# Pilot Study on Atomic Force Microscopy of $CO_2$-TEA Laser-irradiated Enamel Surfaces

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In studies concerning laser applications in oral hard tissues, the tooth surface is usually investigated by scanning electron microscopy and associated microanalysis techniques. In this pilot study, atomic force microscopy (AFM) is evaluated as a technique to visualize and quantify structural and morphological changes in enamel surfaces irradiated by $CO_2$-TEA (transversal electric atmosphere) laser. The AFM was chosen not only because of its high resolution and high contrast imaging capability, but mainly due to the fact that quantitative information can be directly obtained from the samples in their natural state (no need of dehydration, coating, staining or even evacuation). Samples were extracted from human and bovine teeth; only the bovine samples were irradiated with 10 pulses of a $CO_2$-TEA laser (10.6 μm, 5.7 J/cm$^2$). Images of 25 μm x 25 μm and 10 μm x 10 μm of scanning area were obtained from random points of the surfaces, and prismatic areas (with holes) and interprismatic enamel were easily visualized in unlased samples. The mean diameter and the apparent depth of enamel holes and the RMS roughness (Rq) were directly measured. Lased surfaces showed typical changes due to fusion and solidification, and greater roughness in comparison to unlased surfaces. Results indicate that AFM is an excellent tool for the proposed task.

**Key words:** AFM, $CO_2$-TEA laser, tooth imaging, surface roughness.

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Several works in the literature report studies on laser irradiation of dental enamel surfaces in order to decrease its susceptibility to caries. In these studies, tooth surfaces were usually investigated by scanning electron microscopy (SEM) and associated microanalysis techniques.[1,2,3,4]

However, in addition to SEM, there is another powerful investigative technique which also resolves surface structure down to the nanometer scale: atomic force microscopy (AFM).[5] The AFM provides not only high resolution and high contrast three-dimensional imaging but also quantitative information with the same level of precision in the three axes x, y, and z. Moreover, of particular relevance for biomaterials and life sciences, the main attractive feature of the AFM is the fact that samples are preserved in their native state: they do not need to be dehydrated, coated, stained, or even evacuated.

In fact, the AFM has already been used in studies on general features of dental enamel,[6,7,8] dentin hardness and elasticity,[9] dimensional changes during demineralization,[10] the effect of bleaching agents on enamel sur-

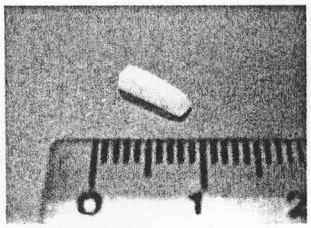


**Fig 1a** Photo of bovine enamel block.

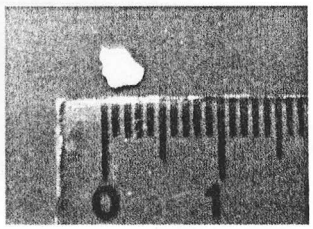


**Fig 1b** Photo of human enamel block.

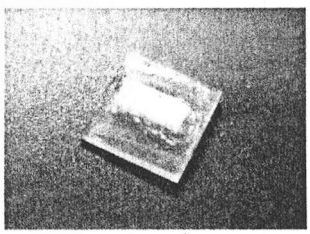


**Fig 2** Photo of the embedded bovine sample.

faces,[11] and on the characterization of adhesives.[12,13] Our group is now working on AFM characterizations in studies concerning laser applications in caries prevention for the first time.

A pilot study is presented here which evaluates AFM as a tool for the structural and morphological characterizations of dental enamel surfaces irradiated by a $CO_2$-TEA laser.

## MATERIALS AND METHODS

This study was approved by the institutional ethics research committee on human beings.

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 x 2 x 2 $mm^3$ (bovine) and 3 x 3 x 1 $mm^3$ (human) enamel blocks were prepared from their vestibular faces, as shown in Fig 1. A low-speed saw (Isomet 11-1180, Buehler, IL, USA) with a diamond blade (10.2 cm diameter x 0.03 cm thick, Buehler) was used.

Half of the bovine sample was partially covered with 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 from a focusing lens (17.2 cm of focal length) and irradiated with 10 pulses of a $CO_2$-TEA laser (10.6 μm, 700 mJ/pulse, 120 ns pulse width, 0.5 pulses per s).

Due to the natural curvature of the bovine enamel surface, the sample was embedded in acrylic resin, attempting to keep the surface to be analyzed nearly parallel to the sample holder of the AFM (Fig 2).

Subsequently, bovine and human samples were ultrasonically cleaned with distilled water for 10 min before AFM observations.

A Nanoscope IIIa atomic force microscope (Digital Instrument, Santa Barbara, CA, USA – FAPESP- Multiusers Proc. No 95/5651-0) was used. Images of 25 x 25 $\mu m^2$ and 10 x 10 $\mu m^2$ of scanning area were obtained from random points of the surface, using a conventional $Si_3N_4$ tip in contact mode and J scanner. Both lased and unlased regions were observed on the bovine sample. Quantitative information was directly obtained using the "section analysis" and "roughness analysis" commands of the equipment.

For the sake of comparison, surface roughness of bovine and human samples was also measured using a focus detection system for topographic measurements (Perthometer S8P – 4.51, Perthen-Mahr, Göttingen, Germany). This equipment uses a diode laser spot (diameter: 1 μm) as a probe, and the smallest scanning length is 0.560 mm.

Finally, after concluding this roughness measurement, the bovine sample was dehydrated and coated with a thin film of gold for SEM observation (model LX30, Phillips, Eindhoven, the Netherlands).

## RESULTS AND DISCUSSION

Images of human and unlased bovine dental enamel surfaces are shown in Fig 3, using the 3D visualization command. Prismatic areas (with holes) and interprismatic enamel can be visualized in both samples; these results are consistent with observations made by Schaad et al.[6]

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

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

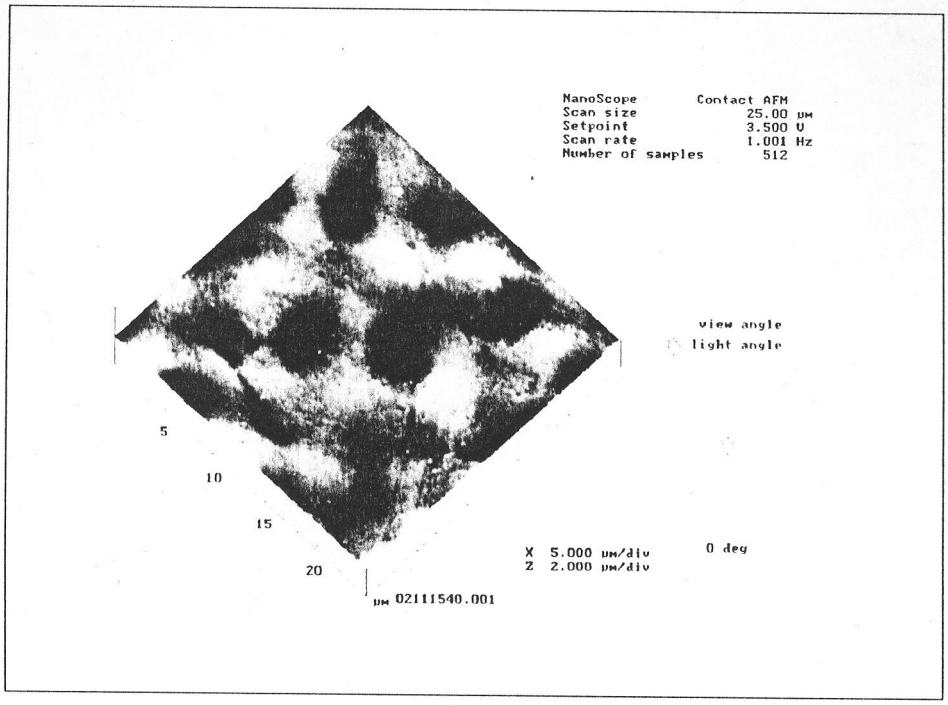



**Fig 3a** 3D AFM image of human dental enamel surface.

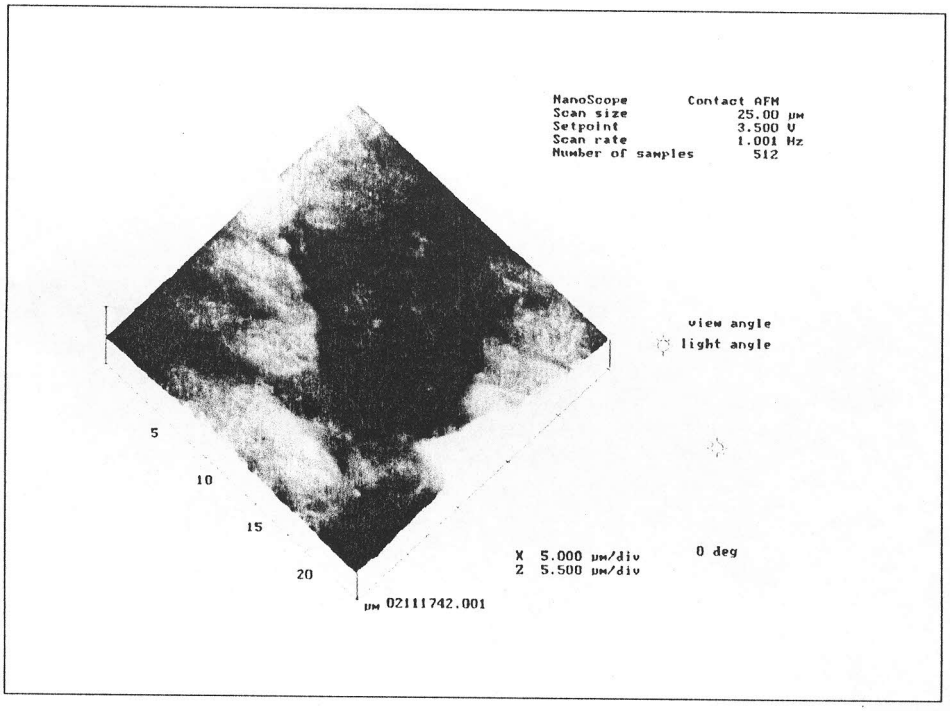



**Fig 3b** 3D AFM image of unlased bovine dental enamel surface.

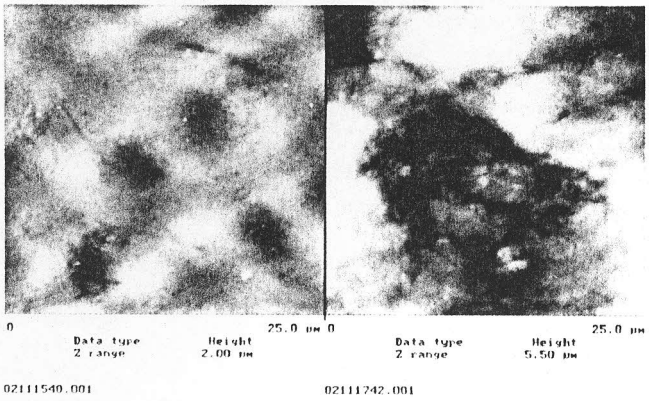



**Fig 4a** 2D image of the prismatic area of human (left) and bovine (right) enamel.

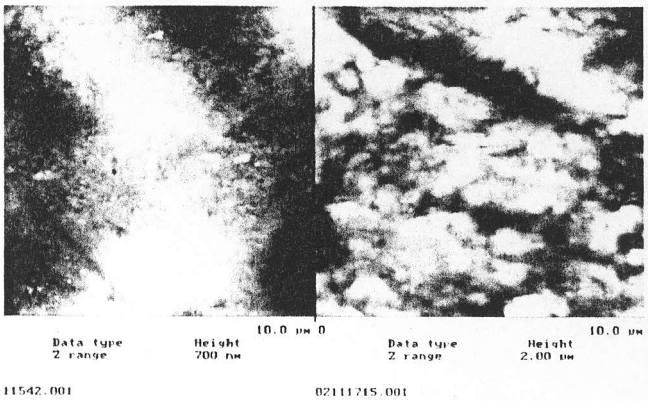



**Fig 4b** Magnification (10 x 10 µm²) of the same image showing the interprismatic enamel details.

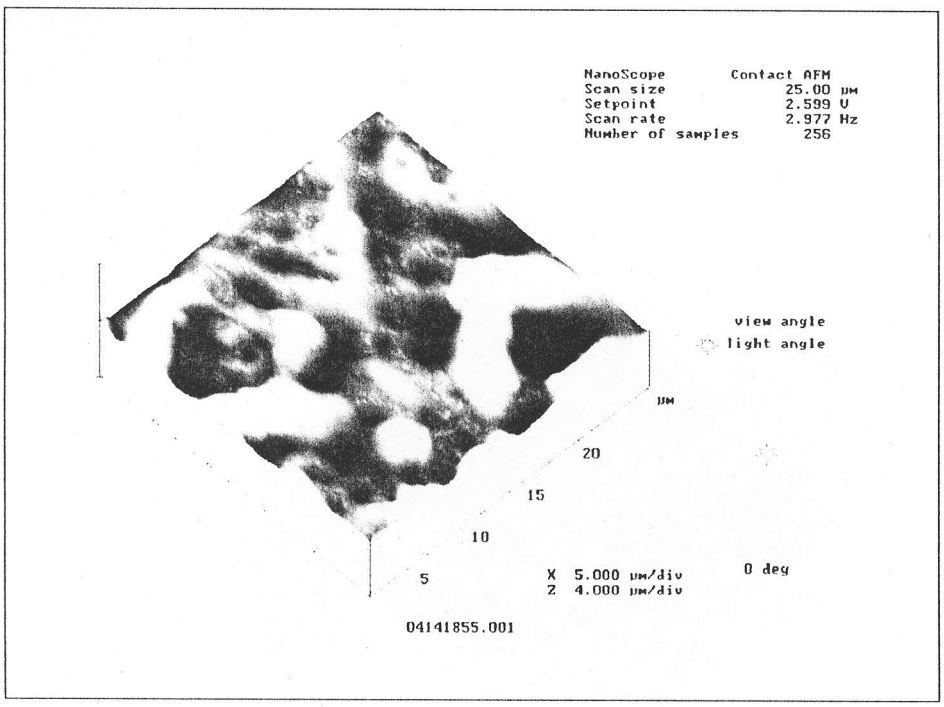


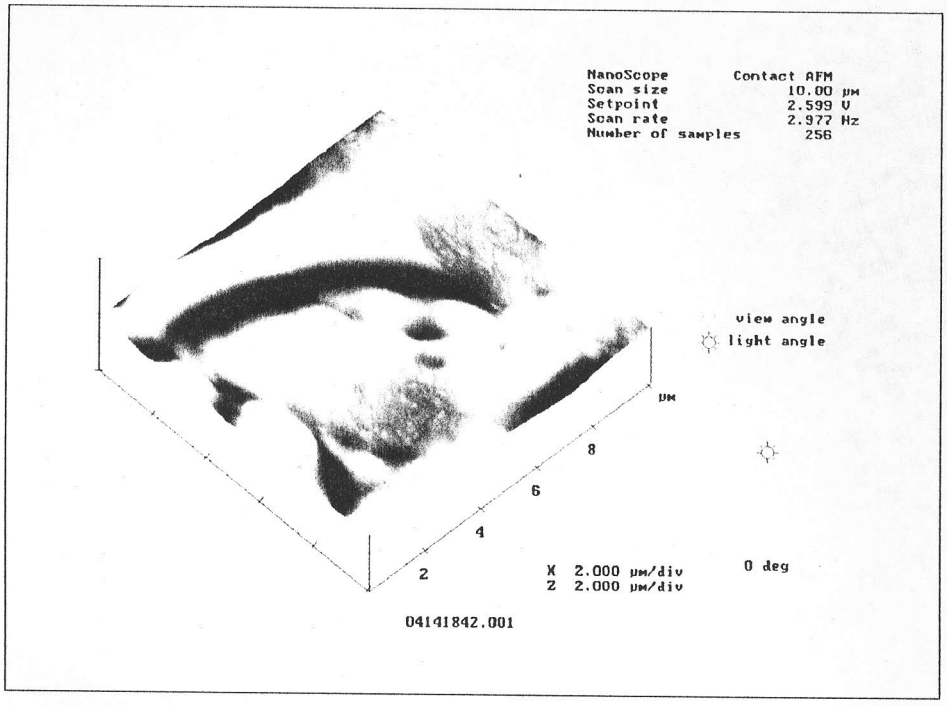

**Fig 5a to c** AFM images obtained from 3 random points of the lased surface of bovine dental enamel.

**Fig 5a**

points (a, b and c) of the bovine sample. In Fig 6, 2D visualizations of 3b and 5a – corresponding to the unlased and the lased halves, respectively – are juxtaposed for visual comparison.

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 over the indicated area. According to the data, surface roughness of human enamel is approximately 45 nm. The unlased bovine sample has a 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 greater, nearly 180 nm; the value 55 nm for the smallest scanning area possibly represents a local characteristic.

Table 2 presents roughness data of the same human and bovine samples, obtained from a topographic measurement device (Perthometer). The apparent discrepancy of values comes from the fact that the AFM tip has 20 to 60 nm of nominal curvature, while the Perthometer runs with a 1 µm laser spot, and the fact that the AFM can measure roughness in areas smaller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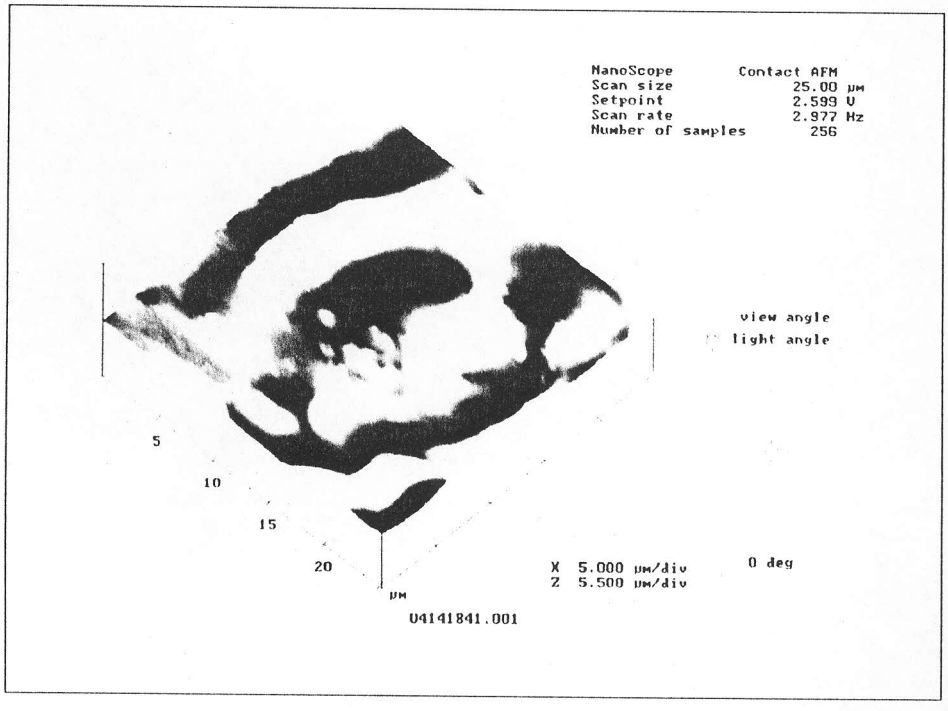
Scan size 10.00 µm
Setpoint 2.599 V
Scan rate 2.977 Hz
Number of samples 256
view angle
light angle
µm
8
6
4
2
X 2.000 µm/div
Z 2.000 µm/div
0 deg
04141842.001

Fig 5b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Setpoint 2.599 V
Scan rate 2.977 Hz
Number of samples 256
view angle
light angle
5
10
15
20
µm
X 5.000 µm/div
Z 5.500 µm/div
0 deg
04141841.001

Fig 5c

**Table 1 Values of RMS roughness (Rq) directly evaluated using the roughness analysis command**

| Sample | Image number | Rq (nm) | Area ($\mu m^2$) |
|---|---|---|---|
| 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 |

**Table 2 Roughness values measured with the Perthometer focus detection system**

| Sample | Surface roughness (µm) |
|---|---|
| Human | 1.14 |
| Unlased bovine | 1.62 |
| Lased bovine | 1.67 |

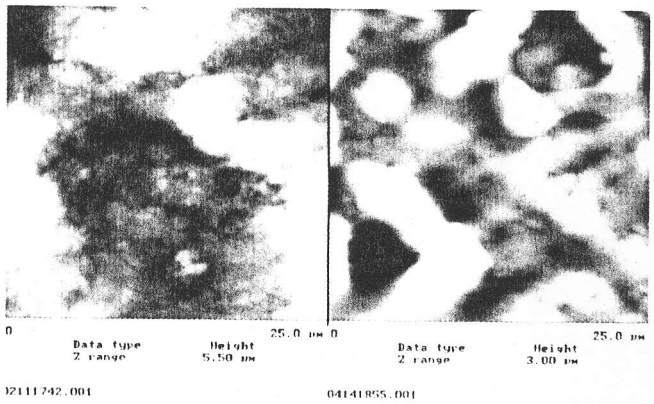


**Fig 6** Comparison between the unlased (left) and lased (right) halves of the bovine dental enamel. AFM images.

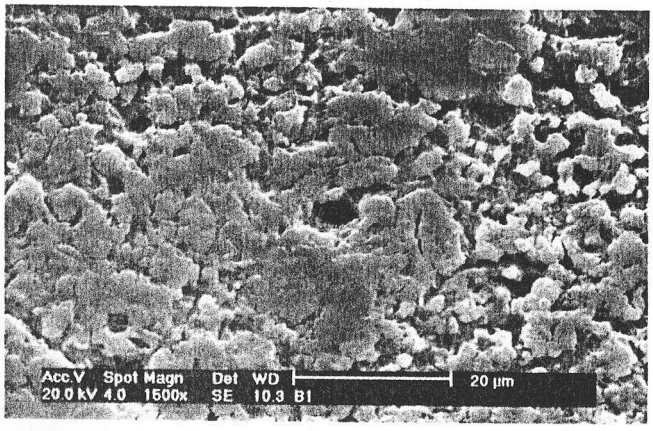


**Fig 7a** SEM image of the unlased half of sample of bovine dental enamel.

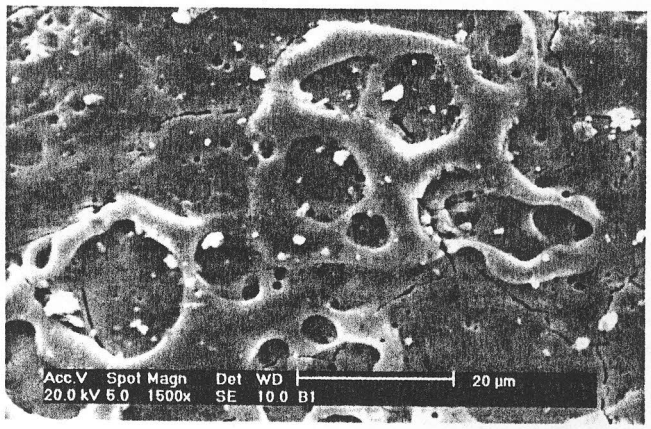


**Fig 7b** SEM image of lased half of the same sample of bovine dental enamel.

than 1 x 1 $\mu m^2$. In contrast, the Perthometer measurement line is at least 0.400 mm long. Hence, these data just emphasize one of the high-resolution capabilities of AFM.

Finally, it is interesting to compare the AFM images of Fig 6 with the SEM images of Fig 7. There are perceptible differences originating in the way these two techniques process vertical changes in topography. Both have probes (a tip or an electron beam) interacting with the surface to form the image, and consequently, both have image artifacts. Hence, professionals should be trained to understand these artifacts and take advantage of this powerful technique.

## CONCLUSION

Using AFM to examine bovine and human enamel, surface structures with prismatic areas and interprismatic enamel were easily visualized in unlased samples, and typical morphological changes were also easily observed in lased samples. Some internal commands, such as "section analysis" and "roughness analysis" were explored for directly obtaining dimensions, depths and surface roughness. These results indicate that atomic force microscopy is an excellent tool for the characterization of laser-irradiated dental surfaces.

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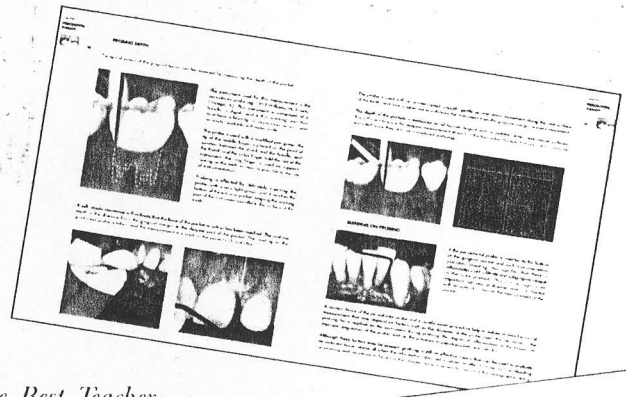


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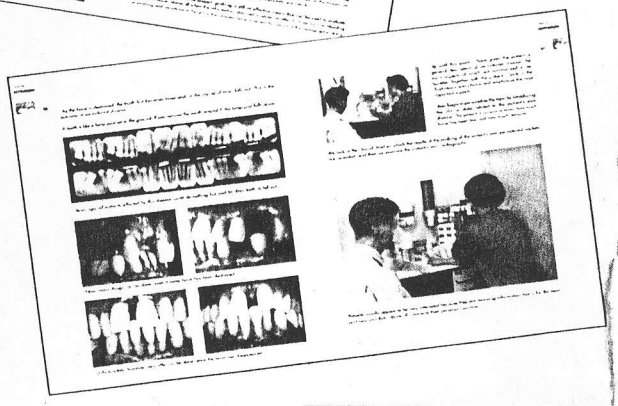

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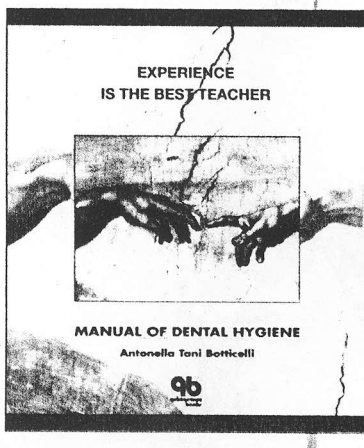


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