POTENTIAL APPLICATIONS OF THE AFM (ATOMIC FORCE MICROSCOPY) IN COSMETOLOGY.

B. Berra, G. Poletti, A. Pozzi, S. Zava
Institute of General Physiology and Biochemistry, University of Milan.

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Summary

The Atomic Force Microscope (AFM) is able to image the surface properties of all types of materials (conductors, insulators, biological and vegetal samples) with a very high resolution and in the native state.
In particular, AFM is used to study a number of biologic system such as DNA-replication and protein/ protein, DNA/protein interactions; furthermore, in cosmetology, it's suitable to study the fundamental characteristics of the skin, like elasticity and wrinkledness, and to compare for example morphological properties of human virgin hair and chemical or polymer treated hair.
The most important characteristics of this instrument in cosmetological analyses are:
• the samples can be directely examined without previus treatment, i.e. in native conditions,
• data are collect in a digital form,
• 3-D image rendering with variable magnification and shading,
• view of the recorded image from any point of the space.
In our laboratory we analyse numerous samples of cosmetic interest and in particular human hair and replica of human skin. In this short paper we present preliminary data on human vergin hairs.

Riassunto

Il Microscopio a Forza Atomica (AFM) è particolarmente indicato nello studio delle proprietà superficiali di materiali di ogni tipo (conduttori, isolanti, materiali biologici, vegetali) con risoluzioni mai raggiunte fino ad ora e senza ricorrere alla necessità di effettuare una complicata preparazione del campione.
In biologia l'AFM può misurare le forze intermolecolari che governano processi biologici quali la replicazione del DNA, le interazioni tra proteine e proteine e tra DNA e proteine; in cosmetica in particolare è in grado di sondare caratteristiche fondamentali della pelle, come l'elasticità e la rugosità, e di mettere a confronto la morfologia di un capello umano vergine e di un campione trattato chimicamente o con polimeri.
Tra le caratteristiche fondamentali che rendono l'AFM uno strumento all'avanguardia per rilevare l'efficacia di un prodotto cosmetico, possiamo enumerare:
• la superficie dei campioni può essere esaminata direttamente senza la necessità di preparare il

campione
- i dati possono essere immagazzinati in forma digitale
- l'immagine può essere ricostruita in tre dimensioni
- l'immagine tridimensionale può essere osservata da differenti angolazioni.

Nel nostro laboratorio è stata messa a punto l'analisi di molti campioni di interesse cosmetico, tra i quali i capelli o la replica di una zona ben individuata della pelle. In questo lavoro breve riportiamo i dati preliminari ottenuti analizzando campioni di capelli non trattati.

INTRODUCTION

An Atomic Force Microscope (AFM) (1,2) is able to image biological molecules and living cells in native conditions at very high resolution. Until recently, biologists have had to use SEM and TEM vacuum technologies to image surface of samples and this prevented in vivo analysis since in general biological materials cannot withstand prolonged vacuum. In addition since SEM and TEM operate only on conducting samples, the surfaces of the samples had to be coated with metals prior to imaging. Unlike traditional microscopes, AFM forms topographic images by detecting the deflection of a sharp-tipped cantilever as it is scanned across the sample surface. Biological samples can be imaged (3-6) fixed and mounted on mica or glass or unfixed in a liquid or gaseous environment without additional preparation. The AFM collects the data in a digital form, in software allows to reconstruct the 3-D surface structure on a computer screen and to calculate and display the main parameters generally used to characterise any rough real surface.

AFM TECHNIQUES

In an AFM the tip is mounted on the end of a flexible cantilever. As the sample is scanned beneath the tip, small forces of interaction with the sample cause the cantilever to deflect, enabling to record the sample surface topography. The AFM can be operated in three different modes: Contact Mode, Non-Contact Mode and Tapping Mode.

In Contact Mode the tip is maintained in contact with the sample during measurements and slides over the surface as the sample is scanned.

In Non-Contact Mode the tip is forced to oscillate at high frequency (100 kilohertz to 1 megahertz) a few nanometers above the surface. Using this technique the sensitivity of the microscope results to be greatly increased so that even weak, long-range forces as attractive van der Waals forces and electrostatic forces can be detected. During scanning, the topography of the surface is tracked by detecting the effect of these forces on the amplitude and/or the frequency of the cantilever oscillation. It is therefore possible to image even the softest sample without damage.

In Tapping Mode the cantilever is oscillated with larger amplitude as compared with the Non Contact Mode, and the tip is allowed to make transient contact with the sample at the bottom of its swing. The Tapping Mode is a compromise between the Contact and the Non Contact Mode so that the resolution is usually almost as good as in Contact Mode, but the damage caused by shear forces is almost completely eliminated since the contact tip-sample is extremely brief.

BIOLOGICAL APPLICATIONS

- DNA protein and chromatin structure;
- Enzyme/substrate interactions;
- Protein/protein, protein/DNA interactions;
- Adsorption properties of cells and molecules to biological or other surfaces;
- Recognition of cell surface antigens;
- Visualisation of antigen/antibody complexes;
- Studies on cell morphology and motility;
- Evaluation of dynamic variation in chemical and mechanical properties of biological materials;
- Synaptic release and signal transduction processes;
- Structural characterisation of planar membranes and liposomes;
- Surface analysis of virus and batteria;
- Particle interactions;
- Evaluation of the performances of biomaterials (e.g., titanium implants, biomedical plastics).

CONTACT AFM

The Contact Mode usually produces stable, high-resolution images, but compression and shear forces generated between the tip and the surface may cause damage. This possibility can be especially troublesome when imaging biomolecules, which are almost always soft and only weakly attached to the substrate.

NON-CONTACT AFM

Non-contact AFM imaging produces high resolution characterisation of surface topography on soft samples using only the lightest interaction forces. Non Contact AFM can even avoid sample and tip damages on surfaces such as protein or nucleic acid complexes adsorbed to a flat substrate or sample. In biological applications NC-AFM is desirable because it provides a means for measuring sample topography with no contact between the tip and the sample. The total force between the tip and the sample in this regime is very low, generally about 10^{-12} N. A further advantage is that both biological samples and tips are not contaminated or degraded due to tbe contact with the tip.

AFM SOFTWARE CHARACTERISTICS

AFM instruments offer a complete imaging processing package and a control language for automated data acquisition and very complete image processing. It includes:

- Line and region analysis of the data;
- Direct measurement of the distances between relevant points of the image;
- Processing and analysis of tbe data to get the surface parameters;
- Exportation of the data files to allow further processing;
- Zoom software;
- Multi image presentation;
- 3-D image rendering with variable magnification and shading;
- View of the recorded image from any point of the space.

REGION ANALYSIS WITH AFM

In Region Analysis only the data pertaining to selected region of the recorded image are taken into consideration for processing. This allows to include/excluded selected regions of the recorded image (possibly even multiple inclusions/exclusions) in the processed data. Therefore all the parameters characterising the wanted involved regions can be evaluated.

SINGLE LINE ANALYSIS WITH AFM

In Single Line Analysis only the data pertaining to a selected cross section of the image (height profile given in different colours) are taken into consideration for processing.

Quantitative measurements of surface features by moving cursors along the line and collection of the surface parameters are possible.

RESULTS, DISCUSSION AND CONCLUSION

In this short paper preliminary data obtained with AFM on vergin hairs and some examples of the images and analyses of the samples produced in our laboratory are presented.

To our knowledge few and scattered data are present in the literature on the use of AFM in cosmetic and in particular on hair analysis (7,8). We believe that from our preliminary results it appears very clear the potential of this tecnique to test cosmetic products and to verify their efficacy.

We are presently analysing more samples and we hope in the near future to present data for comparison between vergin and treated hairs.

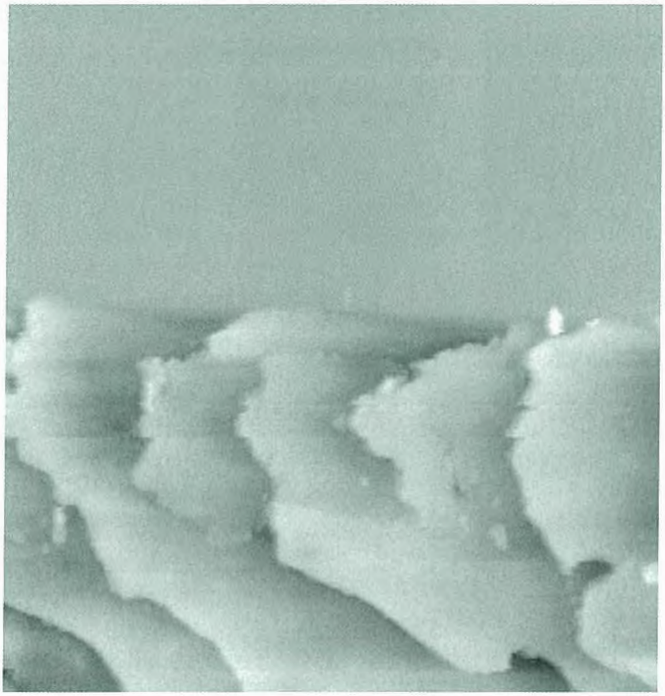


Figure 1: Non-contact AFM image of an human hair (30 μm)

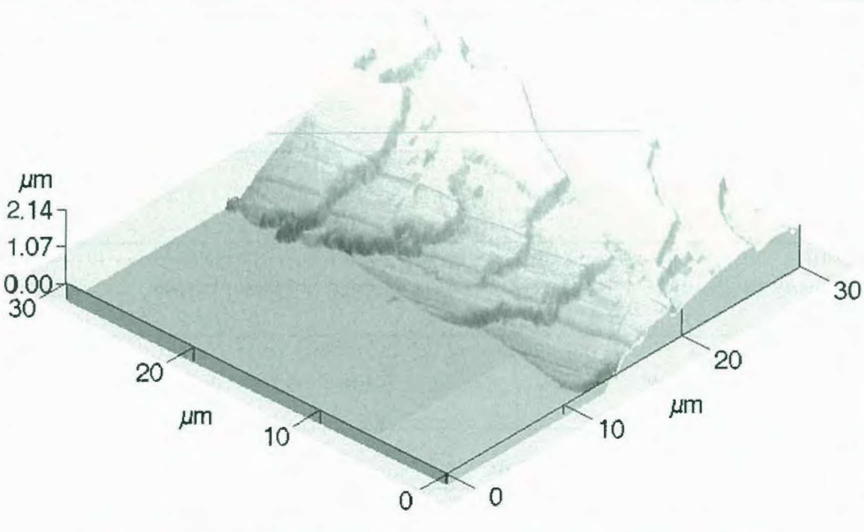
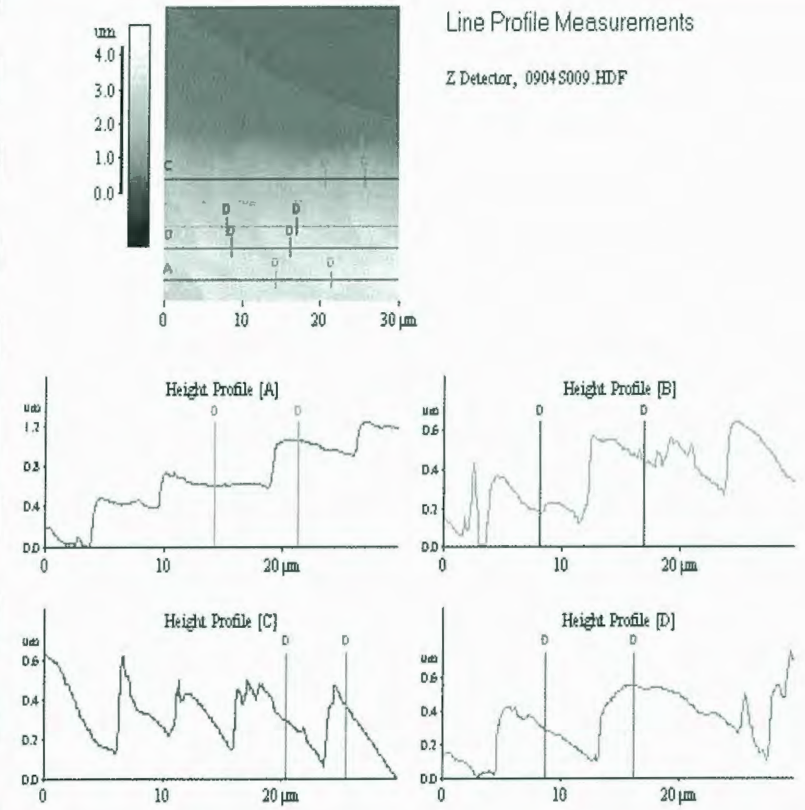


Figure 2: 3-D Non-contact AFM image of an human hair (30 μm)



Line	Height	Distance	Angle	Rp-v	Rms	Ave	Mean Ht	Median Ht	Arc length	Bearing ratio
[A]	0: 0.452 um	0: 7.06 um	0: 3.7°	1.23 um	0.340 um	0.277 um	3.73 um	3.68 um	30.5 um	@30.0 % 0.943 um
[B]	0: 0.258 um	0: 8.82 um	0: 1.7°	0.644 um	0.166 um	0.142 um	3.24 um	3.24 um	30.7 um	@30.0 % 0.489 um
[C]	0: 783 Å	0: 5.06 um	0: 0.9°	0.630 um	0.137 um	0.111 um	2.09 um	2.09 um	30.6 um	@30.0 % 0.394 um
[D]	0: 0.256 um	0: 7.53 um	0: 1.9°	0.738 um	0.170 um	0.144 um	3.48 um	3.51 um	30.5 um	@30.0 % 0.449 um

Figure 3: Single line profile measurements.

Figure 4: Non-contact AFM image of an hair's detail (10 mm).

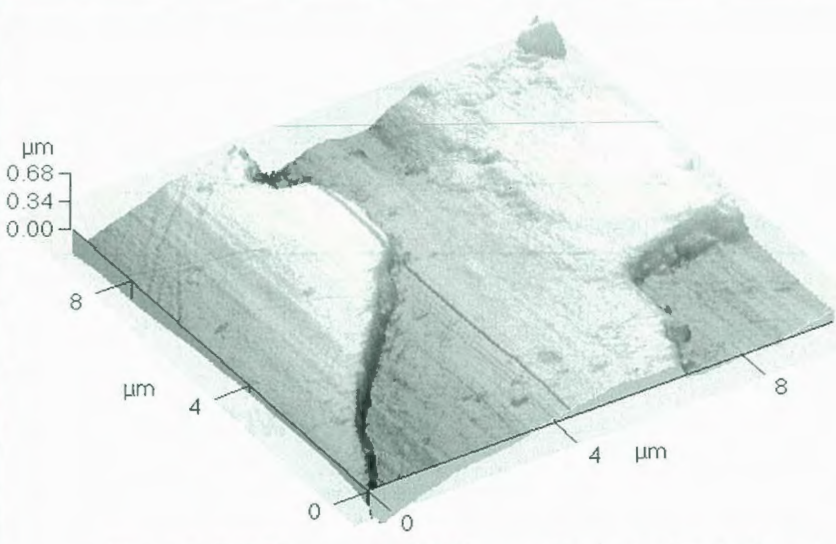
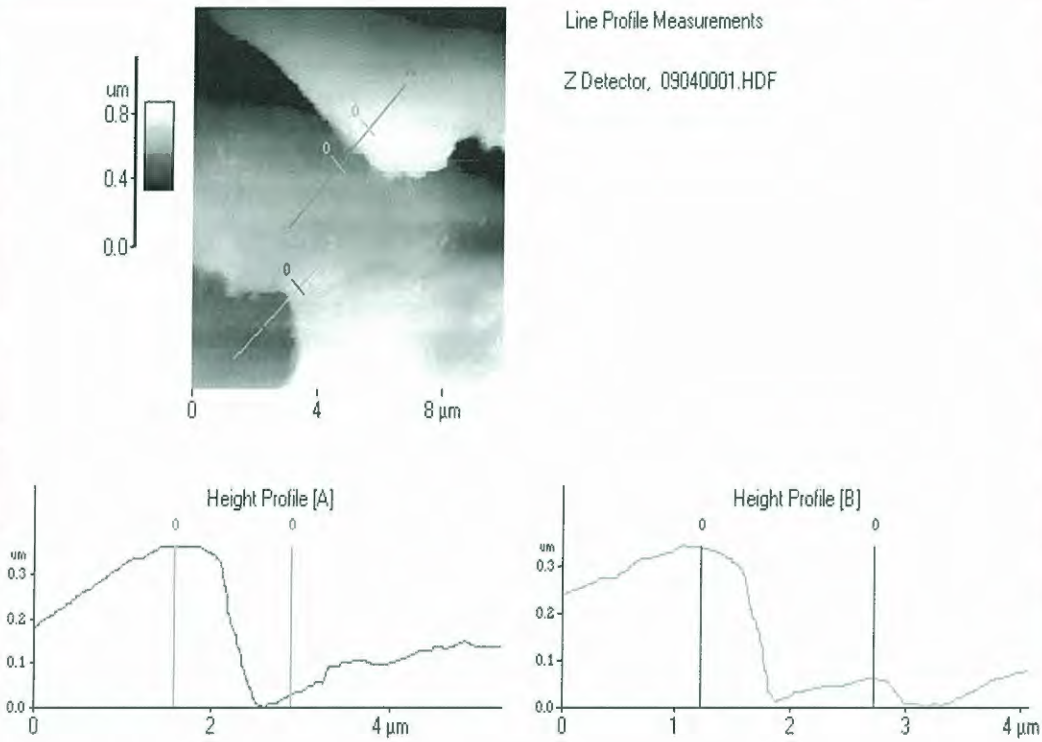


Figure 5: 3-D Non-contact AFM image of an hair's detail (10 mm).



Line	Height	Distance	Angle
[A]	0: 0.337 um	0: 1.30 um	0: 14.5°
[B]	0: 0.276 um	0: 1.47 um	0: 10.6°

Figure 6: Single line profile measurements.

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Author Address:

Prof. B. Berra,
Institute of General Physiology
and Biochemistry, University of Milan,
Via Trentacoste 2, 20134 Milan, Italy
Tel. 0039 02 58355777
Fax. 0039 02 58355813
e-mail: bruno.berra@unimi.it