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

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1. 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)(1) 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 study 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*. (See also Table-I)

2. THE ATOMIC FORCE MICROSCOPE

Detailed cell surface structures are in general resolved using a Scanning Electron Microscope (SEM). This technique requires the arrest of cellular processes and structures using chemical or physical fixation protocols in combination with thin layers of conductive metals. However, imaging cell surface structures using an atomic force microscope (AFM) requires neither chemical nor physical treatment of the samples. Cellular structures of vital cells can be investigated *in situ* and dynamic changes due to *e.g.* gravity may be visualized in living cells.

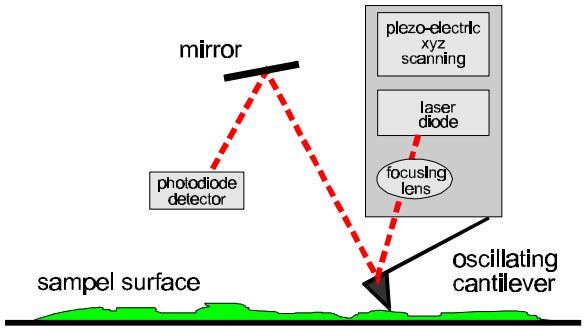

Figure 1: Schematic layout of the main components in an Atomic Force Microscope.

2.1 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 1)

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 [3] that the AFM became useful for the research of living cells.

2.2 Possible Applications

Although the AFM (in tapping mode) is generally used to visualize the surface structure of (living) biological samples [2,3,6], also fragile crystals [4], inorganic materials, or molecular processes [7] 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 individual molecules.[6]

Since the cytoskeleton is of major interest in gravitational biology it is very interesting to note the work of Putman et al. [3], 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.[5] In this system the dynamic *in situ* repair of isolated UV damaged DNA by photolyase is visualized. Although these experiments are very premature (personal communication dr. C. Wyman of the Erasmus University in Rotterdam) it might be possible that in future studies repair processes in DNA after cosmic radiation damage may be studied in more detail.

	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.*

Table I: Differences between Atomic Force Microscopy (AFM) and Confocal Laser Scanning Microscopy (CLSM).

There are also recent developments in which an AFM and a CLSM are combined within one system.[2]

3 DISCUSSION

The Atomic Force Microscope has evolved rapidly in recent years. Although similar microscopes are widely used in material sciences and quality control processes, it may be expected that, in the near future, this kind of imaging technique, in combination with *e.g.* its ability to measure molecular binding forces, will increase popularity in biological research.

Especially in gravitational biology where the study of cell shape and the cell cytoskeleton gains increasing attention, a stand-alone AFM or combined with a CLSM is a very suitable instrument to be used for this research.

A final outcome could be the development of a space qualified AFM to be used for numerous studies planned for the International Space Station. However, before entering this stage, the application of an AFM (and CLSM) for gravitational biological studies must first be demonstrated and used in ground based experiment.

The Dutch Experiment Support Center, DESC, in close collaboration with the University of Twente and various cell biologists, is planning to perform a pilot study in which an AFM will be used to investigate possible cell shape changes due to hypergravity.

4 REFERENCES

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