Confocal Laser Scanning Microscopy (CLSM) has been identified as a nondestructive tool for viewing the subsurface tomography of enamel and dentine hard tissue. This tool is of particular interest if it allows real-time assessment of demineralization and remineralization processes within these tissues. Previously, demineralization effects on subsurface enamel have been demonstrated using CLSM (Ando et al., 1994). It was not known, however, if CLSM could focus through a lesion and into sound enamel underneath. The purpose of this study was to determine if CLSM is capable of focusing not only into but through the depth of a lesion and into the underlying sound enamel. Lesions were prepared by cutting 4mm diameter discs of human enamel from incisors which were free of visual cracks or surface imperfections (10x). Specimens were ground with silica carbide paper (600 grit), removing approximately 50 µm of the natural surface, then polished to a high luster using AB Gamma alumina (particle size < 1.0 µm). Surface hardness of specimens after polishing (Leitz microhardness tester @ 200g load) was ~360 VHN (Vickers hardness). One-half of the specimen was covered with a clear, acid resistant nail varnish. The uncovered half of the specimen was exposed to 25ml of demineralization solution consisting of 0.1 M/L lactic acid, 0.2% Carbopol 907 (B.F. Goodrich, Co.) 50% saturated with respect to hydroxyapatite, pH 5.0 for 24 hours at 37°C. After demineralization, specimens were rinsed with deionized, distilled water and stored in a cool humid environment until analysis. The results of this experiment can be seen in the following series of images from a single specimen. Depth (from the bare enamel and that covered by the nail polish. CLSM images (x,y) were made at 5µm increments from 0-40µm. Changes in the porosity of the enamel were clearly visible, and the junction between exposed/unexposed sides was well defined. Images throughout the body of the lesion were easily distinguishable. At a depth of approximately 40 µm, images on each side of the junction appeared identical. CLSM images (x,z) showed clear definition of the lesion depth as well. Results from this study indicate that CLSM is capable of visualizing not only into but through demineralized lesions into the underlying enamel. This capability is critical to the usefulness of CLSM in assessing further the processes of demineralization and remineralization. Material and methods

Preparation of enamel specimens

Enamel specimens were prepared by cutting 4mm cores from extracted, human incisors using a diamond core drill. The teeth were stored at room temperature in a saturated thymol solution until ready for use. Enamel cores were mounted in 1/4 inch diameter Lucite rods with dental acrylic (Dura Base, Reliance Mfg. Co.). Course polishing with 600 grit silicon carbide-water slurry was used to remove approximately 50 µm of the outer enamel. Specimens were polished with gamma alumina (Linde No. 3, AB Gamma Polishing Alumina) to a mirror finish. Enamel specimens that had surface imperfections were rejected. One half of the surface of each specimen was covered with a clear, acid resistant nail varnish to serve as a control. Each specimen was then suspended in 25ml of a solution containing 0.5M/L lactic acid, 0.2% Carbopol 907 (B.F. Goodrich, Co.) 50% saturated with respect to hydroxyapatite, pH 5.0 for 24 hours at 37°C. After demineralization, specimens were rinsed with deionized, distilled water and stored in a cool humid environment until analysis. Visualization of specimens

Each specimen was mounted on a microscope slide, then placed on the microscope stage in such a way as to simultaneously visualize both control and demineralized areas of the sample. Microscopic tomographs (images parallel to the surface) of the contact zone between the demineralized and control areas were recorded to a depth of 40 µm in incremental steps of 5 µm. RESULTS

The results of this experiment can be seen in the following series of images from a single specimen. Depth (from the anatomical surface) and scale are noted on each image. These images suggest marked differences between control (left, mineral concentrated in the interprismatic areas) and demineralized (right, mineral concentration is less and appears diffused) areas of the sample throughout the first 30µm. Beyond 30 µm the differences between control and demineralized areas are less obvious. The 40 µm image shows no difference between control and demineralization, suggesting the lesion front is located at a depth of ~35 µm. The final image is an x-z image which depicts a transverse view of the lesion.
CONCLUSION

This experiment demonstrates that CLSM does have the ability to focus not only into but through a lesion into the underlying sound enamel. Images were taken with virtually no specimen preparation and with no tissue damage. Clearly, CLSM offers a unique perspective with respect to the process of demineralization. Its potential with regard to remineralization must still be assessed. Efforts are underway to quantify the images acquired in this experiment.