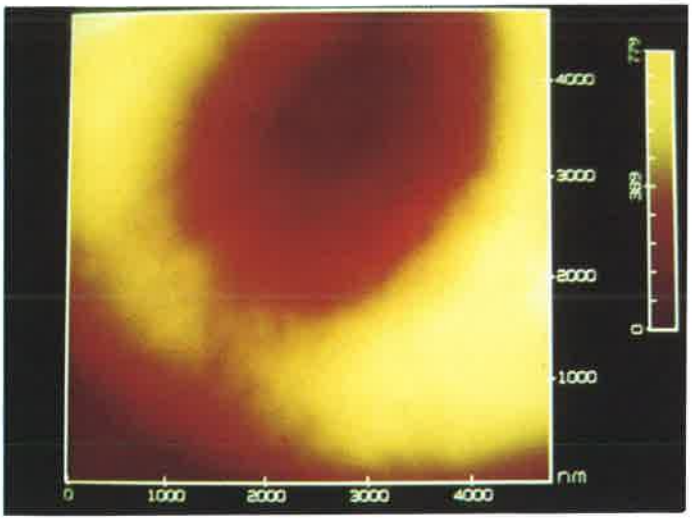

FIGURE 3. - *Erythrocyte imaged in 2.5 % glutaraldehyde in buffer 0,1 M, pH 7.3.*

Institut d'Histologie et d'Embryologie Générale, Université de Fribourg, Suisse.

Another interesting application of AFM in biology is to image DNA or proteins on a molecular resolution. *Figure 4* shows a single laminin molecule imaged in our laboratory. Laminin is a protein that promotes cell adhesion ; its characteristic shape is illustrated on *Figure 5*. A commercially available laminin was diluted in 200 mM ammonium-acetat buffer to a concentration of 2 µg/ml. 3 µl of this solution were deposited on freshly cleaved mica, rinsed 3 times in distilled water, dryed in vacuum and imaged in air. The images were taken in the "force mode".

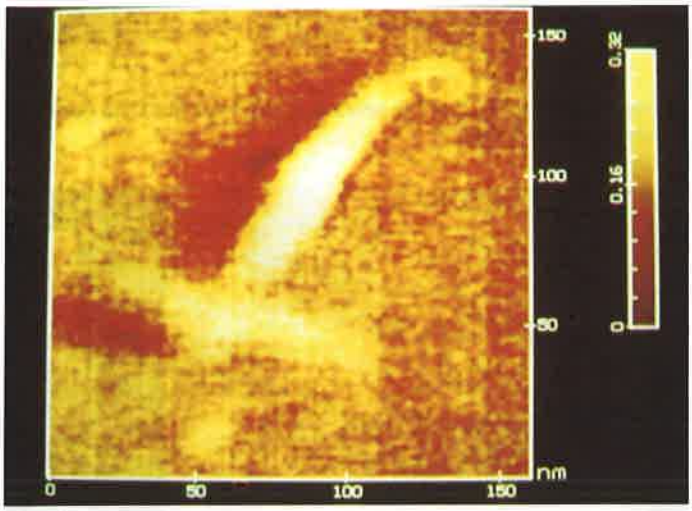

FIGURE 4. - *Laminin molecule.*

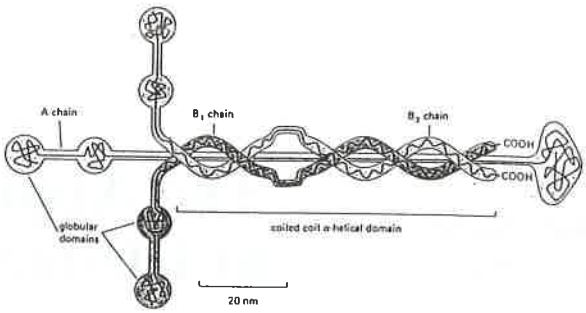

FIGURE 5. - *The theoretical shape of a laminin molecule (from The Molecular Biology of the Cell. p. 820, second edition 1989).*

Conclusion

The atomic force microscope seems to be a very promising instrument in biology and specially in morphology. The images we present here clearly show that it can achieve a good resolution on samples prepared in conventional way for electron microscopy. Despite the impossibility to obtain low magnification on rugged samples, it can image biological structures immersed in a fluid medium. This means that the tissue preparation is very simple and the morphological artefacts caused by other methods are absent. Some of the informations obtained by AFM are difficult or impossible to obtain by electron microscopy for example, the sample size in all three axis. This microscope will never replace the electron microscope but it will certainly be a complementary instrument to it. We believe that in the future it will be used more and more in the study of living materials and in the visualization of dynamic processes.