Observation of living cells using the atomic force microscope

S. Kasas, V. Gotzos, and M. R. Celio
Institute of Histology, University of Fribourg, CH-1700 Fribourg, Switzerland

ABSTRACT We used an atomic force microscope (AFM) to image samples immersed in a fluid in order to study the dynamic behavior of the membranes of living cells. AFM images of cultured cells immersed in a buffer were obtained without any preliminary preparation. We observed surface changes and displacements which suggest that the cells were still alive during the measurements. Some membrane details imaged with the AFM have also been observed using a scanning electron microscope and their dynamic behavior has been confirmed by microcinematography. We believe that the AFM will offer new insights into the exploration of dynamic changes affecting cell membranes.

INTRODUCTION

Before the development of the scanning probe microscopes, the only tool for the investigation of the surface of living cells was the optical microscope. However, the resolution achieved is unfortunately limited to roughly half the wavelength of light and, without the use of the confocal laser microscope, there is no possibility of making precise measurements along the *z*-axis. The atomic force microscope allows us to overcome these two major problems and to image living cells in their culture medium.

This instrument (AFM), which was invented by Binnig, Quate, and Gerber (1, 2, 3), is a scanning probe microscope designed for investigating surfaces. Different materials have been successfully imaged by it with atomic resolution (4, 5). Among biological materials, the AFM has been used to image amino acid crystals (6), proteins (7, 8, 9), DNA (10), organic monolayers (11), and viruses (12). Living plant cells have already been observed in air (13, 14).

The AFM can also be used to image surfaces in an aqueous environment. The use of water as a scanning agent has the advantage of minimizing the adhesion forces between the tip and the surface (15). In addition it allows the imaging of biological systems under more natural conditions. For example, red and white blood cells have been imaged in buffer solutions (15) after fixation. Some biological processes like the polymerization of fibrinogen (7) or the budding of viruses through a cell membrane (16) have also been observed in fluid medium using the AFM.

In this report we demonstrate the possibility of imaging single cells at a medium magnification with minimal preparation. The cells were grown on glass and imaged during one hour while immersed in a physiological buffer. Wave-like structures were observed moving on the surface of the cells and parts of the cells were seen to be moving while the cell was raster-scanned by the AFM. These observations suggest that the cells were still alive during the scan. To confirm that cells are mobile, we made a microcinematographic film of the cell culture. The presence of waves on the surface of the cells was also confirmed by electron scanning microscopic examination of the same sample. Our observations lead us to believe that the AFM may become a very useful tool for studying the surface of living cells.

MATERIAL AND METHODS

Sample

The cells we used were from a line that has been cultivated in our laboratory for 4 yr. They were obtained from a pleural effusion in a 62-yr-old patient with metastatic lung carcinoma. Their flat shape and their tendency to grow in monolayers make them good candidates for AFM studies. They were washed, and cultured in a medium composed of 90% of EMED (enriched Dulbecco's modified Eagle medium) and FMED (modified Eagle's F-12 nutrient mixture) 1:1, 10% of foetal calf serum with 10^4 U of penicillin/liter and 10^4 μg of streptomycin/liter and then maintained at 37°C in 95% air and 5% CO_2. The cells were again cultivated on round glass slides for 3 d in the culture chamber illustrated in Fig. 1, using the same medium.

AFM Imaging

The glass holding the cells was glued onto a steel disc with Cyanolyth®, and deposited on the "G" or "J" *xyz* translator (maximum *xy* scanning size 90 and 130 μm) of a Nanoscope II® AFM (Digital Instruments, Santa Barbara, CA). A few drops of PBS pH 7.2 or culture medium were deposited on the sample and the "fluid cell" (Digital Instruments) dropped onto it. We did not use the O ring delivered with the fluid cell as the PBS capillary forces were strong enough to maintain the fluid in the space between the sample and the plexiglas above it.

We used a 200 μm long, thin, microfabricated Si_3N_4 triangular cantilever with a spring constant of ~0.05 N/m supplied by Digital Instruments. The force applied to the sample was, as a rule, less than 3 nN.

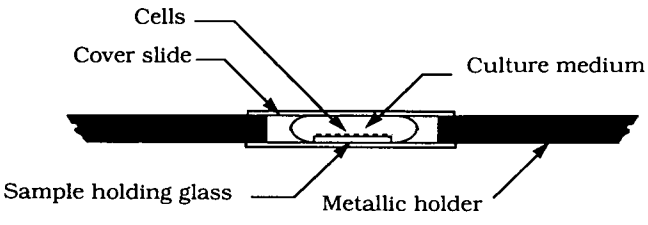


FIGURE 1 The culture chamber.

0006-3495/93/02/539/06 $2.00

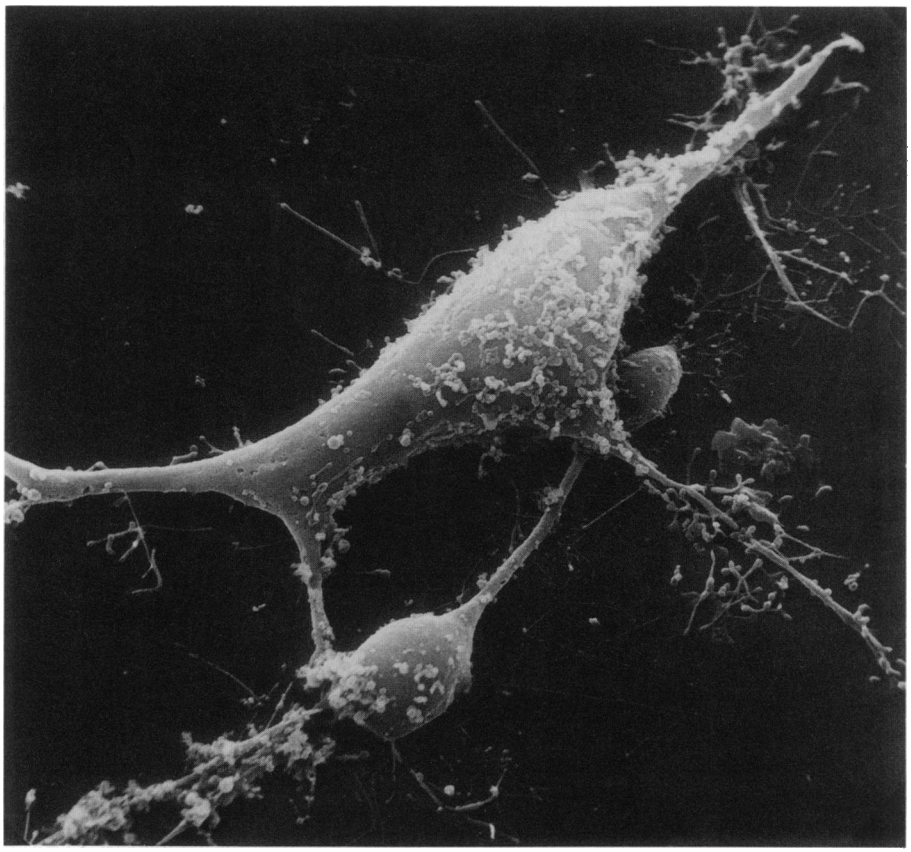


FIGURE 2 Scanning electron microscope image of two cells coming from the 131 cell line.

The images (20 s/image) were taken and stored in a personal computer to be processed later.

Scanning electron microscopy

The sample was removed from the AFM and prepared for critical point drying by passage through increasing acetone-H_2O concentrations: 50% 5 min, 75% 10 min, 90% 2× 15 min, and 100% 4× 15 min. Drying was performed with a CP 20 critical point apparatus (Balzers®). The sample was metallized in a sputter coater S 150 (Edwards®) for 4 min and was finally observed using a Jeol JSM 35 scanning electron microscope at 25 kV.

Microcinematography

The microcinematography was done using a 16 mm microkinocamera mounted at the top of a phase contrast microscope (Zeiss). Cells from the same cell line were cultivated and imaged in the chamber depicted on Fig. 1. Images were taken every 15 and 30 s for a period of 24 h. During this period the cells were maintained at 37°C in cell culture medium.

RESULTS AND DISCUSSION

The cell line we chose, labelled No. 131 in our catalogue, has the advantage of forming monolayers in culture and, except for cells in mitosis, tending to adopt a relatively flat shape. This particularity makes it interesting for AFM imaging.

We did not see significant differences between cells immersed in PBS (Fig. 13) and those immersed in the culture medium (Figs. 3–7).

Some cells (3 of the 20 that we observed) disappeared after they had been imaged once, probably displaced by the tip because they didn't stick to the glass strongly enough.

Images taken in the constant height mode were far better than those taken in the constant force mode. We have no satisfactory explanation for this phenomenon at the moment.

We usually had difficulty in scanning a whole cell. If we focused on a cell extension at high magnification and then increased the scan size, the image became worse and we lost resolution. It is possible that some parts, in particular the central area, were too elevated for successful scanning with the Nanoscope II, or that the rugosity of the cell surface was too important. The Fig. 2 shows two typical cells of the 131 cell line, seen with the scanning electron microscope (SEM). The numerous small cell extensions present on the upper right-hand part of the cell are invisible on the AFM images, probably because these small protrusions were flattened during the scan. However we did not always observe exactly the same cell with the AFM, we cannot affirm this definitely.

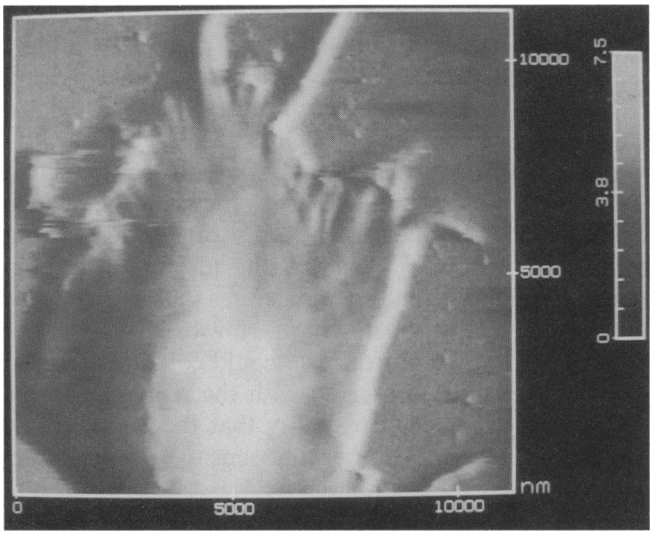
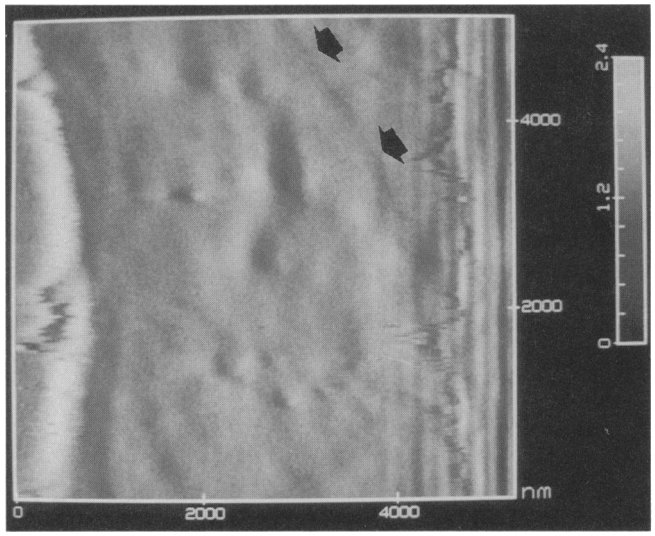
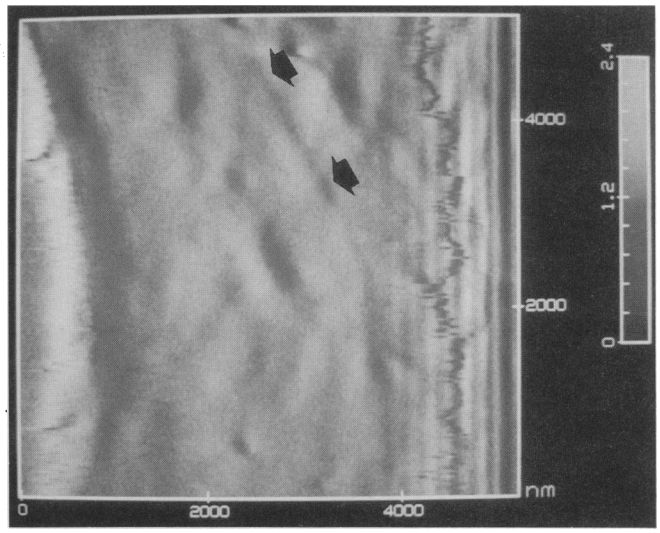
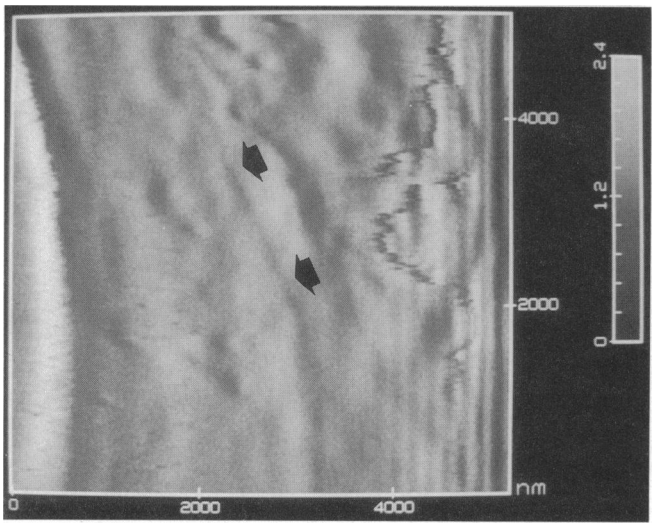
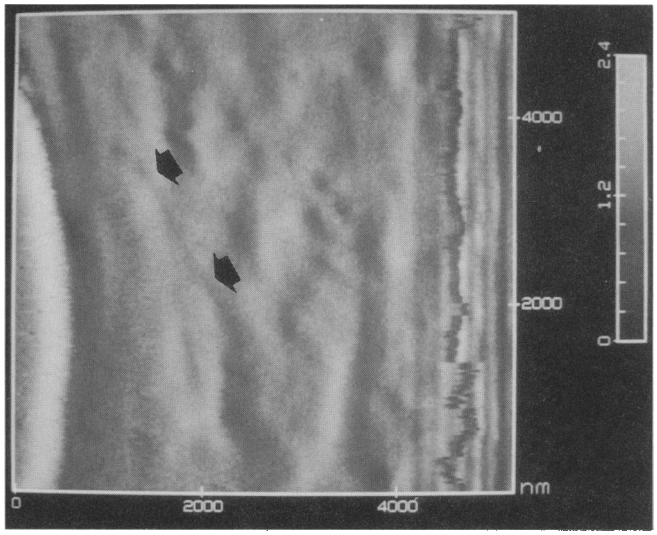


FIGURE 3 A cell imaged with the AFM.









FIGURES 4 to 7 (viewed from *top left* to *bottom left*, in a clockwise direction) An area of 5×5 μm was selected over the surface of a cell and AFM images were taken each 5 min. The arrows show the slow propagation of a wave-like structure from upper-right to lower-left.

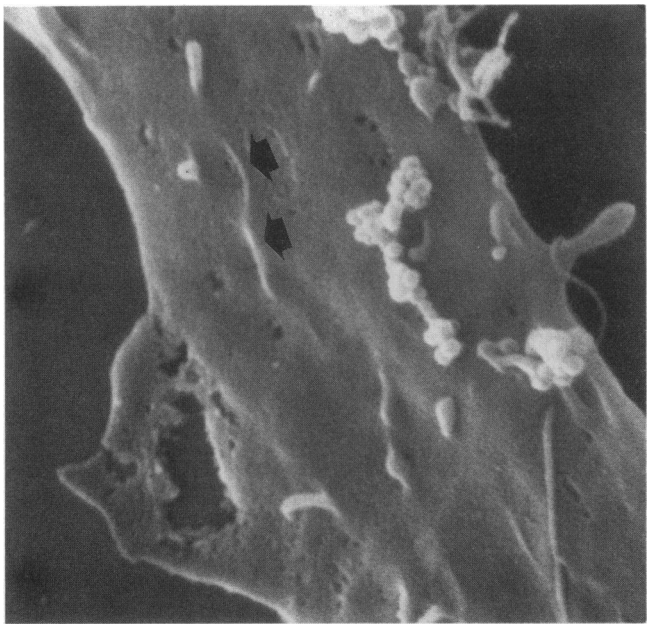


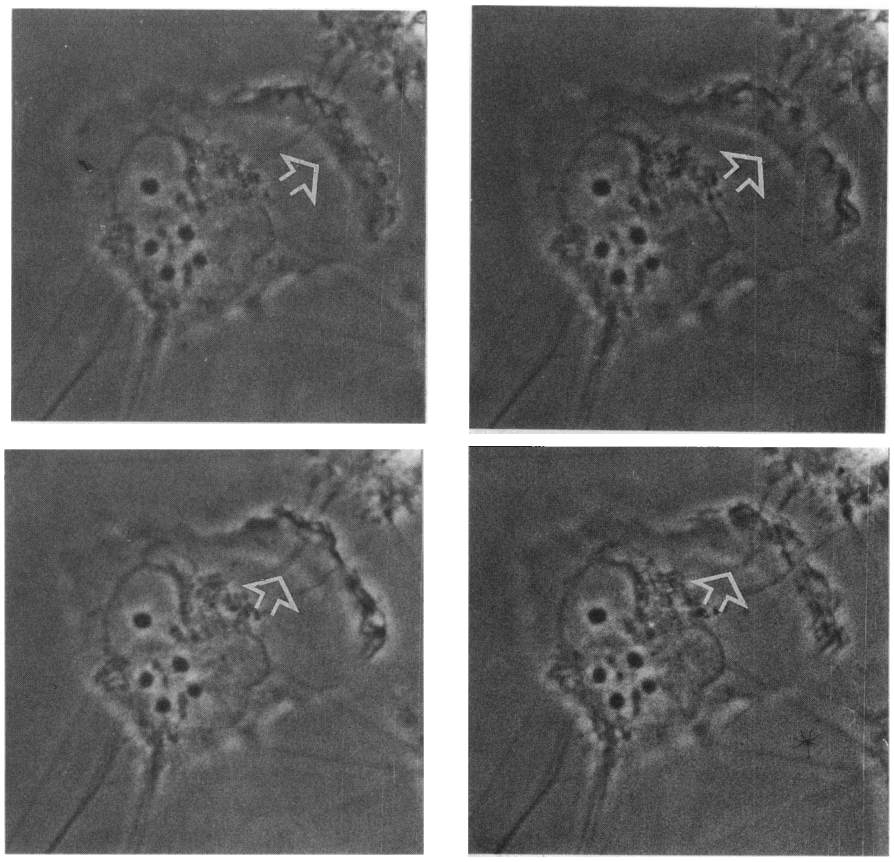
FIGURE 8 This scanning electron microscope image of the same cell line as Figs. 4–7 also shows the presence of wave structures on the cell surface.

Examination of the flattest parts of the cells was more successful. Scanning with the AFM over the same area revealed changes over a few minutes. Wave-like structures were seen crossing the cell surface. The Figs. 4 to 7 show the propagation of such waves (*arrow*). These structures were also present during the scanning electron microscope examination. The arrow on Fig. 8 shows one of them. The microcinematographic film shows the presence of such waves and demonstrates their dynamic behavior. Figs. 9 to 12 represent the slow displacement of a clearly defined structure which could correspond to the features previously observed with the AFM. However, we can't exclude the possibility that the AFM tip, by deforming the surface, became sensitive to structures moving under the membrane.

Fig. 13 shows an entire cell and the displacement of a peaked structure on its surface.

The exact identification of all the dynamical structures observed with the AFM on this cell line is difficult for the moment. We hope that in the close future we will be able to give precise responses concerning the identification of these structures.

From our experience, we think that the observation of unfixed cells is possible with the actual generation of



FIGURES 9 to 12 (viewed from *top left* to *bottom left*, in a clockwise direction) Microcinematographic images taken each 15 s show the presence of numerous lamellipods at the upper-right side of the cell. The arrow points to a white, slow moving wave.

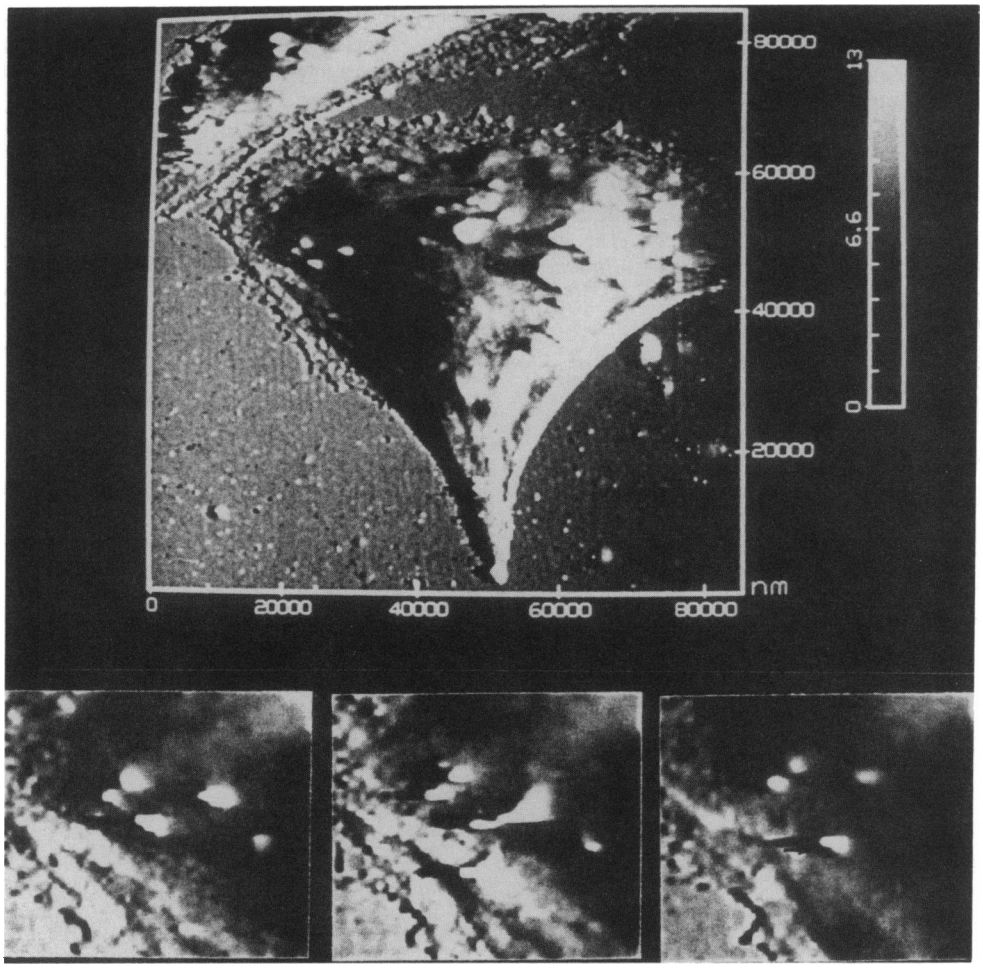


FIGURE 13 AFM image of a cell. Note the displacements of a peaked structure during 9 min (*arrows*).

AFM. The technique presented allows observation of dynamic changes of the cell surface with a minimum of preparation and a minimum of traumatism for the cells.

Financial support was provided by the Sandoz and Roche Foundations.

Received for publication and in final form 22 June 1992.

REFERENCES

1. Binnig, G., C. F. Quate, and Ch. Gerber. 1986. Atomic force microscope. *Phys. Rev. Lett.* 12:930–933.
2. Wickramasinghe, H. K. 1989. Scanned-probe microscopes. *Sci. Am.* 260:98–105.
3. Rugar, D., and P. K. Hansma. 1990. Atomic force microscopy. *Physics Today.* 43:23–30.
4. Binnig, G., Ch. Gerber, E. Stoll, T. R. Albrecht, and C. F. Quate. 1987. Atomic resolution with Atomic Force Microscope. *Europhys. Lett.* 3:1281–1286.
5. Albrecht, T. R., and C. F. Quate. 1987. Atomic resolution imaging of a nonconductor by atomic force microscopy. *J. Appl. Phys.* 62:2599–2602.
6. Gould, S. A. C., O. Marti, B. Drake, L. Hellemans, C. E. Bracker, P. K. Hansma, N. L. Keder, M. M. Eddy, and G. D. Stucky. 1988. Molecular resolution images of amino acid crystals with the atomic force microscope. *Nature (Lond.).* 332:332–334.
7. Drake, B., C. B. Prater, A. L. Weisenhorn, S. A. C. Gould, T. R. Albrecht, C. F. Quate, D. S. Cannell, H. G. Hansma, and P. K. Hansma. 1989. Imaging crystals, polymers and processes in water with the Atomic Force Microscope. *Science (Wash. DC).* 243:1586–1589.
8. Worcester, D. L., H. S. Kim, R. G. Miller, and P. J. Bryant. 1990. Imaging bacteriorhodopsin lattices in purple membranes with atomic force microscopy. *J. Vac. Sci. Technol.* 8:403–405.
9. Kasas, S. 1992. La microscopie à force atomique dans la recherche en biologie. *Médecine/Sciences.* 8:140–148.
10. Weisenhorn, A. L., H. E. Gaub, H. G. Hansma, R. L. Sinsheimer, G. L. Kelderman, and P. K. Hansma. 1990. Imaging single-stranded DNA, antibody-antigen reaction and polymerized Langmuir-Blodgett films with an Atomic Force Microscope. *Scanning Microscopy.* 4:511–516.
11. Marti, O., H. O. Ribi, B. Drake, T. R. Albrecht, C. F. Quate, and

P. K. Hansma. 1988. Atomic force microscopy of an organic monolayer. *Science (Wash. DC)*. 239:50–52.

12. Zenhausern, F., M. Adrian, R. Emch, M. Jobin, M. Taborelli, and P. Descouts. 1991. Scanning force microscopy on cryoelectron microscopy of tobacco mozaic virus as a test specimen. *Ultramicroscopy*. 42–44:1168–1172.
13. Gould, S. A. C., B. Drake, C. B. Prater, A. L. Weisenhorn, S. Manne, G. L. Kelderman, H.-J. Butt, H. G. Hansma, P. K. Hansma, S. Magonov, and H.-J. Cantow. 1990. The atomic force microscope: a tool for science and industry. *Ultramicroscopy*. 33:93–98.
14. Butt, H.-J., E. K. Wolff, S. A. C. Gould, B. Dixon Northern, C. M. Peterson, and P. K. Hansma. 1990. Imaging cells with the atomic force microscope. *J. Struct. Biol.* 105:54–61.
15. Weisenhorn, A. L., P. K. Hansma, T. R. Albrecht, and C. F. Quate. 1989. Forces in atomic force microscopy in air and water. *Appl. Phys. Lett.* 54:2651–2653.
16. Häberle, W., J. K. H. Hörber, F. Ohnesorge, D. P. E. Smith, and G. Binnig. 1992. In situ investigation of single living cells infected by viruses. *Ultramicroscopy*. 42–44:1161–1167.