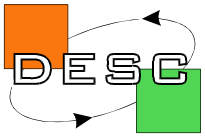
ATOMIC FORCE MICROSCOPY AS TOOL IN CELL BIOLOGICAL RESEARCH FOR GROUND BASED AND IN-FLIGHT STUDIES



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INTRODUCTION

From previous investigations it has become clear that cells behave differently under conditions of hypergravity (centrifuges), simulated hypogravity (clinostats, Random Positioning Machine, Free Fall Machine) and spaceflight compared to their appropriate 1×g controls. Changes in gene expression, signal transduction, energy consumption or general cell differentiation are measured using (bio-) chemical assays. Morphological or (bio-) mechanical changes in cells such as general cell shape, intracellular architecture (cytoskeleton, location / shape of cell organelles), cell-cell interactions, or cell motility require imaging techniques such as light microscopic or transmission electron microscopy (TEM).

Since gravity acts on mass, it might be expected that changes in cells due to (micro-) gravity are due to intracellular mass displacements and / or changes in general cell shape. Both processes involve the cytoskeleton and this might be a focal point for future gravity studies both on ground as well as for the international space station.

For light microscopic observations the more advanced Confocal Laser Scanning Microscope (CLSM) has been used to s tudy intracellular static or dynamic processes. Most of these studies still require some way of chemical fixation. For the CLSM a sample has to be stained with a fluorescent probe and may then be studied in a three-dimensional way by reconstructing a series of optical sections.

In recent years the Atomic Force Microscope (AFM) has become available to study biological samples. Although both systems have their particularities, the AFM has some advantages over a CLSM. The AFM is a very compact system and provides high spatial resolutions as well as the possibility to visualize living cells *in vitro*

POSSIBLE APPLICATIONS OF AFM

Although the AFM (in tapping mode) is generally used to visualize the surface structure of (living) biological samples [1,2,5], also fragile crystals [3], inorganic materials, or molecular processes [6] can be studied using an AFM.

In recent studies dynamic interactions between individual molecules have been reported. In these studies the topographic AFM image is supplemented by a binding specific image that displays the distribution of molecular recognition sites down to the level of individualmolecules. [5]

Since the cytoskeleton is of major interest in gravitational biology it is very interesti ng to note the work of Putman et al. [2], in which the authors report on the visualization of cytoskeleton elements directly lined by the cell membrane.

Very recent studies are undertaken in which the AFM is used to visualize DNA repair processes. [4] In this system the dynamic in situ repair of isolated UV damaged DNA by photolyase is visualized. Although these experiments are very premature, it might be possible that in future studies repair processes in DNA after cosmic radiation damagemaybestudiedinmoredetail.

1: Putman, C.A.J., van Leeuwen, A.M., de Grooth, B.G., Radosevic, K., van der Werf, K.O., van Hulst, N.F., Greve, J. (1 993) Atomic force microscopy combined with confocal laser scanningmicroscopy: a new look at cells. Bioimaging, 1, 63-70.

2: Putman, C.A.J., van der Werf, K.O., de Grooth, B.G., van Hulst, N.F., Greve, J. (1994). Viscoelasticity of living c ells allows high resolution imaging by tappingmodeatomicforce microscopy. Biophysical J., 67, 1749-1753.

3: Kuznetsov, Y.G., Malkin, A.J., Land, T.A., Deyoreo, J.J., Barba, A.P., Konnert, J., McPherson, A. (1997) Molecul ar reso lution imaging ofmacromolecular crystals by atomic forcemicroscopy. Biophys. J., 72, 2357-2364.

4: Van Noort, S.J.T., van der Werf, K.O., Eker, A.P.M., Wyman, C., de Grooth, B.G., van Hulst, N.F., Greve, J. Direct vi sualization of dynamic protein-DNA interaction with a dedicated atomic forcemicroscope. Biophys. J., 74, 2840-2849.

5: Dammer, U., Popescu, O., Guntherodt, H.-J. (1995) Binding strength between cell adhesion proteoglycans measure d by AFM. Science, 267, 1173-1175.

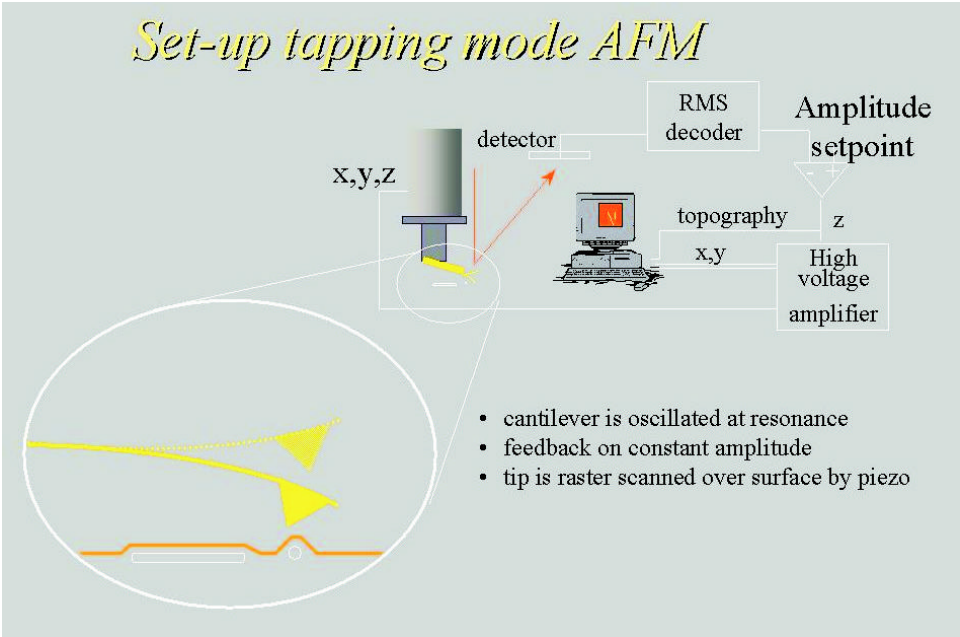
6: Moy, V.T., Florin, E.L., Gaub, H.E. (1994) Intermolecular forces and energies between ligands and receptors. Science, 266, 257-259.

AFM WORKING PRINCIPLE

Although the name may be somewhat deceptive, the imaging of a sample by AFM involves hardly any optics.
The working principle of an AFM is based on the deflection of a very sensitive cantilever due to repulsive forces between atoms on the sample surface and atoms at the cantilever tip. This deflection is measured using a laser beam while the sample is scanned. The scanning in x, y and z position is performed by a piezo-electric translator. (See also Figure)

The computer subsystem controls the xyz translations and records the reflected laser beam signal. Dedicated software reconstructs these data into a topographic picture of the sample.

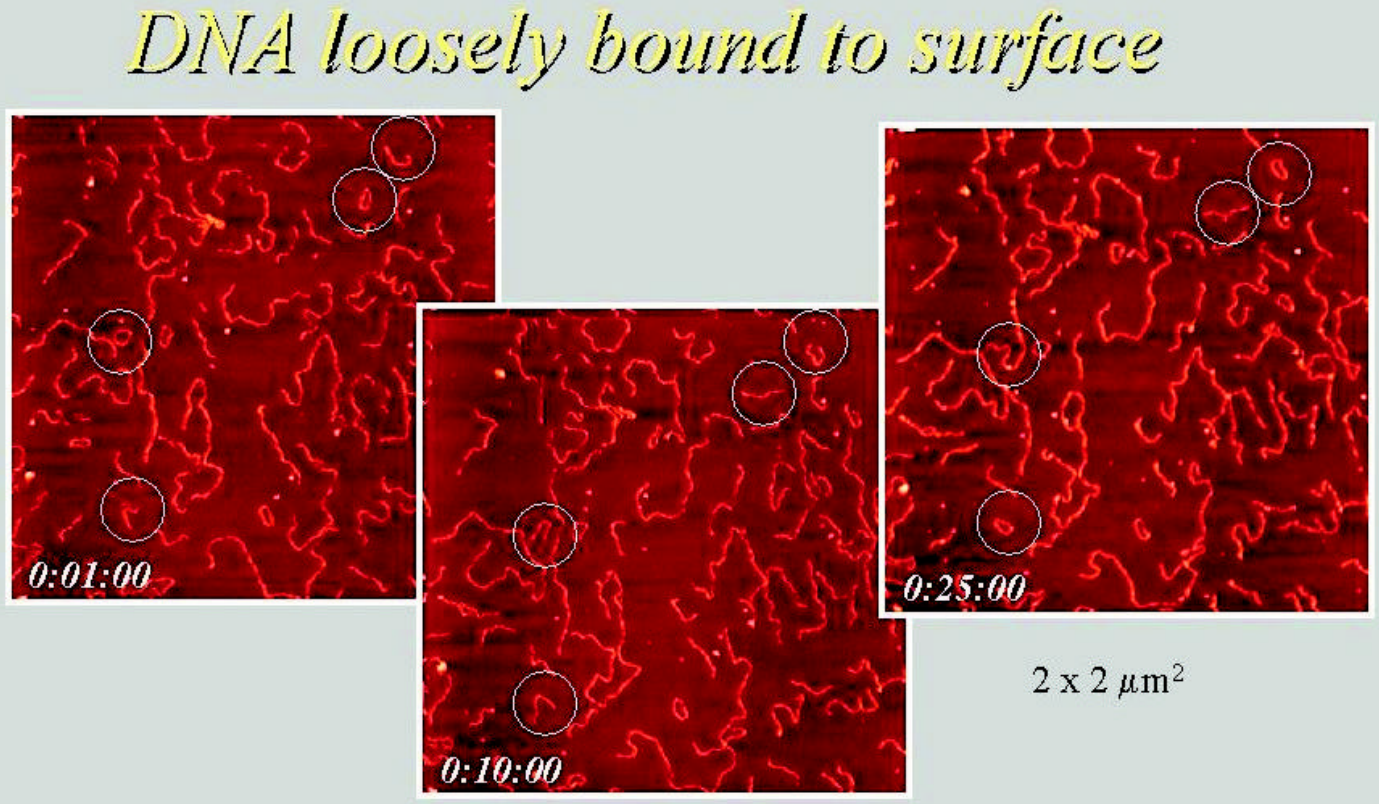
Since the onset of this type of microscopy starting with the 1987 Nobel Prize winning Scanning Tunneling Microscope by Binning and Rohrer, solely the surfaces of inorganic materials could be visualized. After improvement of the various scanning techniques, it was only with the development of more gentle surface scanning methods, such as 'tapping mode' operations that the AFM became useful for the research of living cells.



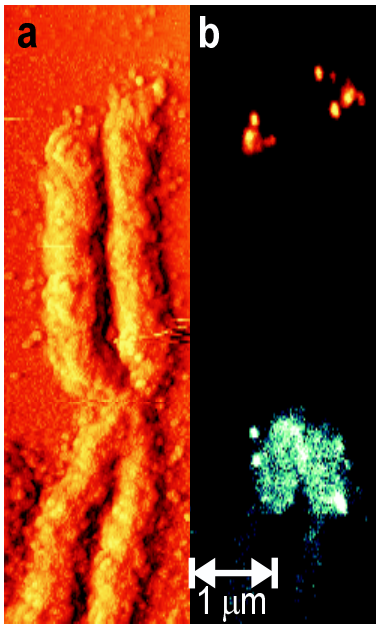
	AFM	CLSM
Sample prep.	No	Yes
System Complexity	+	++
Resolution: Z (nm) XY	0.1 < 25	800 300
Size (cm^3)*	head: < 750 e-box: ~15000	> 70000 > 50000
Mass (g)*	head: < 200 e-box: ~ 4000	> 15000 > 15000
Power (W)*	<= 300	> 1000

*Values without computer subsystem.

IN SITU DNA REPAIR PROCESSES



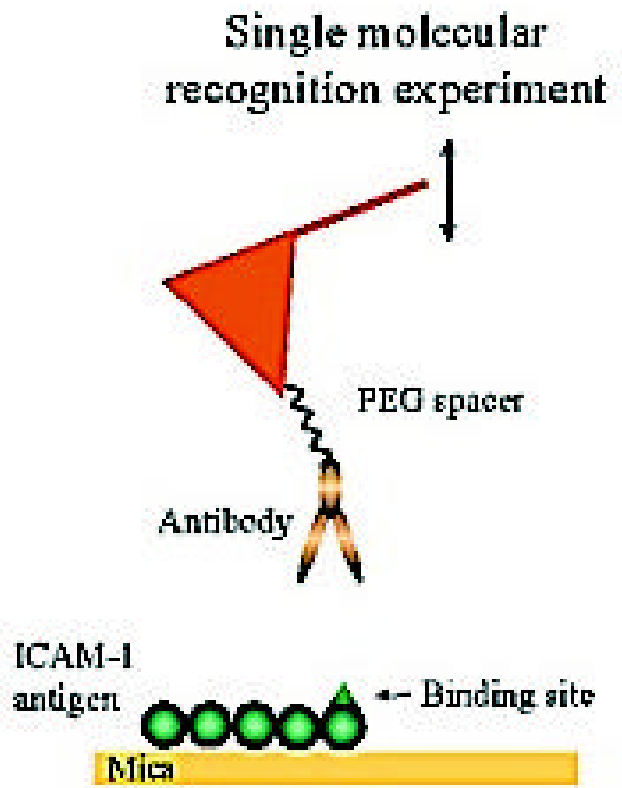
John van Noort, BioPhysical Journal **74** (1998) 2840-2849

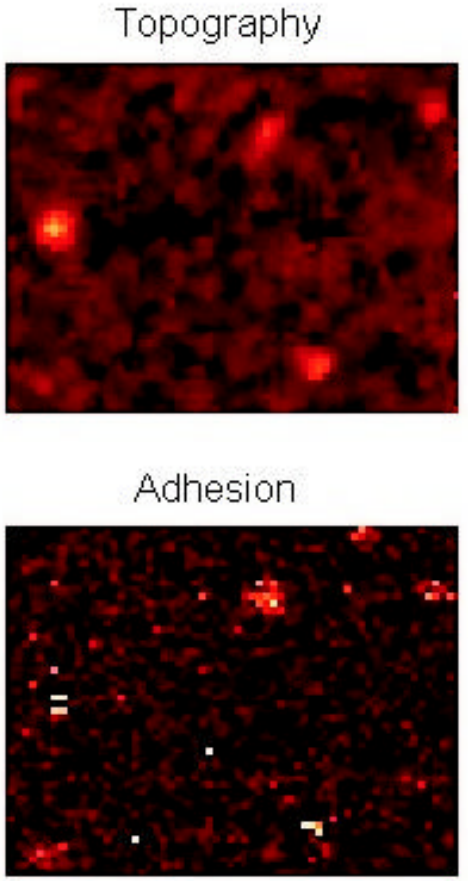


Topography (AFM) and Fish label (near field optics)

Very recent studies are undertaken in which the AFM is used to visualize DNA repair processes. In this system the dynamic *in situ* repair of isolated UV damaged DNA by photolyase is visualized. Although these experiments are very premature, it might be possible that in future studies repair processes in DNA after cosmic radiation damage may be studied in more detail.

DYNAMICS OF INDIVIDUAL MOLECULES





Data: Oscar Willemsen et.al.

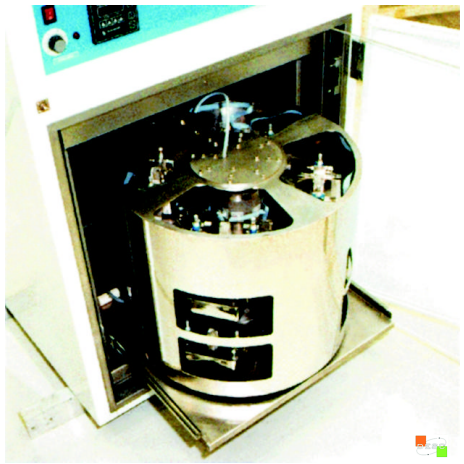
In recent studies dynamic interactions between individual molecules have been reported. In these studies the topographic AFM image is supplemented by a binding specific image that displays the distribution of molecular recognition sites down to the level of individualmolecules.

‘SURFACE’ TOPOGRAPHY OF A LIVING CELL

Since the cytoskeleton is of major interest in gravitational biology it is very interesting to note the work at the University of Twente. They are able to visualise cytoskeleton elements directly lined by the cell membrane in a living cell.

STATUS

The results as presented in this paper are gained from the ongoing research at the Applied Optics Group at University of Twente.
For futue studies a modified AFM instrument will be accommodated in a cell / tissue culture centrifuge (MidiCAR) to investigate cell behaviour at hypergravaity conditions.



MidiCAR cell / tissue culture centrifuge

ACKNOWLEDGEMENTS

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