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UV radiation: Aggressive agent to the hair—AFM, a new methodology of evaluation

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Synopsis

A new method for morphological hair analysis at high resolution and under ambient conditions is presented in this paper. The AFM has been used in these experiments to analyze morphological changes in hair roughness and thickness after UV radiation. Through the powerful analytical AFM tools, changes in hair morphology can be proven. A new quantitative methodology to evaluate hair structure is presented in this paper.

INTRODUCTION

Constant investigations into technological development of hair care products that exceed consumers' expectance have long represented a challenge for researchers and scientists. Many investigations have been carried out to develop effective cosmetic products for human hair, and scientists have employed a variety of methods in their attempts to understand the alterations and to prove the cosmetic efficacy of the products.

Recent reports on the harmful effects of sunlight on human skin have raised the awareness of the deleterious effect of sunlight on biological tissues in general. Hair can also suffer photo degradation (1), which can contribute significantly to overall hair damage (2). The influence of sunlight on untreated human hair has been extensively analyzed by examination of the protein, melanin, and lipid components as well as the morphological areas on irradiated hair (3). These studies have demonstrated that the physical and chemical modifications of hair are marked by intrinsic patterns of damage correlated to its pigmentation and to the amount and intensity of sunlight to which it has been exposed. UV-A and the visible part of sunlight (VIS) damage hair significantly; however, hair's properties are also influenced to a minor extent by UV-B and IR irradiation. Black hair is largely protected from damage by the pigment eumelanin. In contrast, blonde hair is detectably photochemically damaged due to its low pigment content or pigmentation with pheomelanin.

Hair damage causes a reduction in tensile and fatigue properties (3). According to Robbins and Bahl (4) and Speakman and McMahon (5), the reduction is largely attributed to chemical damage to the protein chains, especially the S-S covalent crosslinks in cystine. They have found that photochemical damage, the loss of crosslinks, leads to an increase in solvent swelling, a finding not shared by Wolfram (6), who argues that crosslinking between amino acid residues is induced by irradiation and that these new crosslinks would limit the solvent swelling in the case of keratin fibers.

According to the hair photolysis mechanism proposed by Tolgyesi (1), cystine, tyrosine, phenylalanine, and tryptophan residues absorb UV radiation, resulting in the formation of free radicals. Homolytic scission of disulfide bonds occurs. Melanin, the natural coloring matter in hair, provides partial protection to the hair fiber. It acts in a sacrificial manner, resulting in lightening of hair color. Tolgyesi explained that melanin, by its comprehensive system of double bonds and conjugated carbonyl groups, protects hair by scavenging free radicals generated by exposure to light. Wei (7) furthered the concept of the free-radical mechanism, showing that, all other factors being equal, the extent of photodamage is proportional to the square root of the amount of free-radical initiators present in the fiber. The mechanism assumes that the S-S group is the initiator, giving two –S free radicals by homolytic scission.

A study using field emission scanning electron microscopy (FESEM) was carried out by Ruetsch *et al.* (8) to monitor the effects of UV irradiation on the physical nature of hair fibers. Long-term UV irradiation-humidification cycling causes thinning and fusion of the surface cuticle cell, as well as fusion of the cuticular sheath into a solid, rigid, and brittle unit.

Swift and Smith (9) reported the advantages of the use of AFM, such as the greater details in the hair's cuticular surfaces that appear not to be as smooth as had been previously supposed when evaluated by SEM. They observed that the surface of the hair gradually changes as a function of its length, measured from the hair root. This technique and the effects of subsequent damage are herein discussed.

The study reported in this paper verified these initial findings on the damage of human hair exposed to sunlight, and also determined the photochemical damages associated with the physical morphological changes in hair structure. Scanning electronic microscopy (SEM) analyzed the damage of UV radiation on hair fibers. This technique was employed to observe the damage induced on hair fibers by UV radiation. The use of atomic force microscopy (AFM) provides a new understanding of the mechanism of UV radiation damage to hair by physical quantification of the nature and damage of hair cuticle as roughness and thinning.

MATERIALS AND METHODS

MATERIALS

Untreated black Caucasian hair was obtained from De Meo Bros., New York. Hair samples were pre-treated using 3% LAS (lauryl ammonium sulfate).

IRRADIATION OF HAIR

Hair tresses weighing 2.0 g were exposed to simulated solar radiation in the Atlas Weather-Ometer, model 65 XW-WR1. It utilized a 6500W xenon lamp. The specific exposure condition of hair fibers to UV/visible radiation was 160 hours. The programming used was 102 minutes of insolation at relative humidity (RH) levels of 50 + 5% and 18 minutes of insolation with rain simulation with 100% RH. The mean temperature in a cycle of two hours was 60°C. The energy density at the 340-nm wavelength was maintained at 0.35 W/m^2. In the wavelength range of 250–800 nm, the energy densities were 397 W/m^2, resulting in a total energy density of 41.27 mW/cm^2.

TESTING

Two different techniques were employed to assess hair photodamage: (1) the scanning electronic microscopy (SEM) technique was used to identify morphological changes on the hair surface; and (2) the atomic force microscopy (AFM) technique was used to analyze hair morphology as the environmental conditions changed, allowing the direct study of roughness and cuticle thickness.

SCANNING ELECTRON MICROSCOPY (SEM)

SEM has been widely used over many years for claim substantiation of various toiletry treatments on the architecture of the hair's surface. It has been used to evaluate the effectiveness of hair shampoos in the removal of sebum and detritus, to investigate the effects of permanent waving systems, and to observe the deposition of polymeric materials from hair products. Although SEM analyses provide valuable information about the hair surface, which helps to understand some of the existing hair products and aids in the development of new ones, it should not be seen as a key technique to understand all product systems. Its use is limited by many factors because pictures produced by SEM usually cause a high visual impact, but the technique is poor in terms of quantitative analysis.

Longitudinal segments of untreated and UV-exposed fibers were mounted on an aluminum substrate and coated with an approximately 90 Å thickness of gold. The hair fiber topography was examined in a ZEISS 940-A spectrometer digital scanning electron microscope coupled to EDS and PGT, Digital Prism model. The objective was to analyze the morphological alterations in hair exposed to UV radiation.

ATOMIC FORCE MICROSCOPY (AFM)

The atomic force microscopy (AFM) technique has received a great deal of attention in recent years due to its enormous potential for the study of the surface of materials. AFM can be used for imaging non-conductive surfaces. The equipment consists of a probe, a laser beam, and a photodiode used to detect the movement of the probe. The probe, in the form of a sharp tip attached to a cantilever, scans the surface by using force in the order of 10 to 20 nN in the contact mode, and 0.1 nN in the tapping mode. The tapping mode is used to measure the surface characteristics of soft materials such as some polymers. The equipment used was Digital Instruments model Nanoscope IIIa®. Figure 1 presents a schematic diagram of the hair surface and how AFM, in its contact mode, was used to evaluate hair characteristics.

Mattoso *et al.* (10) described several advantages of the AFM over common electron microscopy techniques, including:

- Greater resolution, which can achieve the molecular or even atomic scale for some materials.
- Generation of digital images from three-dimensional topographic data, which can be quantitatively analyzed in 3D.
- Quantification of variables such as roughness, surface area, and morphology heights.
- Absence of the need for any conductive coating on the sample to be analyzed, but just a little sample preparation, which allows the discernment of surface features that are undetectable by scanning electron microscopy.
- Depiction of variations of surface properties, which can be directly and statistically studied.

This paper presents results of AFM use to investigate the morphology of hair fibers exposed and not exposed to UV radiation as well as measurement of the roughness and thickness of cuticle layers.

RESULTS AND DISCUSSION

SCANNING ELECTRON MICROSCOPY (SEM)

Hair damage is the breakdown or removal of structural components or parts of hair that are more vulnerable to chemical breakdown. The exposure to sunlight over prolonged periods induces changes in hair that can be detected at the morphological level, as shown in Figure 2.

It can be observed that the unexposed hair (Figure 2a) has more cuticle layers, that the cuticles have a uniform and regular shape, and that they are oriented longitudinally and

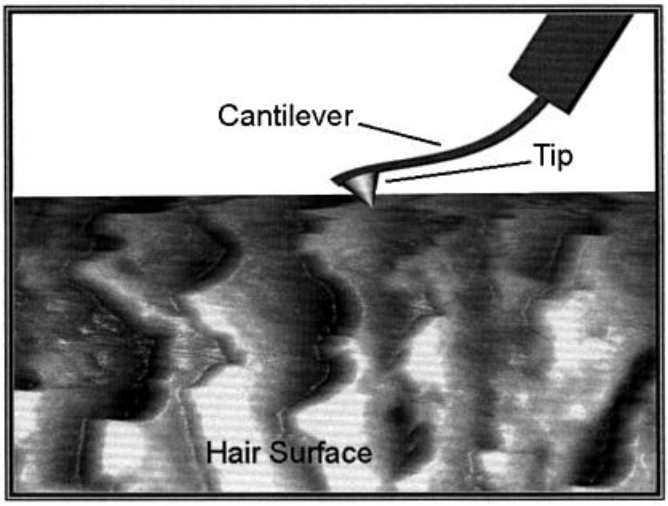


Figure 1. Schematic diagram of the hair surface and evaluation by AFM.

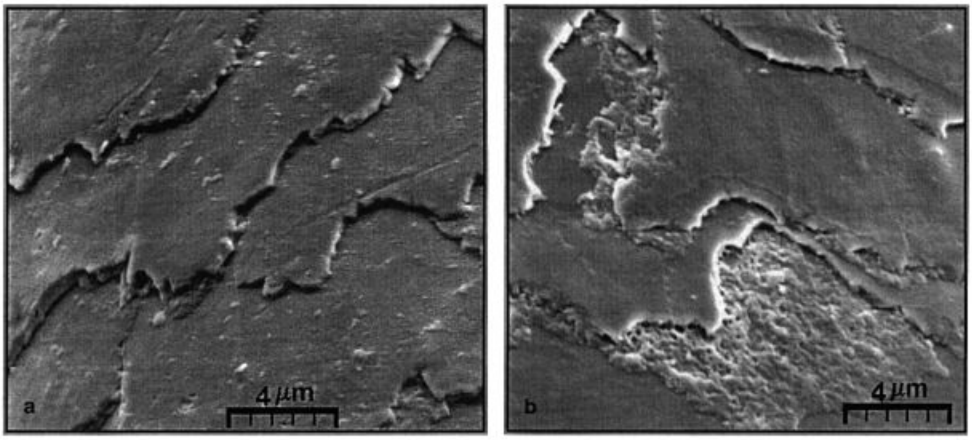


Figure 2. SEM micrographs comparing hair fibers after exposure to UV radiation. (a) Control: virgin hair, 0 h (5 KV, 5000×); (b) 160 h of UV exposure (5 KV, 5000×).

aligned to the direction of the fiber. When exposed to UV radiation, the cuticle undergoes a process of chipping, extraction, and erosion, causing dryness of the hair and greater susceptibility to further damaging action, which may involve large segments or sections of scales being ripped from the hair.

These observations are further elucidated in the profile in Figure 3, which shows the breakdown mechanism of the cuticles when exposed to UV radiation. The physical and mechanical damages induced by irradiation from UV light are illustrated in these micrographs, where we can deduce that the energy photons cause bubble formation and breakdown and cracking of the cuticle.

These results are in agreement with those of Tate *et al.* (11), who observed changes in cuticle topography after UV irradiation, including a decrease in scale thickness. In the present study, we have identified one physical breakdown mechanism for the cuticle. The first event is the expansion of the superficial layer and the formation of bubbles,

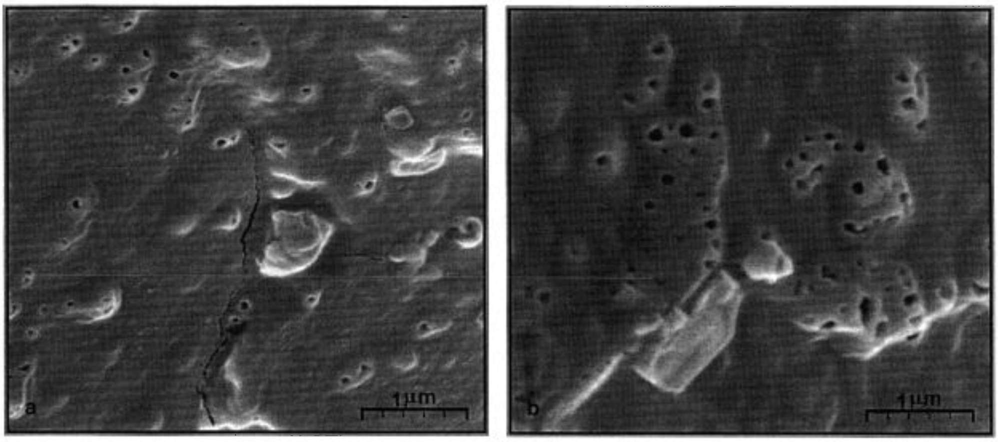


Figure 3. Breakdown and mechanical damage to cuticle irradiated for 160 hours. (a) and (b) 160 h of UV exposure (5 KV, 20000×).

followed by the removal of the structure, leading to a decrease in cuticle thickness or even to the total breakdown of the cuticle.

ATOMIC FORCE MICROSCOPY (AFM)

This section discusses the results of the use of AFM to investigate the morphology of hair fibers exposed to UV radiation. We were also interested in exploring the changes in the physical nature of the cuticular sheath, which displayed thinning and fusion of the surface cuticle cell, forming bubbles during long-term UV exposure.

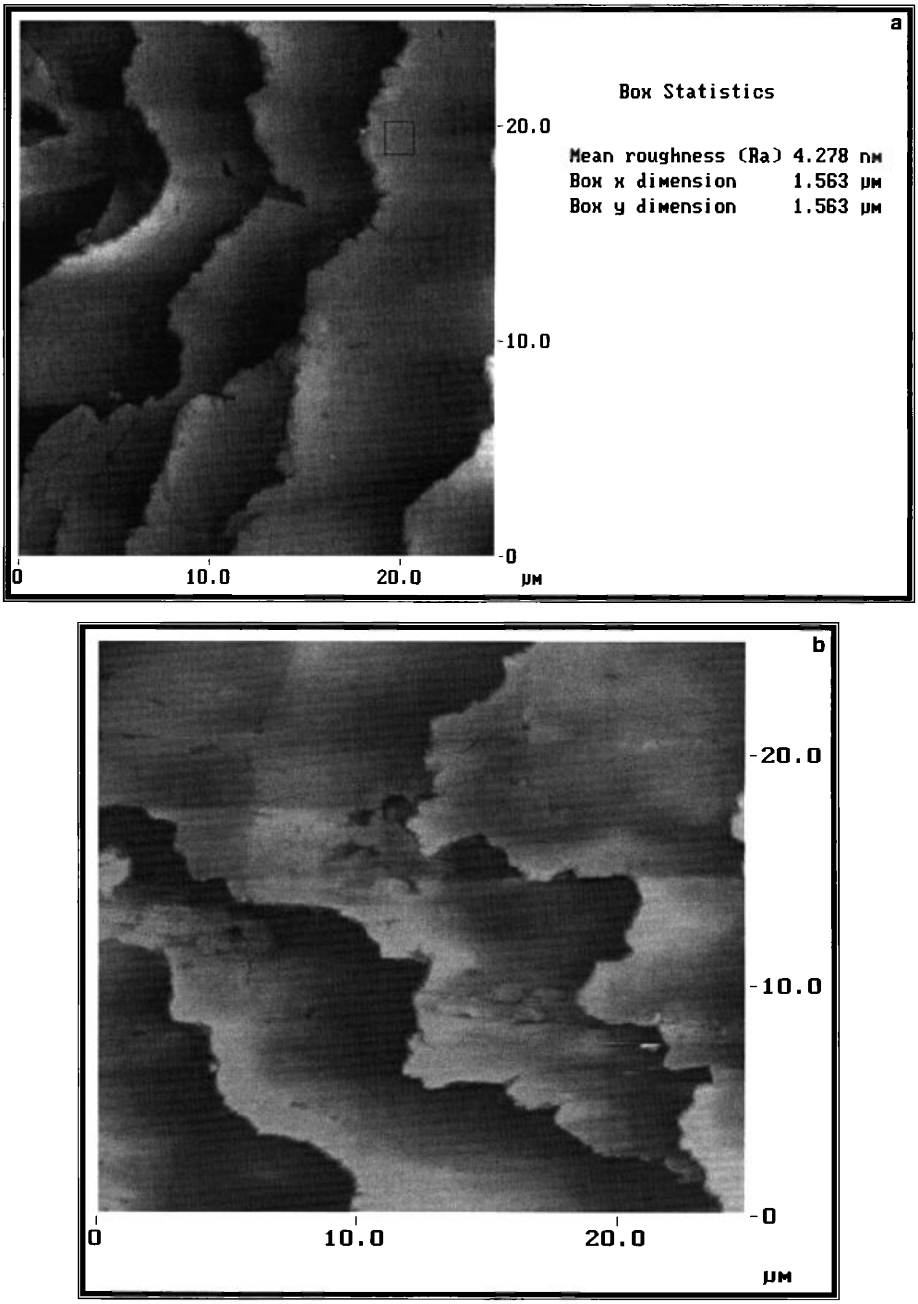
The AFM technique is a better tool to evaluate thickness, roughness, and scale lifting because of several advantages over SEM, among which are its higher resolution, which can reach the molecular or even atomic scale, and the generation of digital images from three-dimensional topographic data, which can be quantitatively analyzed in 3D.

Figure 4a presents a photomicrograph of unexposed hair presenting a schematic box cursor used for the AFM measurements. A previous observation allows us to place the box cursors significantly away from the cuticle edges. In this figure, we can observe that the unexposed hair consists of short cuticles oriented longitudinally and aligned in the direction of the fiber in a regular manner, devoid of the presence of fractures or hole-like depressions in low relief. Figure 4b illustrates the effect of UV radiation (160 hours) on the surface morphology of the hair fibers. Although the cuticular structure is still discernible, its appearance indicates considerable change. As can be seen, the UV-exposed hair presents large cuticle scales, which resulted from the breakdown of the other layers. The roughness of these fibers can be estimated based on the images in Figures 4a and 4b. The surface roughness of unexposed and UV-exposed hair was calculated by the average roughness (Ra) for 11 regions, using the Nanoscope® software of the AFM instrument. The average roughness, Ra, is defined as: the arithmetic average of the absolute values of the surface height deviations, measured from the mean plane within the box cursor.

The data shown in Table I were treated statically by means of the SAS software, using the GLM procedure, which includes the Student *t*-test. An increase in roughness from 5.4 to 16.0 was observed from unexposed to UV-exposed hair fibers, and such an increase is statistically significant, with $p < 0.0001$. The symbol p represents the probability that the damaged hair is statically equal to the unexposed hair. One minus p represents the level of confidence that the hair sets are really different. These data confirm the irregular topography shown by the SEM technique for hair exposed to UV radiation for 160 hours.

This study showed that AFM is an excellent analytical technique to provide quantitative information on morphological changes in hair exposed to UV radiation. Supported by the Nanoscope® software, we evaluated the thinning of the cuticle through section analysis in two ways:

First, as illustrated in Figures 5a and 5b, three cross sections were made for each one of the eleven regions evaluated. The cross sections of the hair fibers give origin to different profiles, for the unexposed and exposed hair. The overall perimeter of a studied profile, representing the length that would be obtained by stretching the fibers, is called surface distance. The cross sections of the hair samples in Figures 5a and 5b show that the profile of unexposed hair presents higher peaks than the profile of exposed hair. The natural consequence of this, taking into account the definition of surface distance, is that



Box Statistics

Mean roughness (Ra) 4.278 nm
Box x dimension 1.563 μm
Box y dimension 1.563 μm

Figure 4. AFM images of hair. (a) Control: virgin hair (0 hours) showing the schematic position of a box cursor. (b) Exposed (160 hours) to UV radiation.

unexposed hair samples present higher values of surface distance. These higher peaks are related to the profile that one might expect from a cross section of the unexposed hair cuticles. On the other hand, the loss of protein from the damaged hair leads to a smaller thickness of the cuticle and a smoother profile and thus to a smaller surface distance. The

Table I
AFM Section Analysis

Roughness (nm)		Surface distance (µm)		Thickness (nm)	
Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed
4.30	13.5	26.596	24.999	406.61	267.18
4.40	8.84	26.465	25.076	450.45	249.68
6.14	18.8	26.300	25.044	548.88	349.18
5.65	15.46	26.460	25.027	490.06	249.02
4.61	18.4	26.746	25.342	392.94	252.36
6.30	15.68	26.507	25.609	465.75	241.08
6.10	21.45	26.393	25.290	364.40	203.84
5.60	23.6	26.544	25.395	308.19	398.58
4.82	24.7	26.869	25.454	436.78	434.15
6.11	14.67	26.488	25.528	402.05	391.10
4.46	10.36	26.250	25.308	425.60	292.77
5.33*	16.86*	26.51*	25.28*	426.52*	302.63*
0.78**	5.1**	0.18**	0.21**	64.2**	77.20**
$p < 0.0001$		$p < 0.0001$		$p = 0.0006$	

* Mean.
** Standard deviation.

data presented in Table I were again analyzed statistically by the same procedure. These observations demonstrated and proved quantitatively that long-term UV irradiation causes thinning and fusion of the surface cuticle cell.

Second, as shown in Figures 6a and 6b, another parameter used to evaluate thinning was cuticle thickness. The cuticle thickness values were evaluated by measuring the maximum peak distance in each cross section presented in Figures 6a and 6b. The results of this evaluation showed lower values for UV-exposed hair, as indicated in Table I. These findings ratify the observation verified in the surface distance parameter, confirming that long-term exposure to UV radiation results in a decrease in the surface modulus. This observation is consistent with the destruction of the cuticle structure due to the deterioration caused by protein (cistine) degradation, which leads to the decrease in cuticle thickness.

The surface distance test, developed by our team members, was proven to be able to separate hair samples into unexposed and degraded populations. The Student t-test, as already mentioned, was applied to the data and supported such a separation. The average value of surface distance for the unexposed hair was 26.51 µm against 25.28 µm for the damaged hair (see Table I).

The Student t-test was also applied to the results of roughness and thickness, obtaining the same result in discriminating unexposed and exposed hair. The average value of roughness was 5.33 nm for the unexposed hair, increasing to 16.86 nm for the exposed hair. As for the thickness test, the average value of the unexposed hair was 426.52 nm, decreasing to 302.63 nm for the exposed hair.

CONCLUSIONS

In this study, we presented a new methodology for evaluating morphological changes in human hair. We believe that this technique is superior to any other available, combining

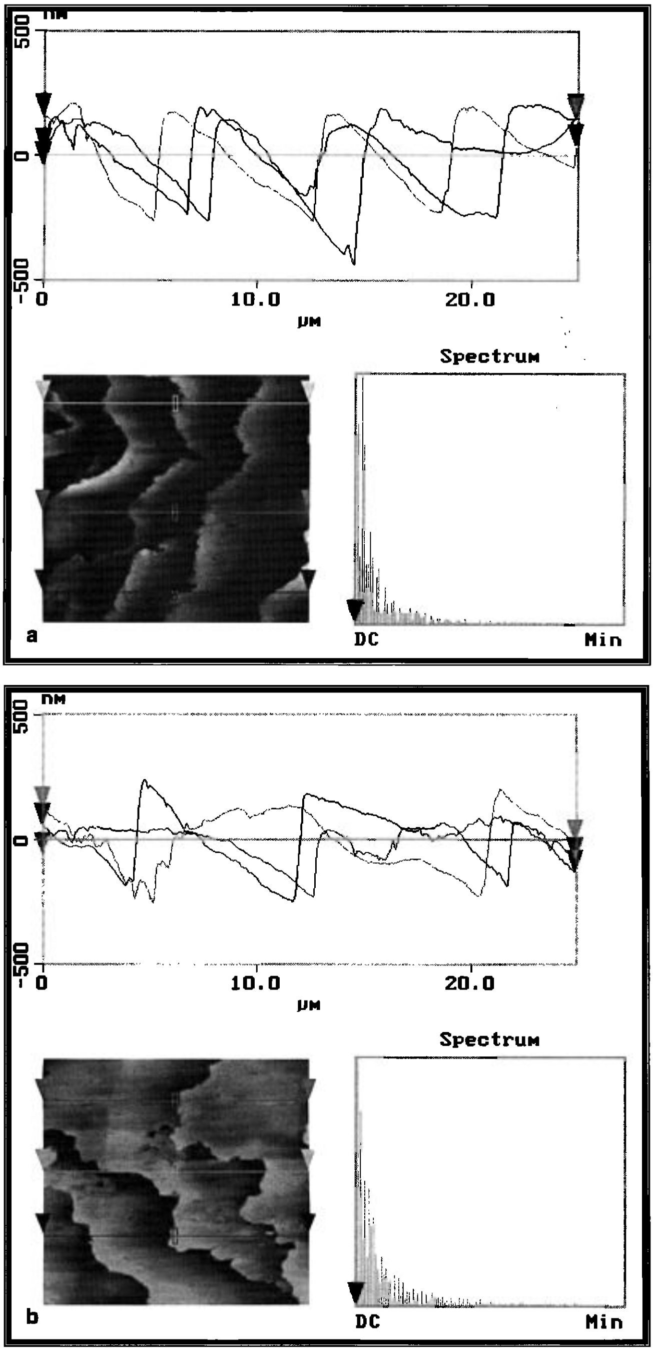


Figure 5. AFM: surface distance analysis. (a) Control: virgin hair (0 hours). (b) Exposed (160 hours) to UV radiation.

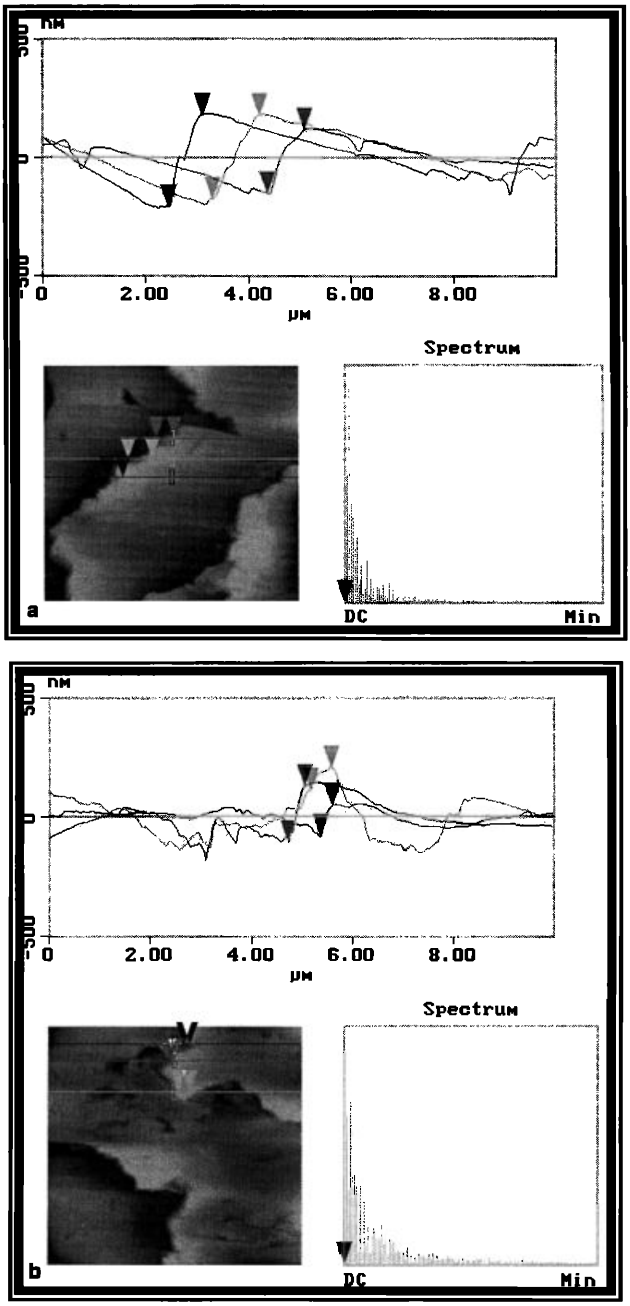


Figure 6. AFM: surface distance analysis. (a) Control: virgin hair (0 hours). (b) Exposed (160 hours) to UV radiation.

the high resolution of electron microscopy with the environmental and simple flexibility of light microscopy. The dual advantage that AFM affords may render it the ideal choice for studies of roughness, thickness, and any other changes in the topography of hair structure.

AFM was also successfully employed to investigate the changes in hair surface roughness when hair fibers undergo long-term exposure to UV radiation. By means of the Student *t*-test, it was possible to prove statistically that the AFM parameters of roughness and surface distance developed in this work, and cuticle thickness, are able to discriminate the two sets of samples—a set of unexposed hair samples and a set of damaged hair samples.

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