Atomic Force Microscopy Of Laser Irradiated Dental Enamel

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Abstract

In Dentistry, the Atomic Force Microscopy (AFM) has been used in studies on tooth general surface features, on hardness and elasticity, on dimensional changes during demineralization and so on, but has never been used in structural and morphological characterizations of dental surface irradiated by laser. In this pilot essay, the AFM potential was evaluated to imaging dental enamel blocks irradiated by laser. Samples were extracted from human and bovine tooth and only the bovine sample was irradiated with 10 pulses of a CO_2-TEA laser. Images of 25 • m x 25 • m and 10 • m x 10 • m of scanning area were obtained from random points of the surfaces and in addition to these images some quantitative results were obtained. Prismatic areas (with holes) and interprismatic enamel were visualized in human and bovine samples. The mean diameter and the apparent depth of enamel holes were directly measured using section analysis command. Surface roughness was also evaluated for both lased and unlased samples. Results indicate that AFM is an excellent tool for the

Introduction

Investigations on surface properties of biological tissues have provided useful information for several branches of life sciences and experienced great development in recent years mainly due to the progress of the scanning technologies.

Amongst these scanning technologies, the Atomic Force Microscopy[1] (AFM) stands out as a powerful investigative technique, which provides not only a high resolution and high contrast three-dimensional imaging, but also quantitative information with the same level of precision in the three axis *x-y-z*. But the main attractive feature of the AFM, specially for biomaterials and life sciences, is the fact that samples are preserved in their native state: they do not need be dehydrated, coated, stained or even evacuated.

The dental enamel is one of the biological tissues of interest in Dentistry. It is made up of an extremely hard inorganic mineral called hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$. The crystalline structure of the enamel has prismatic geometry with hexagonal, oval or polygonal cross section and is formed of densely packed parallel rods, arranged perpendicularly to the surface.

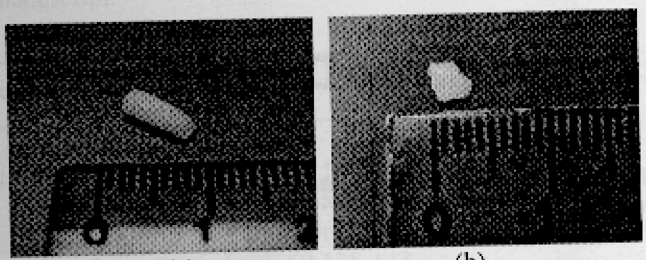
In fact, the AFM has already been used in studies on dental enamel general features[2],[3],[4], on dentin hardness and elasticity[5], on dimensional changes during demineralization[6], on the effect of bleaching agents on enamel surface[7] and on the characterization of adhesives[8],[9]. However, in studies concerning laser applications in caries prevention, tooth surface is usually investigated by scanning electron microscopy and associated microanalysis techniques[10],[11],[12],[13].

Hence, this work reports a pilot essay where the Atomic Force Microscopy (AFM) is evaluated as a tool for the structural and morphological characterizations of dental enamel surfaces irradiated by laser

Experimental Procedures

Samples were extracted from a single noncarious, fully erupted bovine tooth and a single noncarious, fully erupted human tooth. These teeth were cut to remove crowns from roots and 6 mm x 2 mm x 2 mm (bovine) and 3 mm x 3 mm x 1 mm (human) enamel blocks were prepared from their vestibular faces, as shown in Figure 1. A low speed saw (ISOMET 11-1180, Buehler Ltd., IL, USA) with a diamond blade (4 in diam. x 0.012 in long, Buehler Ltd., IL, USA) was used.

Half of the bovine sample was partially covered by a thin aluminum foil in order to avoid transmitting the laser energy during irradiation. This shielded half was used as the unlased control. The other half was positioned 22 cm far from a focusing lens (17.2 cm of focal length) and irradiated with 10 pulses of a CO_2-TEA laser (10.6 • m, 700mJ/pulse, 120 ns pulse width, 0.5 pulses per second).



(a) (b)

Fig. 2: photos of bovine (a) and human (b) enamel blocks.

Due to the natural curvature of the bovine enamel surface, the sample was embedded in acrylic resin aiming to keep the surface to be analyzed nearly parallel to the sample holder of the atomic force microscope (Figure 2).

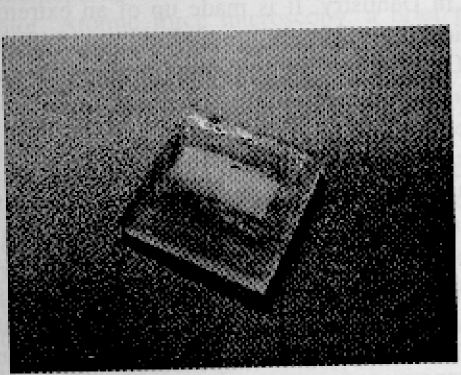


Fig. 3: photo of the embedded bovine sample.

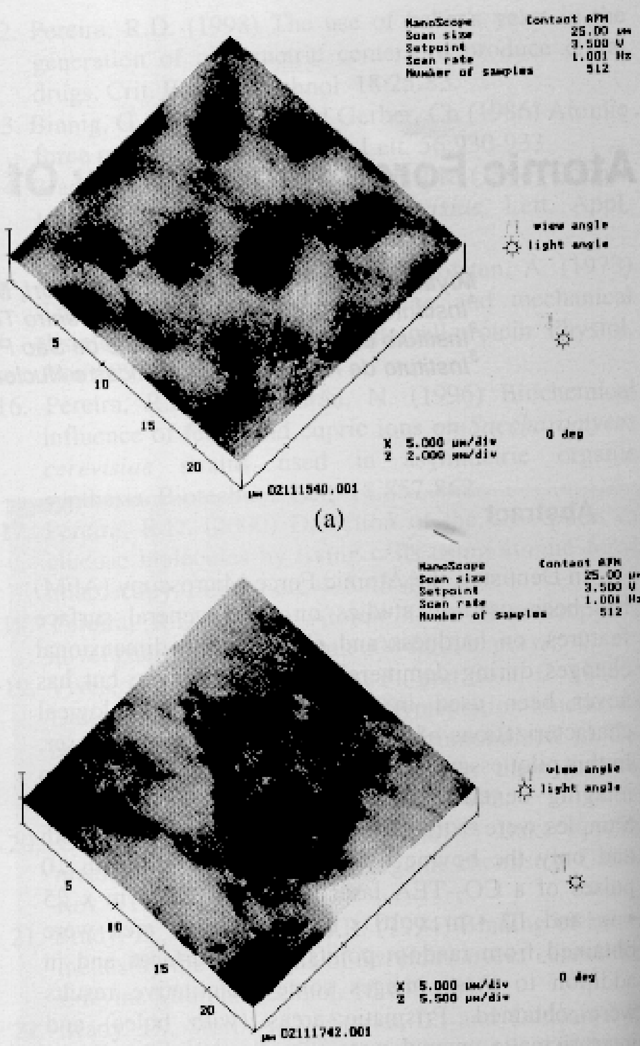
Before observations, bovine and human samples were ultrasonically cleaned with distilled water for 10 minutes.

For this work, a Nanoscope IIIa atomic force microscope (Digital Instrument, Santa Barbara, CA, USA – FAPESP- Multi-users Proc. Nº 95/5651-0) was used. Images of 25 • m x 25 • m and 10 • m x 10 • m of scanning area were obtained from random points of the surface, using a conventional Si_3N_4 tip in contact mode and *J* scanner. For bovine sample, both lased and unlased regions were observed.

Quantitative information was directly obtained using the *section analysis* and the *roughness analysis* commands of the equipment.

Results and Discussion

Images of human (a) and unlased bovine dental enamel (b) surfaces are shown in Figure 3. using the 3D visualization command. Prismatic areas (with holes) and interprismatic enamel can be visualized in both samples and these results are consistent with observations made by Schaad et al.[2].



(b)

Fig. 4: 3D image of human (a) and unlased bovine (b) dental enamel surface.

Figure 4 (a) presents these same images in 2D visualization and disposed side by side in order to facilitate comparison: human holes have round shape, with (3.6±0.4) • m of mean diameter (Schaad et al.[2] found the value (2.80±0.95) nm in their experiments) and nearly 150 nm of apparent depth. Bovine holes are bigger and more irregular, resembling an ellipse with 14.0 • m of major axis and 9.5 • m of minor axis, and approximately 6 times deeper. A magnified image of the interprismatic enamel region (10 • m x 10 • m of scanned area) can be seen in Figure 4 (b).

Figure 5 illustrates typical morphological changes in the enamel surface due to fusion and solidification induced by CO_2-TEA laser, observed from three random points (a, b and c) of the bovine sample. In Figure 6, 2D visualizations of Figure 3 b and Figure 5 a, which correspond to, respectively, the unlased and the lased halves are disposed close together just for visual comparison.

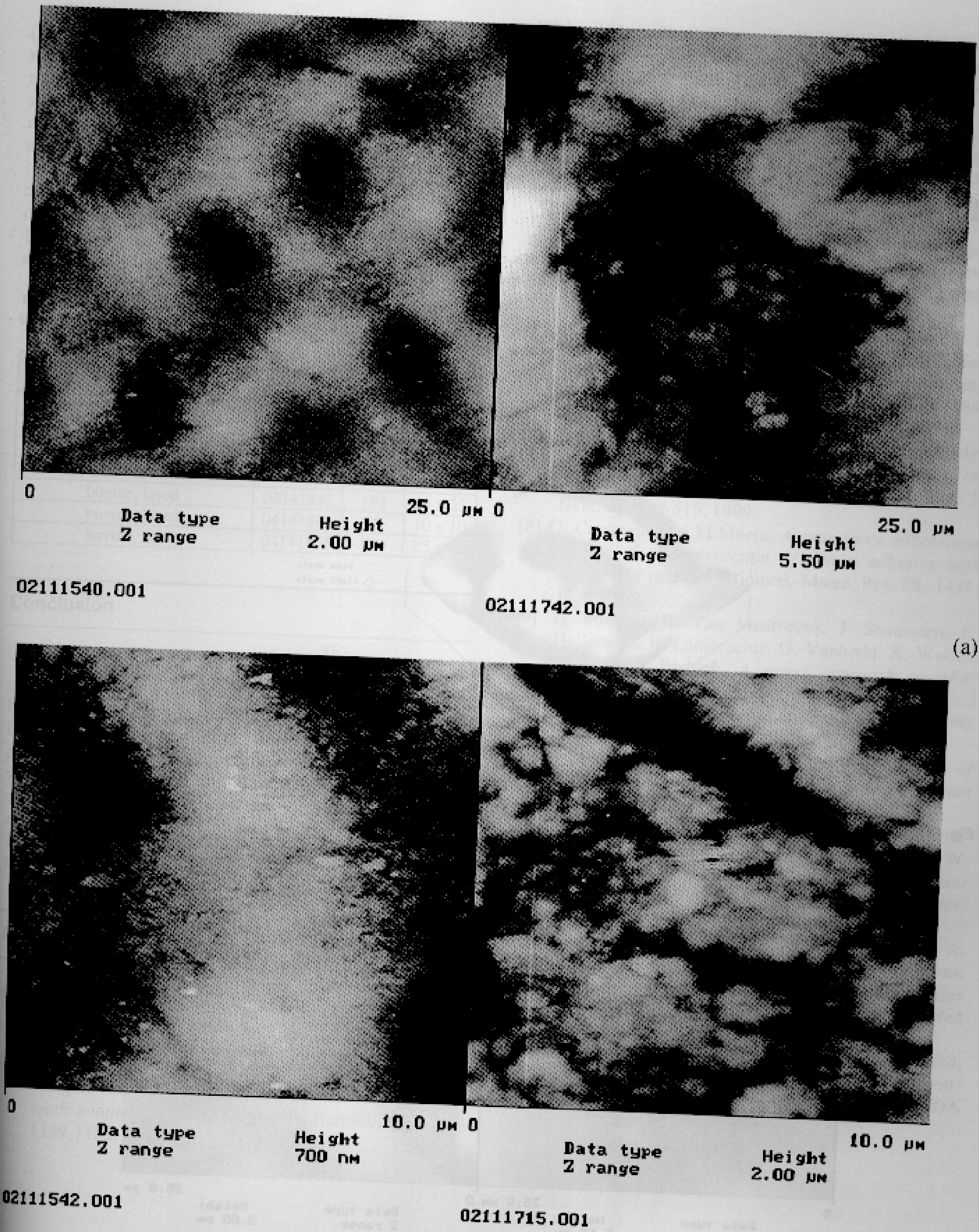


Fig. 5: (a) 2D image of the prismatic area of human (left) and bovine (right) enamel and (b) magnification (10 • m x 10 • m) of the same image showing the interprismatic enamel details.

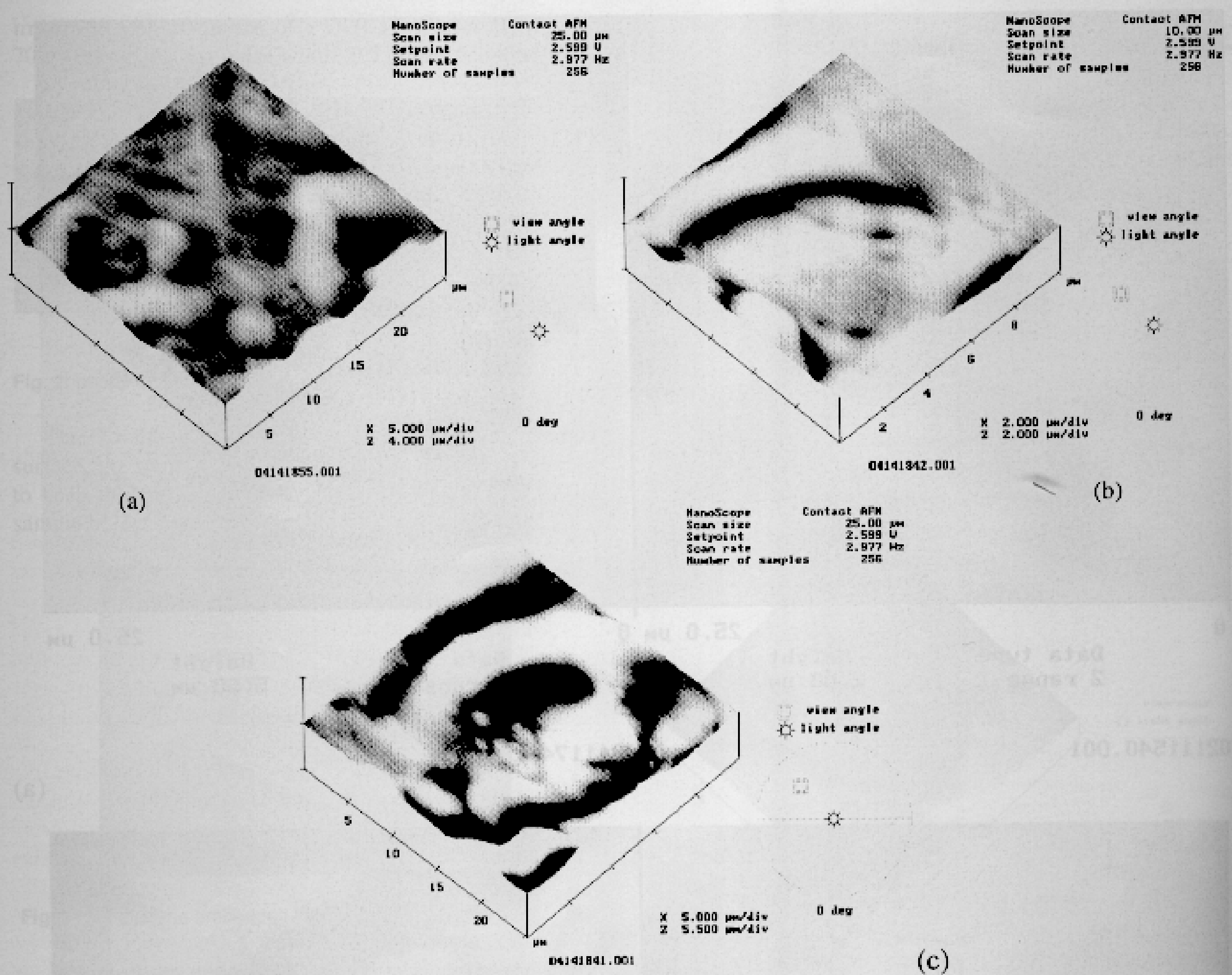


Fig. 6: images obtained from three random points of the lased surface of bovine dental enamel.

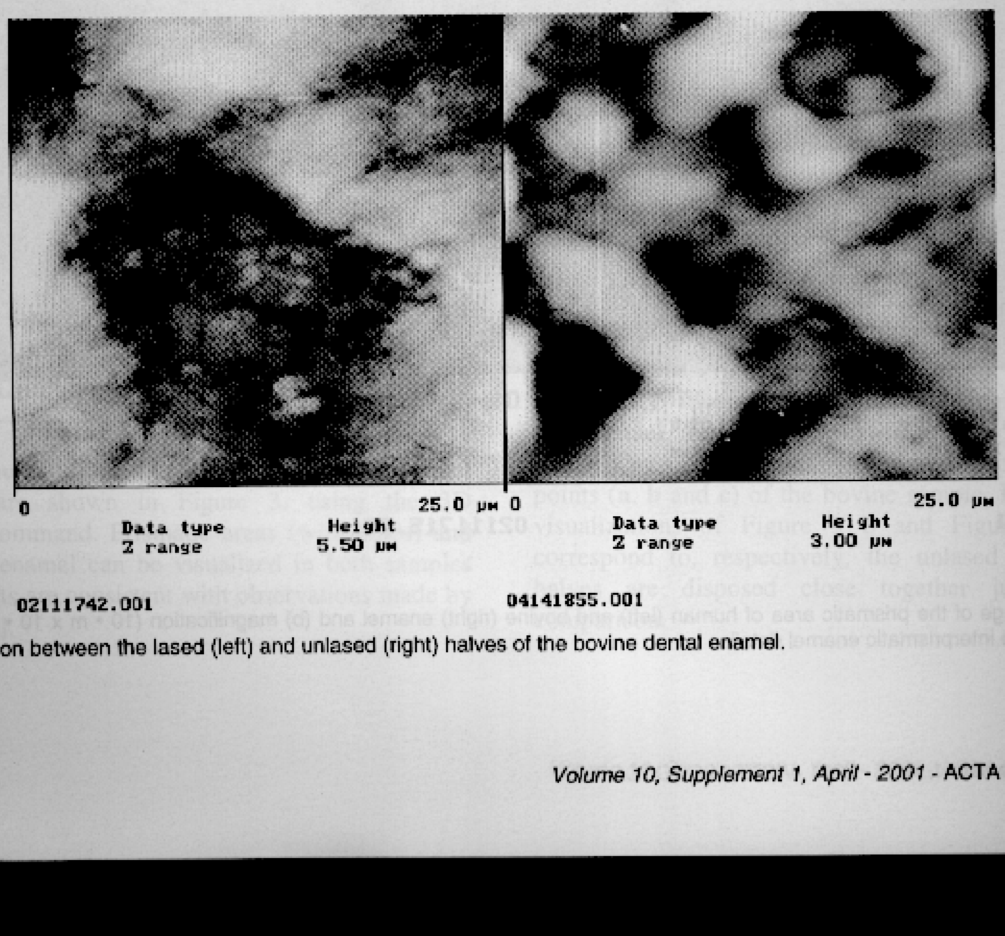


Fig. 7: comparison between the lased (left) and unlased (right) halves of the bovine dental enamel.

Table 1 summarizes the values of RMS roughness (Rq) of some images, identified by the internal reference number given by the microscope, and evaluated using the roughness analysis command. According to the data, surface roughness of human enamel is approximately 45 nm. Unlased bovine sample has surface roughness of about 95 nm for interprismatic enamel. The value 322 nm obtained for the prismatic areas probably included hole depths in the evaluation. In lased bovine samples, on the other hand, surface roughness is relatively greater, near 180 nm and the value 55 nm, for the smallest scanning area, possibly represents a local characteristic.

Tab. 1: values of RMS roughness (Rq) directly evaluated using the roughness analysis command.

sample	image number	Rq (nm)	Area (• m²)
human	02111540	47	25 x 25
human	02111542	42	10 x 10
bovine, unlased, interprismatic	02111715	95	25 x 25
bovine, unlased, enamel hole	02111742	322	10 x 10
bovine, lased	04141841	182	25 x 25
bovine, lased	04141842	55	10 x 10
bovine, lased	04141855	174	25 x 25

Conclusion

Images of human and bovine dental enamel were obtained in order to evaluate the potential of the Atomic Force Microscopy in the characterization of laser irradiated dental surfaces. Surface structures with prismatic areas and interprismatic enamel were easily visualized. Some internal commands, such as *section analysis* and *roughness analysis* were explored in the evaluation of dimensions, depths and surface roughness. The obtained results indicate that the Atomic Force Microscopy is an excellent tool for the proposed task.

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