Published in final edited form as:

Lasers Surg Med. 2011 December ; 43(10): 951–959. doi:10.1002/lsm.21139.

Nondestructive Clinical Assessment of Occlusal Caries Lesions using Near-IR Imaging Methods

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Abstract

Objective—Enamel is highly transparent in the near-IR (NIR) at wavelengths near 1300-nm, and stains are not visible. The purpose of this study was to use NIR transillumination and optical coherence tomography (OCT) to estimate the severity of caries lesions on occlusal surfaces both *in vivo* and on extracted teeth.

Methods—Extracted molars with suspected occlusal lesions were examined with OCT and polarization sensitive OCT (PS-OCT), and subsequently sectioned and examined with polarized light microscopy (PLM) and transverse microradiography (TMR). Teeth in test subjects with occlusal caries lesions that were not cavitated or visible on radiographs were examined using NIR transillumination at 1310 nm using a custom built probe attached to an indium gallium arsenide (InGaAs) camera and a linear OCT scanner. After imaging, cavities were prepared using dye staining to guide caries removal and physical impressions of the cavities were taken.

Results—The lesion severity determined from OCT and PS-OCT scans *in vitro* correlated with the depth determined using polarized light microscopy (PLM) and transverse microradiography (TMR). Occlusal caries lesions appeared in NIR images with high contrast *in vivo*. OCT scans showed that most of the lesions penetrated to dentin and spread laterally below the sound enamel.

Conclusion—This study demonstrates that both NIR transillumination and OCT are promising new methods for the clinical diagnosis of occlusal caries.

Keywords

NIR imaging; optical coherence tomography; dental caries; caries detection; occlusal caries

INTRODUCTION

Caries lesions on occlusal surfaces are routinely detected clinically using visual/tactile (explorer) methods coupled with radiography. Unfortunately, these methods have numerous shortcomings and are inadequate for the detection of the early stages of the caries process. Radiographic methods use ionizing radiation and do not have the sensitivity for early lesions, particularly occlusal lesions and by the time an occlusal lesion is visible on a radiograph, it has likely penetrated deep into the dentin [1-3]. At that stage in the decay process, it is far too late for preventive and conservative intervention and a large portion of carious and healthy tissue will need to be removed, often compromising the mechanical integrity of the tooth. If left untreated the decay could eventually infect the pulp, leading to

loss of tooth vitality and possible extraction. The caries process is potentially preventable and reversible in the early stages. If lesions are detected early enough, it is likely that they can be arrested/reversed by non-surgical means through fluoride therapy, anti-bacterial therapy, dietary changes, or by low intensity laser irradiation [4,5].

Light scattering in sound enamel decreases markedly from the visible to the NIR [6,7] and enamel is nearly transparent in the NIR around 1310-nm which makes this wavelength ideal for imaging demineralization [8,9]. NIR images have been acquired of natural interproximal and occlusal lesions through transillumination of extracted human teeth [10,11]. Recently, NIR imaging at 1310-nm was compared with radiography in an *in vivo* study of lesions on proximal tooth surfaces. That study showed that NIR imaging was as sensitive as radiographs for approximal lesions. Occlusal lesions can be imaged in the NIR using a novel approach in which light is delivered just above the gumline while the occlusal surface of the tooth is imaged. NIR light diffuses up through the crown, and lesion areas attenuate that light to a greater degree than surrounding sound tissues [12]. Areas of demineralization appear darker than the sound areas and are visible with high contrast. Moreover, stains are not visible in the NIR since the organic molecules responsible for pigmentation absorb poorly in the NIR making it easier to identify areas of demineralization. Mild developmental defects [13] and shallow demineralization [14] appeared lighter while deeper more severe demineralization appeared darker. Recent studies have shown that the area and contrast of occlusal lesions in NIR images can be correlated with the lesion severity and that lesions which penetrate into dentin have significantly higher contrast than those that are limited to enamel [15-17].

Optical coherence tomography (OCT) is a noninvasive technique for creating cross-sectional images of biological structures [18]. Most OCT systems operate at 1310-nm and benefit from the high transparency of enamel at this wavelength. Several groups have used OCT and PS-OCT to image caries lesions [19-27] on both smooth surfaces and occlusal surfaces. Polarization-sensitivity is invaluable for providing measurements of the severity of demineralization both *in vitro* and *in vivo* [28-31]. Polarization sensitivity is also useful for differentiating subsurface artifacts produced by the birefringence of sound enamel from caries lesions. The most obvious approach for quantifying the lesion severity using OCT is to directly integrate the reflectivity from the lesion area over the lesion depth. However, the strong reflectivity of the tooth surface can produce a signal of greater magnitude than that produced due to the overall scattering/reflectivity of the lesion. If the incident light is linearly polarized, surface reflections do not depolarize light, so the surface reflection does not interfere with the signal in the orthogonal ($\perp$) polarization state. This can also be called the cross polarization OCT image. Caries lesions strongly depolarize incident linearly polarized light and the reflectivity in the cross polarization image provides increased contrast. Therefore, lesion areas can be directly integrated in the cross polarization OCT image, including lesion areas close to the tooth surface. The lesion surface zone is particularly important because it can provide information about lesion activity.

Quantitative depth resolved measurements are useful for clinical studies and for monitoring the state of early lesions and studies indicate that polarization sensitivity provides considerable advantages for these types of measurements particularly for early demineralization near tooth surfaces [28-31]. However, many clinicians are mainly interested in knowing how deep the occlusal lesions have actually penetrated into the tooth so that they can decide whether a restoration is necessary. The Diagnodent which employs fluorescence from bacteria porphyrin molecules was developed for this purpose, however the reading only reflects the amount of fluorescence and does not indicate the depth [32-35]. Previous studies have explored the use of PS-OCT to measure demineralization and remineralization in the occlusal surfaces. Ngaotheppitak et al. [36] showed that the

integrated reflectivity for simulated and natural lesions of less than 500- μ m deep correlated with the mineral loss. Jones et al. [29] also demonstrated that the demineralization could be measured under sealants and that better images could be acquired in occlusal surfaces by use of index matching agents applied to the fissure areas [30]. It is not clear, however, that polarization sensitivity is necessary to measure the depth of deeply penetrating lesions.

In the first part of this study, extracted teeth with suspected occlusal lesions were imaged using only OCT and those images were compared to images of serial sections obtained by polarized light microscopy (PLM) and transverse digital microradiography (TMR) to determine how well the OCT images reflect the actual lesion depth. In addition, the correlation of lesion depth was calculated with and without polarization sensitivity with the lesion depth determined from PLM and TMR. This comparison was carried out to determine if polarization sensitivity is necessary for imaging deeply penetrating lesions in which the interference from reflected light at the tooth surface is not as important. In the second part of this study, occlusal lesions on teeth scheduled for restoration were imaged using both NIR transillumination and OCT and the images were compared with a physical impression of the cavity required after actual caries excavation guided by a caries indicator dye.

In both parts we decided to focus on lesions that penetrate to or beyond the dentino-enamel junction (DEJ), as such lesions are of greatest interest to the clinician and also demonstrate the diagnostic capabilities of these imaging systems at greater depth.

MATERIALS AND METHODS

In Vitro Sample Preparation

Teeth extracted from patients in the San Francisco Bay area were collected, cleaned and sterilized with gamma - radiation. Molars and premolars were visually inspected for caries lesions. On extracted molars suspected lesions appear as white or brown/black stained areas on the tooth surface and specimens are readily available. Those samples with suspected lesions were further screened using a NIR imaging system operating at 1310-nm. In the visible range it is difficult to differentiate between stains and actual demineralization and many of the teeth selected by visual inspection were only stained without demineralization. The organic molecules that cause pigmentation apparently do not strongly absorb NIR light and staining does not interfere in the NIR (28, 29). Twenty samples were selected with suspected deep natural existing occlusal lesions using this screening technique.

The roots were sectioned and removed and the teeth were mounted on $1.2 \times 1.2 \times 3$ cm rectangular blocks of black orthodontic composite resin with the occlusal surface containing the lesion facing out from the square surface of the block. Each rectangular block fit precisely in an optomechanical assembly that could be positioned with micron accuracy. The rectangular symmetry of the mount facilitates matching the position of plano-parallel OCT b-scans to the serial thin sections produced for mineral density determination using a digital transverse microradiography system (TMR) and for histological examination using polarized light microscopy (PLM). Figure 1 shows one sample before and after sectioning. Additionally, the teeth were etched with a CO₂ laser to produce fiducial points for more precise matching of the PS-OCT scans with the sections. The CO₂ laser etched a 2 $\times$ 2 mm square with the incision approximately 200- μ m across and 50-100- μ m deep. Similar rectangular sample holders were mounted on both the OCT scanning system and the microtome. Digital micrometers on both the microtome and the OCT scanning stages were used to position the microtome saw blade and the OCT scans.

PS-OCT System

A single-mode fiber, autocorrelator-based Optical Coherence Domain Reflectometry (OCDR) system with polarization switching probe, high efficiency piezoelectric fiber-stretchers and two balanced InGaAs receivers that was designed and fabricated by Optiphase, Inc. (Van Nuys, CA) was integrated with a broadband high power superluminescent diode (SLD), Denselight (Jessup, MD) with an output power of 45-mW and a bandwidth of 35 nm and a high-speed XY-scanning system, ESP 300 controller & 850-HS stages, Newport (Irvine, CA) and used for in vitro optical tomography. The system was configured to provide an axial resolution at 22- μm in air and 14- μm in enamel and a lateral resolution of approximately 50- μm over the depth of focus of 10 mm. The all-fiber OCDR system has been previously described in greater detail (30, 31). The PS-OCT system was completely controlled using LabVIEW™ software, National Instruments (Austin TX). Figure 2 shows the scanning stage, sample holders and probe used for this study. Images were acquired in both orthogonal polarizations that were parallel ($\parallel$) and perpendicular ($\perp$) to the light incident on the tooth surface. The orthogonal polarization ($\perp$) is also called the cross polarization image and this image provides the best contrast between sound and demineralized enamel and dentin. We used the sum of both images to represent a “conventional” non-polarization sensitive OCT scan.

Polarized Light Microscopy (PLM) and Transverse Microradiography (TMR)

After the PS-OCT scanning, the teeth were serially cut into sections of ~200 μm thickness for polarized light examination and digital quantitative microradiography as shown in Fig. 1 using a linear precision saw, Isomet 5000 (Buehler, Lake Buff, IL). The thickness of each section was measured with a digital micrometer with 1- μm resolution. Ideally, thin sections of 80-100- μm thickness should be used for both PLM and TMR to minimize edge effects, and thinner sections are more desirable for comparison with the 20- μm thick PS-OCT scans. However, enamel is very brittle and difficult to cut into thin sections without fracture, more so for carious sections, and attempts to produce such thin sections would result in an unacceptable high loss rate of samples/sections, therefore we chose a more reliable section thickness of 200- μm . Thin sections were imbibed in water and examined at up to 500x with a polarizing microscope interfaced to a high-resolution digital camera. Demineralization due to strong scattering causes loss of birefringence in the lesion and it appears dark. Measurements of lesion depth were made with calibrated image analysis software that is capable of direct length and area measurements. Polarized light microscopy (PLM) was carried out using a Meiji Techno RZT microscope (Saitama, Japan) with an integrated digital camera, Canon EOS Digital Rebel XT from Canon Inc. (Tokyo, Japan). The sample sections were imbibed in water and examined in the brightfield mode with cross polarizers and a red I plate with 500-nm retardation.

Thin sections used in PLM were also imaged using transverse microradiography (TMR). A custom-built digital TMR system was used to measure mineral loss in the different partitions of the sample (32). A high-speed motion control system with Newport (Irvine, CA) UTM150 and 850G stages and an ESP300 controller coupled to a video microscopy and laser targeting system was used for precise positioning of the tooth samples in the field of view of the imaging system. The volume percent mineral for each sample thin section was determined by comparison with a calibration curve of X-ray intensity vs. sample thickness created using sound enamel sections of 86.3 ± 1.9 vol.% mineral varying from 50 to 300 μm in thickness. The calibration curve was validated via comparison with cross-sectional microhardness measurements. The volume percent mineral was determined using microradiography for section thickness ranging from 50 to 300- μm highly correlated with the volume percent mineral determined using microhardness $r^2 = 0.99$ [37].

In vivo procedures

Subjects were recruited from the patient population of the University of California at San Francisco School of Dentistry who had occlusal lesions that were scheduled for restoration after undergoing a routine dental examination. Occlusal lesions without gross cavitation that were judged by visual/tactile examination to extend to dentin were included. Large lesions that were visible on bitewing radiographs were excluded. Informed consent was obtained in accordance with the protocol approved by the Institutional Committee on Human Research.

NIR Imaging

An imaging probe was employed to acquire images from the occlusal surface. It consisted of a 25-mm objective lens, a ½ inch in diameter tube 5" long with a relay lens, a mirror and light delivery optics. A high sensitivity InGaAs (Indium gallium arsenide) imaging camera, Model SU320KTSX (Sensors Unlimited, Princeton, NJ) was used to collect all the images. For comfort and stability, the video camera/handpiece assembly was mounted onto the examiner's forearm as shown in Fig. 3 and the probes were held by the ½ tube of the probe. Since lesions are detected by differences in optical contrast uniform illumination is critical for NIR imaging. The probe shown in Fig. 3 is placed directly over the tooth and optical fibers coupled to Teflon optical diffusers in two arms directed the NIR light to just above the gingival tissues on the facial and lingual side of the tooth and the mirror directed the light to the imager. The system provided uniform illumination of the crown and the sound enamel was visible as a ring of higher intensity around the central dentin core of the tooth. Occlusal lesions were visible as dark areas in the occlusal pits and fissures. Light was provided by two 1310-nm superluminescent diodes (SLD) from (Optospeed, Zurich, Switzerland), with an output power of 15 mW and a 35-nm bandwidth. The power was determined empirically by experimenting with various settings and the bandwidth was chosen because the use of broadband SLD's reduces speckle noise and the related image degradation that is common with narrow bandwidth light sources. If it was necessary to remove bubbles on the teeth to be examined, they were gently dried with a stream of air from a dental unit air syringe and video (8-bit) was acquired as the imaging handpiece was passed over the tooth.

OCT Imaging

A single-mode fiber, autocorrelator-based Optical Coherence Domain Reflectometry (OCDR) system similar to the PS-OCT system described above was used for *in vivo* imaging. However, this system did not have polarization sensitivity. To enable the acquisition of *in vivo* images a low profile scanning stage was integrated with an optical-fiber probe, Fig. 4. The MM-3M-F-05 mini-stage from National Aperture Inc, was used with a MS-4CA Servo Amplifier system and Labview™ Software and the National Instruments PCI-7344 motion controller. This stage has a lateral scan range of 12.7-mm, sufficient to scan across the entire tooth with a speed of 6 mm/sec to avoid motion artifacts. The scanner has an outer sheath of delrin that is removable and autoclavable. A picture of a subject being scanned using the system is also shown in Fig. 4.

Restorative procedures

The teeth were restored with conventional dental instrumentation under local anesthesia, taking extra care to keep the cavity preparation minimal. This was accomplished by using the smallest possible size of the bur for the initial opening and using caries indicator dye (Caries Detector, Kuraray Co., LTD) to guide the operator in extending the cavity along the DEJ to just include the demineralized dentin and no additional tooth structure. The cavity preparation was not extended into neighboring pits and fissures (extension for prevention). Photographs of the tooth were taken before preparation, when the dentin caries lesion was visible and again when caries removal was complete. Additionally, an impression was taken

of the preparation for measurement of the size of the lesion. We were not able to make impressions of the smallest cavities.

RESULTS

OCT Studies on Extracted Teeth

PS-OCT scans show deep lesion zones penetrating to the DEJ for many of the samples. Cross polarization b-scan images along with the corresponding PLM and TMR images of the matching thin section are shown in Fig. 5. Each figure represents a cross polarization OCT b-scan, and PLM and TMR images from one matching section from one tooth sample. The black and gray boxes demarcate the areas of the OCT b-scan and the PLM image represented in the corresponding TMR image. In the OCT images demineralized zones or areas of high reflectance appear as white-red in this color scheme. In the PLM images demineralized zones appear as dark black areas.

One section from each tooth that was cut through the 2×2 mm window matching the position of the PS-OCT scan was selected for PLM and TMR analysis. The most severe lesion areas of the OCT scans were chosen for analysis. It is important to point out that in many areas of each section the OCT scans did not show the full depth of penetration of the lesion. A comparison of OCT and PS-OCT scans for each lesion showed that position of the maximum lesion depth was similar in both cases. The lesion contrast in the combined OCT image was not compared with the contrast of the cross polarization and co-polarization images. For most of the samples the corresponding OCT and TMR images correspond well showing that the increase in reflectivity follows the increase in mineral loss. Significant reflectivity is observed from below the DEJ in most of the images. Fig. 6 shows linear correlation plots comparing the lesion depth measured using OCT with the lesion depth measured using PLM and TMR. The correlation coefficient (Pearson) was $r = 0.63$ ($P < 0.01$) for OCT and PLM, and $r = 0.75$ ($P < 0.001$) between OCT & TMR.

In vivo NIR Transillumination and OCT of Occlusal Caries Lesions

Fifteen lesions in fifteen test subjects were examined using the occlusal NIR imaging system. All the lesions had sufficient contrast in the NIR to be visible in the recorded video. NIR images of two of the lesions are shown in Fig. 7. Since stains do not interfere in the NIR the dark areas are indicative of demineralization in depth. It is important to point out that the lesion contrast appears higher for the visible light photographs but that contrast is provided solely by stain, which is not a reliable indicator of lesion depth or severity.

Fourteen of the lesions were scanned using OCT. The scanner malfunctioned with one of the test subjects and we were unable to scan that particular lesion. In 12 out of the 14 test subjects (86%) with occlusal lesions that penetrated to the dentin, one or more of the OCT scans showed an area of high reflectivity at the center of the suspected lesion in the fissure with loss of reflectivity directly below the lesion in the OCT image along with a subsurface increase in reflectivity well below the surface to the left or right of the fissure near the location of the dentin-enamel junction (DEJ). Examples of this can be seen in the four OCT images shown in Fig. 8. There is typically high reflectivity from the top of the lesion in the fissure and the reflectivity rapidly drops off with depth in that area. However, there is a distinct rise in reflectivity to the left of the lesion well below the surface at the position of the DEJ as indicated by the arrows.

All of the lesions in the study were found to penetrate to the DEJ with stainable dentin demineralization present, but in some cases the penetration was minimal, with the final diameter of the preparation less than 1 mm. In the minimal cases it was not possible to obtain an impression of the cavity, as the impression material would not penetrate to the

bottom of the cavity. We obtained usable impressions of 11 cavities and measured the largest lateral dimension of the cavity on these, obtaining an average of 3.03 mm.

DISCUSSION

This study demonstrated that NIR transillumination and optical coherence tomography have great clinical potential for the nondestructive assessment of the severity of occlusal lesions and for the detection of hidden lesions under the enamel. Both techniques appear to be useful, in complementary ways. Transillumination appeared to be most useful for detecting the presence or absence of an occlusal lesion and differentiating lesions from stain. OCT appeared to be most useful for resolving the depth of enamel lesions and the spread of lesions below intact enamel.

All the lesions were visible in the NIR images. The approach of delivering the light at the gum line of the tooth and allowing it to diffuse up through the enamel works *in vivo* in vital teeth in a similar fashion to our *in vitro* studies. The underlying dentin and pulp do not appear to be overly dark due to excessive absorption of the incident NIR light indicating that the vital pulp does not interfere with the distribution of light under the crown. At other NIR wavelengths where water absorption is higher, such as 1460-nm, the strong absorption in dentin does cause it to appear very dark and reduces the contrast of occlusal lesions [38]. The contrast appeared lower than we had observed *in vitro* and we had difficulty focusing on the lesions due to the topography of the occlusal surfaces and the limited depth of focus of the optical system. In our previous *in vivo* NIR imaging study, we had less trouble viewing approximal lesions from the occlusal surface using the same probe, therefore we didn't modify our imaging probe to enhance the visibility of occlusal lesions for this study. However, we believe the performance can be increased considerably by increasing the depth of focus and adjustment of the illumination system. Moreover, we have achieved high contrast for higher resolution imagers and a recent study showed significantly higher contrast for a (640 × 400) focal plane array versus the (320 × 256) FPA used in this study [39].

The requisite optical penetration/imaging depth for the detection and diagnosis of occlusal lesions is to the DEJ. If the lesion is present in the underlying dentin and the enamel above is sound, OCT works quite well in resolving that lesion and the images confirm penetration to the DEJ. If extensive demineralization is present from the enamel surface all the way down to the DEJ the results are quite mixed, i.e., sometimes the entire lesion is visible from the enamel surface to the DEJ, while more typically only the outer surface of the lesion is visible or the area where the lesion has reached the DEJ (lower part) can be seen. Additional studies are needed to determine why the optical penetration depth through the lesions is so variable. Most lesions extend laterally along the DEJ upon reaching the underlying dentin, therefore we anticipate that OCT should be able to determine whether most lesions have reached the DEJ. However, because of the complex nature of the OCT images it will probably be necessary to acquire 3D images of each lesion or several b-scans across different regions of the lesion in order to make a reliable assessment of the lesion. Improvements in performance should be attainable with higher sensitivity systems along with a better signal to noise ratio. It is anticipated that future improvements in OCT technology in such areas should allow better resolution of the reflectivity from deeper layers within the tooth for even better performance.

One limitation of radiography is that ionizing radiation is required, however it is even more important to emphasize that radiography is insensitive for the detection of early occlusal lesions and that by the time they show up on a radiograph they have typically spread extensively throughout the dentin and are far too late for preventive intervention. For 12 out

of 14 of the lesions examined *in vivo* using OCT there was increased reflectivity below the DEJ which suggested that the lesions had spread to the dentin, considering that none of the lesions showed up on a radiograph, this is a remarkable improvement in sensitivity over existing technology. Therefore, it is likely that OCT can be used in a similar fashion as the Diagnodent, namely to show that there is hidden demineralization under the sound enamel and confirm that the lesion has penetrated beyond the DEJ. Moreover, OCT has the added advantage of producing an image showing the spread of demineralization as opposed to a single reading that does not indicate the depth or area of demineralization and stains do not absorb NIR light so there is minimal interference from stain.

The utility of PS-OCT for the measurement of early demineralization on tooth surfaces has been clearly established from previous studies [28,30,31,40]. However, the advantage of having polarization sensitivity was less evident for assessing the depth of deeply penetrating caries lesions, the aim of this study. Although the polarization sensitivity did provide an apparent improvement in contrast, most of the benefit is near the tooth surface. Polarization sensitivity also helps differentiate subsurface artifacts from lesions. However, PS-OCT systems are more expensive and difficult to use and this study suggests that it is not absolutely necessary to have polarization sensitivity to image deep occlusal lesions.

The correlation of OCT with PLM and TMR was encouraging. But it is important to stress the point that the comparison is based on the deepest lesion penetration in the OCT image and we did not match the specific volume or area of the lesions for each imaging modality. If the comparison was based on a random position of each lesion initially chosen from the PLM images the correlation coefficients are likely to be lower.

The dye was originally developed to differentiate between affected and infected dentin [41-43]. Degree of staining was found to be correlated with micromechanical properties of dentin [44], but there can be discrepancies between the color perception and the degree of demineralization [45]. Concern has also been expressed about over-excavation due to the dye staining dentin beyond the infected layer [46].

CONCLUSIONS

In summary, these measurements show that these NIR imaging methods can be used to show the structure, depth and severity of natural carious lesions in occlusal surfaces and they have considerable potential for non-destructive assessment of the severity of caries lesions in the important occlusal surfaces where most caries lesions are found.

Acknowledgments

This study was supported by NIH R01 grant R01-DE14698. We are grateful to Dr. Roger Pelzner and Dr. Ann Wei for help with patient recruitment.

This study was supported by NIH R01 grants R01-DE017869 and R01-DE14698

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Figure 1.

An extracted tooth with occlusal lesions mounted on the acrylic base is shown with a laser etched 2×2 mm square area for analysis before and after serial sections were cut for histological examination with PLM and TMR.

Figure 2.

The PS-OCT system showing the relative positions of the sample holder with the tooth, scanning stages and probe. The incident beam was aligned with one corner of the brass holder so that the scan position could be matched to the position of serial sectioning for histology.

Figure 3. Photograph of the NIR clinical imaging system with InGaAs imager with probe in use. Inset in upper right shows a diagram of the occlusal probe, which directs light via two teflon diffusers near the tooth roots and the light diffuses up through the crown.

Figure 4. Photograph of the clinical PS-OCT system in action. The handheld linear scanner is also shown without the outer Delrin sterilizable sheath.

Figure 5.

Image series of PS-OCT b-scans ($\perp$ -axis) (left), PLM (center), and TMR (right) for four different teeth, A-D. The lesion is demarcated by the black boxes in the OCT and PLM images. OCT images are shown in a red-white-blue false color scale in dB with high reflectivity in red.

Figure 6.

Lesion depths measured using OCT, PLM and TMR for 20 teeth. Correlation plots are also shown for (OCT & PLM, solid line) $r=0.63$ (Pearson), $P < 0.01$ and (OCT & TMR – dotted line) $r = 0.75$, $P < 0.001$.

Figure 7.
Photographs and NIR images of two of the occlusal lesions imaged *in vivo*.

Figure 8. Four OCT b-scans of occlusal lesions showing the higher reflectivity at the DEJ peripheral to each fissure shown by the upward pointing arrows. Downward pointing arrows demarcate the center of the fissure and lesion.