Title

Near Infrared Imaging as a Diagnostic Tool for Detecting Enamel Demineralization: An in vivo Study

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Near Infrared Imaging as a Diagnostic Tool for Detecting Enamel Demineralization: An *in vivo* Study

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THESIS

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[redacted]

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ABSTRACT

Background and Objectives: For decades there has been an effort to develop alternative optical methods of imaging dental decay utilizing non-ionizing radiation methods. The purpose of this *in-vivo study* was to demonstrate whether NIR can be used as a diagnostic tool to evaluate dental caries and to compare the sensitivity and specificity of this method with that of conventional methods, including bitewing x-rays and visual inspection.

Materials and Methods: 31 test subjects (n=31) from the UCSF orthodontic clinic undergoing orthodontic treatment with planned premolar extractions were recruited. Calibrated examiners performed caries detection examinations using conventional methods: bitewing radiographs and visual inspection. These findings were compared with the results from NIR examinations: transillumination and reflectance. To confirm the results found in the two different detection methods, a gold standard was used. After teeth were extracted, polarized light microscopy and transverse microradiography were performed.

Results: A total of 87 premolars were used in the study. NIR identified the occlusal lesions with a sensitivity of 71% and a specificity of 77%, whereas, the visual examination had a sensitivity of only 40% and a specifity of 39%. For interproximal lesions < halfway to DEJ, both NIR and x-rays were comparable. For lesions > halfway to DEJ, specificity remained constant, but sensitivity improved to 100% for NIR and 75% for x-rays.

Conclusions: The results of this preliminary study demonstrate that NIR is just as effective at detecting enamel interproximal lesions as standard dental x-rays. NIR was more effective at detecting occlusal lesions than visual examination alone. NIR shows promise as an alternative diagnostic tool to the conventional methods of x-rays and visual examination and provides a non-ionizing radiation technique.

TABLE OF CONTENTS

FIGURES AND TABLES

1. INTRODUCTION

1.1 History

Diagnostic methods for detecting dental decay have changed little over the years. Although nearly ubiquitous as the standard for dental offices, the conventional methods of detecting the presence of active dental decay in patients consists primarily of a combination of x-ray evaluation as well as a visual/tactile examination performed by the clinician. Some of the issues that have been discussed concerning these methods include the fact that x-rays tend to underestimate the extent of dental decay, not to mention that the diagnostic accuracy of radiographic caries is only around 70%.[1] In fact, one study demonstrated that the sensitivity for detecting interproximal lesions with bitewings was as low as 54%.[2] The diagnostic accuracy and sensitivity of radiographic occlusal caries is much worse, and because of this x-rays are not routinely used for detecting occlusal lesions. Perhaps more concerning to the patient, however, is the potentially harmful exposure to ionizing radiation that accompanies x-rays.[1] These conventional methods are outdated and the advent of new technology begs the question of whether there is not a more effective diagnostic tool that can be made available to the dental clinician.

Over the past several decades there has been a concerted effort to develop alternative optical methods for detecting dental caries. These methods include, but are not limited to, the following: 1) fiber optic transillumination; 2) quantitative light fluorescence; 3) optical coherence tomography; and 4) near-Infrared imaging (NIR).[3]

DiFOTI™ is an FDA-approved digital fiber optic system (Electro-Optical Sciences, Inc., New York) that transilluminates the tooth with visible light to capture images of dental caries. However, there is a strong scattering of light in enamel in the 400-700 nm wavelength (visible light), inhibiting imaging through the entire tooth. This makes DiFOTI™ of limited clinical use, and certainly not a replacement for our conventional methods, but perhaps as an ancillary tool. Another more recent fiber optic transillumination device is the DIAGNOcam™ (Kavo, Germany), which transmits light in the 780 nm wavelength through the tooth. However, there is still strong scattering in this wavelength.

The Diagnodent™ (Kavo, Germany) is a form of quantitative light fluorescence that uses red light to excite porphyrin fluorescence from bacterial byproducts in the pits and fissures of teeth. This can be used to discriminate between sound and demineralized tissues and quantify the result.[4] The major limitation of this technology is that it tends to detect lesions at the later stages of development, rather than discovering these lesions at an earlier stage so that they can be arrested before surgical intervention is necessary. It also has poor sensitivity (0.4) for early lesions that are confined to the enamel.[5] This is due to the fact that it does not directly measure or detect demineralization, but rather it identifies the presence of bacteria.

Optical coherence tomography (OCT) is an imaging modality that allows the clinician to capture cross-sectional images of the tooth in a non-invasive manner. Demineralization of the enamel was resolvable to a depth of up to 2-3 mm.[6] The limitation of OCT is that it is not well-suited for imaging interproximal lesions, and in order to acquire quality images of the entire tooth surface, would require too much time and an enormity of data. It may be best used to examine lesions after detection with some other diagnostic system, but it is not well-suited for caries screening in general. OCT may have a niche as an auxiliary tool to obtain specific depth measurements of identified lesions.[7]

Much like OCT, near-infrared imaging (NIR) is another recent development in the search for alternative imaging modalities. NIR imaging shows great promise as an effective diagnostic tool and can be used to capture high quality, high contrast images of both interproximal and occlusal enamel lesions. As mentioned earlier, the DIAGNOcam is a recently introduced commercially available NIR transillumination imaging system that utilizes light in the 780 nm range, but this is not ideally suited for imaging enamel lesions.

1.2 NIR Description

To understand how NIR can be used to evaluate enamel lesions, it is important to understand how NIR light propagates through the tooth and how the optical properties of the tooth change as a result of demineralization or tooth decay.[8] The fundamental factor that limits caries detection using visible light (400 to 700 nm) as an imaging modality (*i.e.,* DiFOTI), is light scattering. Light scattering in sound enamel in the visible range is enough to obscure the transmission of light through the tooth. The near-IR region from 780 to 1550 nm offers a potential for new optical imaging modalities due to the weak scattering and absorption in dental enamel. In fact, ballistic transmission measurements have demonstrated that enamel has the highest transparency in the near-IR region near 1310 nm.[9] This means that NIR light transmitted through a tooth will not be absorbed or scattered by the enamel unless there is an area of demineralization in the enamel, in which case, the light will be scattered and will show up as a corresponding darkened area in the affected site. Therefore, at junctions where sound enamel meets demineralization, there is a sharp contrast due to light scattering.

NIR can be used to detect carious lesions primarily in two different ways; 1) transillumination, and 2) reflectance. NIR transillumination involves the transmission of near-infrared light through the tooth. Interproximal caries lesions can be imaged by NIR transillumination of the proximal contact points between teeth and by directing NIR light below the crown while imaging the occlusal surface. The same approach can be used to image occlusal lesions. Recent studies show that NIR wavelengths longer than 1400-nm are likely to have better performance for imaging in reflectance while 1300-nm appears best for the transillumination. Wu and Fried [10] demonstrated that a reflectance imaging configuration is better suited to acquire high contrast images of early shallow lesions that are confined to the enamel surface. In the reflectance imaging configuration, the imaging system (camera) and the light source are positioned on the same side of the tooth.[7] Significantly higher contrast was attained for reflectance measurements at wavelengths that have higher water absorption, namely 1460-nm[9] as well as the 1500-1700 nm range.

The high transparency of NIR makes it a valuable tool for imaging carious lesions. However, there are many other advantages to NIR that make it an effective tool for detecting enamel demineralization. In NIR images of occlusal surfaces, stains are not visible since the organic molecules responsible for pigmentation absorb poorly. This makes it easier to differentiate occlusal stains from actual demineralization or occlusal caries.[2] In NIR imaging, slight developmental defects and shallow lesions appear differently than deeper and more severe lesions. This suggests that NIR may be used to estimate the depth or severity of a lesion by analyzing the tooth surface with both methods of NIR: transillumination and reflectance.[11] Lesions which appear with high contrast in reflectance images and do not appear in transillumination images are likely superficial or very shallow.

1.3 Caries and Orthodontics

A major concern of taking routine x-rays for evaluating dental decay is the ionizing radiation that the patient is exposed to in the process. For this reason, the American Dental Association (Chicago, IL, (ADA) in conjunction with the Federal Drug Administration (Washington DC, FDA) has developed recommendations for dental radiographic examinations. The frequency of prescribed x-rays for at-risk adults should be limited to only once every six to eighteen months. Children and pregnant mothers are advised to avoid exposure due to their high-risk.[12] This can reduce the effectiveness of using x-rays for evaluating dental lesions, especially in patients that are at high-risk for dental caries, because small lesions can propagate quickly in these patients.

A major advantage of NIR is that it employs non-ionizing radiation, therefore, there is no harm in taking images more frequently. With the field of dentistry moving towards a more preventive approach to treatment, this is a logical step in the right direction. Patients who are at high risk can have images taken more frequently. For small lesions that are identified early on, a remineralization regimen can be prescribed and serial scans can be taken at subsequent visits to monitor its progress

We see this as being especially beneficial for orthodontic patients, a dental population that is typically at higher risk due to the orthodontic appliances trapping plaque on the facial surfaces of the teeth.[13] Early “white spot” lesions can form on the facial surfaces of their teeth. *In vitro* studies have shown that NIR reflectance can be an effective tool for detecting these early lesions and possibly monitoring them throughout the course of treatment. The orthodontic wire can make flossing and brushing more difficult for patients, increasing their risk of interproximal caries. The brackets placed on the teeth may obstruct the x-ray images of the interproximal surfaces on teeth. NIR transillumination can be an effective tool to image these interproximal lesions from multiple angles, and of course, without the increased risk of radiation

1.4 Previous Studies

Many *in vitro* studies have been performed demonstrating the effectiveness of NIR imaging. However, clinical *in vivo* studies are lacking. To our knowledge, the first clinical study employing NIR imaging was completed by Staninec *et al.,* 2010; "In Vivo Near-IR Imaging of Approximal Dental Decay at 1,310 nm."[14] All previous studies were completed *in vitro. In vivo* NIR images of 33 proximal lesions on 15 test subjects at UCSF were taken. 32 out of 33 lesions (97%) that appeared on the bitewing (BW) radiographs appeared in the NIR image.[15] However, there were many areas on the NIR image that appeared demineralized that did not show up in the bitewing radiographs. These findings suggest that NIR may be more sensitive than BW radiographs. However, there is no present method to determine if these areas are truly demineralized without extracting the teeth, sectioning and examining them with microradiography.

A second *in vivo* study aimed to determine if NIR techniques can be used to effectively image occlusal lesions.[15] All similar previous studies were done *in vitro* on extracted teeth. Fifteen subjects that had occlusal lesions (which were already diagnosed) were recruited and all 15 lesions were visible in the NIR. The author concluded that NIR may serve as a powerful diagnostic tool for detecting occlusal caries, compared with BW x-rays, which are very poor at imaging them.

The results of these studies suggest that NIR light can be used as a clinical diagnostic tool and that it may also be more effective than conventional methods such as radiographic and visual inspection. The next logical step is to conduct *in vivo* studies in which NIR is used to find and detect lesions, which is also the objective of this thesis. The diagnostic performance will be evaluated and compared to conventional methods; this would include *sensitivity* and *specificity*.

1.4 Objective

These previous studies suggest that NIR images are more sensitive than radiographs. Therefore, to properly assess the diagnostic performance of these NIR imaging methods, we will require teeth to be extracted for analysis by transverse microradiography (TMR) and polarized light microscopy (PLM), the gold standards for evaluating and quantifying demineralization.

The objective of this study is to evaluate the efficacy of NIR as a clinical diagnostic tool and determine if it can be used as an alternative or auxiliary to our more conventional methods of evaluating demineralization.

2. MATERIAL AND METHODS

2.1 Patient Recruitment

We planned to recruit 40 human subjects from the San Francisco Bay Area to participate in our study. All the test subjects were solicited from UCSF clinics. The demographics of the patient population for the UCSF school of dentistry are listed as follows: By gender 50.7% are male and 49.3% female; the age distribution is 1.5% age 1-17, 9.1% age 18-24, 21.1% age 25-34, 24.3% age 35-44, 17.3% age 45-54, 10.5% 55-64, and 16.2% 65+; the Ethnic distribution is 49% White, 12% Black, 10% Asian and Pacific Islander, 11 % Hispanic (any race), and 18% other. Permission to carry out the testing was obtained from the Committee on Human Research (CHR) at UCSF and subjects were fully informed of the procedures. Risks and discomforts associated with imaging or exposure to NIR light should be negligible or absent.

A power analysis scaled from previous studies was performed to determine that 40 patients would be more than sufficient to detect a difference in demineralization if 10-20% of the premolars had demineralization in the occlusal grooves. However, due to time constraints only 31 of the recruited subjects were included for purposes of this thesis. Informed consent was obtained in accordance with the protocol approved by the Institutional Committee on Human Research (IRB # 11-08003). All subjects were current patients at the orthodontic clinic at the University of California San Francisco School of Dentistry and were scheduled to have premolars extracted for orthodontic purposes (for reasons unrelated to the study).

The inclusion criteria for the subjects were that the subjects must be within the ages of 13 to 60; they must be scheduled to have at least two premolars extracted; and the premolars must be erupted for at least one year. Exclusion criteria were subjects with any obvious rampant decay, and children 12 years of age or under. Third molars were not well suited for the study due to accessibility issues and concerns over failed eruption and impactions.

With all of the extracted premolars combined, we anticipated between 80 to 160 teeth to be used in this study.

2.2 Visual Examination

Before the teeth were extracted, a detailed visual examination was performed on each of the subjects to evaluate and discern the presence of occlusal lesions on each of the teeth that were planned for extraction.

In order to prevent bias and to blind our examiners to the subjects, high-definition still images were taken on each of the teeth to be evaluated at random at a later time. The examiner was calibrated using ICDAS (International Caries Detection & Assessment System) to evaluate the presence, absence, and severity of occlusal lesions.

Images were taken using a handheld digital microscope, Dino-Lite Premier AD7013MZT (AnMo Electronics Corporation, Taipei, Taiwan), which was equipped with cross polarizers. The sensor was a color Complementary Metal Oxide Semiconductor (CMOS) with a product resolution of 5.0 megapixels (2592 x 1944). Two occlusal images were taken of each tooth: one moist with saliva and the other was air-dried with an air-syringe for 5-seconds to eliminate bubbles and saliva from the occlusal grooves and fissures, according to ICDAS protocol.

ICDAS classification by visual examination was determined by calibrated examiners. Results from the visual examination were used to compare against the results from the NIR examination.

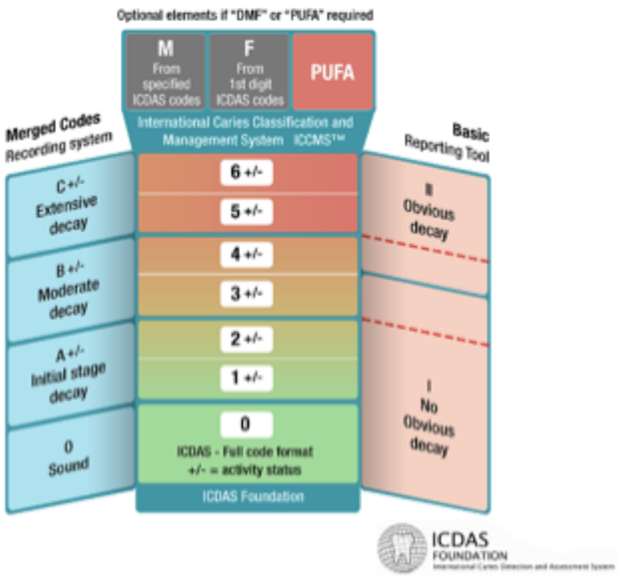

Figure 1. ICDAS tooth grading criteria

The International Caries Detection and Assessment System is an internationally recognized visual assessment scoring system that allows a tooth's health status to be graded numerically. Its application can be used in clinical practice, public health and clinical research, making it another tool in our systematic diagnostic examination (Figure 1). [16]

An ordinal scale of 0-6 is used to classify the severity of occlusal lesions. Lesions scored from 0-3 are confined to enamel, whereas lesions scored from 4-6 are dentinal lesions. Examiners were given images of the teeth in their wet and dry states and asked to give each tooth a score or code corresponding to the ICDAS criteria. The following caries codes were used: (0) no lesion present in wet or dry state (extrinsic stains do not count); (1) lesion limited to the pit or fissure; opacity not seen when surface is wet but is seen when lesion is air-dried for 5 seconds; (2) lesion can be seen in both wet and dry states; the white spot lesion or discoloration is wider than the natural fissure or fossa and not consistent with sound enamel; (3) localized enamel breakdown due to caries with no visible dentin; when wet the lesion appears white or discolored; when dry there is carious loss of tooth structure but no visible dentin; (4) underlying dark shadow from dentin is present and the shadow may appear grey, blue or brown; shadow of discolored dentin is visible through intact enamel that may or may not be broken down; more noticeable when surface is wet; (5) cavitation due to caries and opaque or discolored enamel exposing the dentin; extends less than half of the tooth surface; when wet there is darkening of the dentin that may be visible through enamel; when dry the tooth structure loss and demineralization can be seen at the pit or the fissure; (6) extensive, distinct cavity with visible dentin involving at least half of the tooth surface; obvious loss of tooth structure in the pit and fissure that may be deep.

As a basic reporting tool, a simple *I= no obvious decay*, or *II= obvious decay present* can be used. According to the ICDAS grading system, any tooth surface that is given a caries code of 3 or less is considered a I; whereas, any tooth that is given a caries code of 4 or greater is considered a II. This corresponds similarly with the World Health Organization's (WHO) basic reporting system that uses 0=no decay and 1=decay.

Examination protocol included the following steps: 1) ask the patient to remove any removable appliances; 2) clean the teeth of any visible plaque; 3) place cotton in the vestibule (if needed); 4) remove excess frothy saliva; 5) do a visual examination of surface as wet, 6) as a dry surface for 5 seconds; 7) visual inspection of the dry surface; 8) and explorers are not used.

No tactile examination using a dental explorer was used for the clinical examination. Although this is considered a conventional routine part of the examination for many dentists, it is no longer advised since it may cause damage to reversible initial lesions, thereby exacerbating an already existing condition. [17]

2.3 NIR Examination

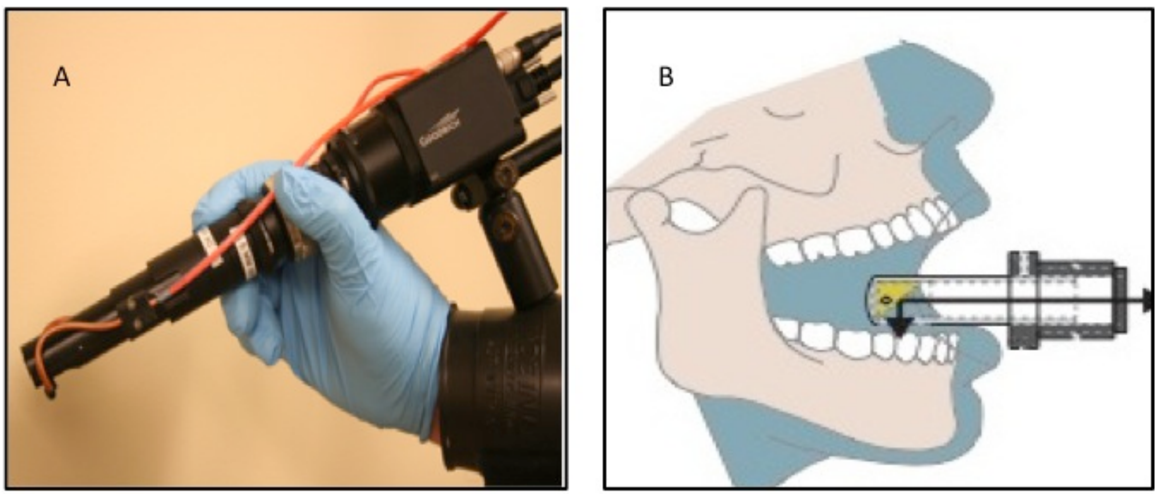

Figure 2. (**A**) Camera probe and handpiece mounted on examiner's forearm.
(**B**) Illustration of probe used intra-orally on subject

Before the teeth were extracted, a detailed NIR examination was performed *in-vivo* on the subject using three different probe configurations. The individual probes are designed to work interchangeably by attaching to a set of universal components connecting the probes to the NIR camera through relay optics. Two iris diaphragms, a 60-nm NIR achromatic doublet AC254-060-C and 150-nm NIR achromatic doublet AC254-150-C (Thor Labs, Newton, New Jersey) lenses project the captured light onto a high sensitivity SWIR (short-wave infrared) InGaAs (indium gallium arsenide) camera, Model GA1280J (Sensors Unlimited, Princeton, NK) with a 1280 x 1024 pixel format and 15-μm pixel pitch. A 360-degree rotation element allows simple flipping of the imaging probe to access the upper and lower teeth. The assembly is physically supported via an aluminum post attached to an elastic band wrapped around the forearm of the clinician. A power supply and output cables attached to the backside of the camera are connected to the data acquisition system through a flexible mesh bundle. Fiber optic cables and waveguides employed for optical delivery are carried in the mesh bundle to the imaging probes.

For comfort and stability, the video camera/handpiece assembly was mounted onto the examiner's forearm as shown in Figure 2-A and the probe end was held over each target premolar intraorally (Figure 2-B). Images were displayed on a monitor in real-time and the 12-bit video was acquired using a camera link interface up to 30 frames per second.

Reflectance

The first probe configuration was used to capture occlusal lesions using NIR-reflectance. With this configuration, the light source and the camera focal-plane array sensor were located on the same side of the tooth as shown in Figure 3.

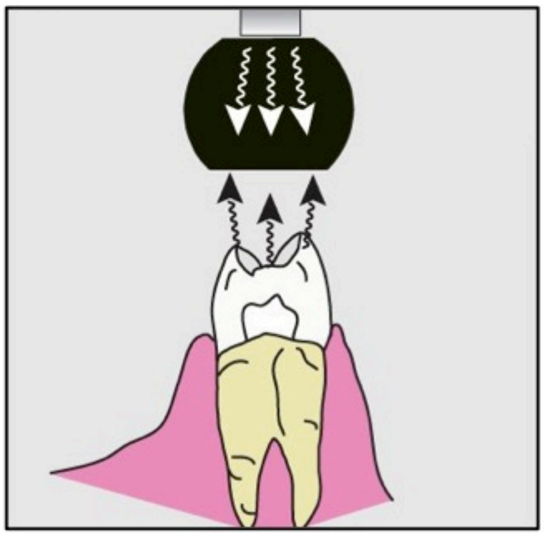

Figure 3. NIR imaging geometry of occlusal reflectance.

Initially, a NIR reflectance probe without cross-polarization elements was used to acquire *in vivo* images. For this probe, light centered at 1600-nm was generated using a super-luminescent laser diode with 50-nm bandwidth and Newport modular controller model 8008 and a fiber optic cable was used to deliver NIR light into a Teflon diffusing element. Light passed through the hollow the imaging probe and interacted with surface of the tooth where it was reflected or scattered back towards the relay right-angled mirror. Specular reflections from the tooth surfaces make identification of demineralized areas difficult. To overcome this phenomenon, crossed polarizers were used to reduce the amount of reflected light reaching the detector.

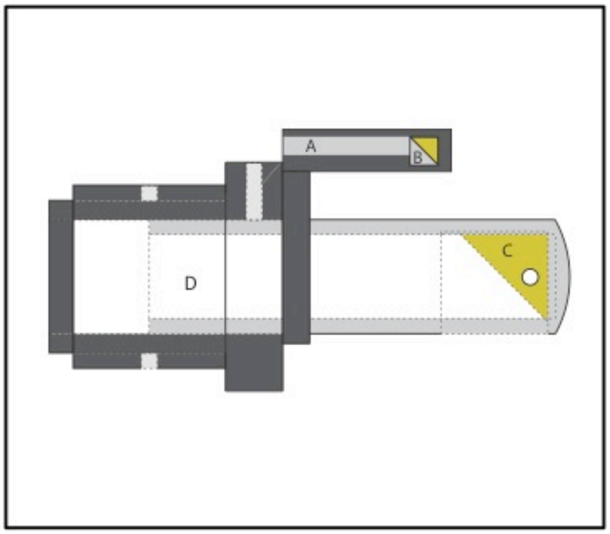

Figure 4. Reflectance probe with crossed polarizers (**A**) fiber optic bundle, (**B**) polarizing beamsplitter and (**C**) right-angle gold plated mirror, and (**D**) linear polarizer

A schematic drawing of the reflectance probe with crossed polarizers is shown in Fig. 4. Light from a tungsten-halogen lamp (model HL-2000, Ocean Optics, Dunedin, Florida) equipped with a long pass 1500-nm filter (FEL1500, Thor Labs) was used. The tungsten-halogen source replaced the SLD source used by the previous model in order to reduce speckle noise resulting from coherent inference of the polarized light. Wavelengths longer than 1500-nm were delivered to the probe through a glass fiber optic waveguide (Dolan-Jenner, Boxborough, MA) into the holder labeled A in Figure 4. The light from the waveguide passes through a 5-mm polarizing beam splitter model PBS053 (Thor Labs, Newton, NJ) (B) and linearly polarized light is reflected down through the hollow probe and onto the sample surface. The light interacts with the tooth and is reflected or scattered back on to a right-angled mirror (C) and the light is directed down the imaging tube and onto the focal plane array. A second 1" linear polarizer LPIRE-100-C (Thor Labs) oriented perpendicular to the first prevents the specular reflected light from reaching the detector. [18]

Snapshot images were extracted from the video for analysis by examiners. Areas of demineralization in the grooves and developmental defects on the cusps appeared in the images as gray-white in contrast to the sound enamel, which appeared black (Fig. 5).

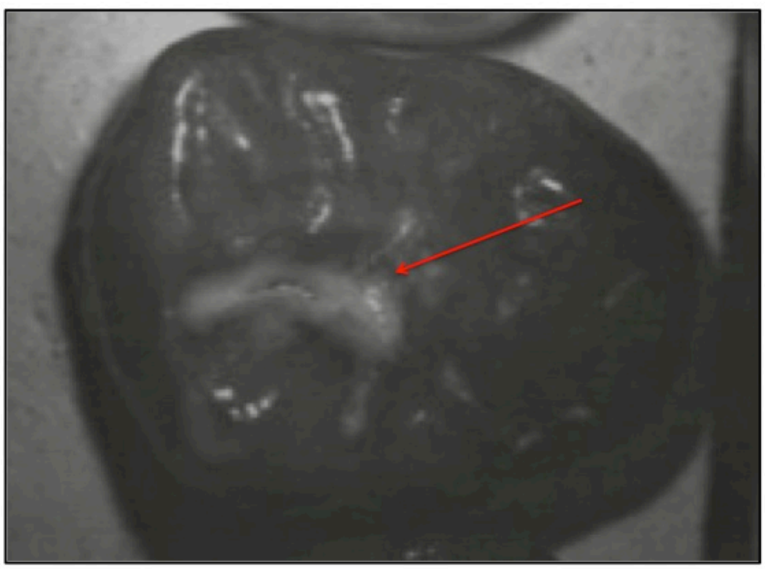

Figure 5. Occlusal image of premolar with NIR-reflectance. Heavy demineralization in grooves and fissures appears gray-white.

Occlusal-Transillumination

The optical design of Fig. 6 is used to uniformly illuminate the occlusal surface of the tooth. The probe is placed directly over the tooth and optical fibers coupled to Teflon optical diffusers in two arms, direct the NIR light to the gingival tissues on the facial and lingual side of the tooth and the mirror directs the light to the camera (see Fig. 7). NIR light at 1310 nm light is transmitted from the light source.

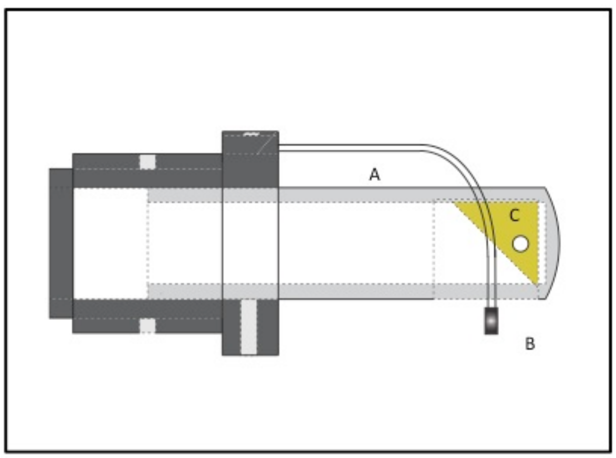

Figure 6. Occlusal transillumination probe with (**A**) optical fiber guide (copper tubing), (**B**) Teflon diffuser and (**C**) right-angle gold plated mirror.

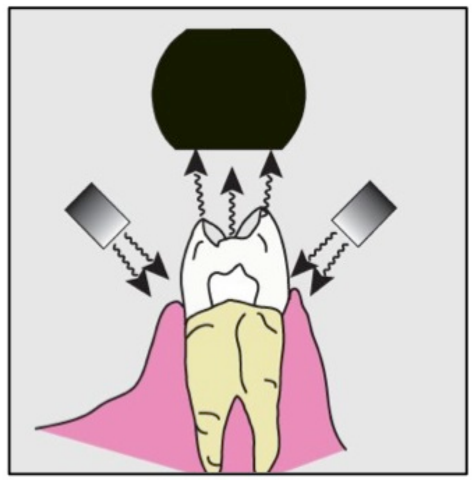

Figure 7. NIR imaging geometry of occlusal transillumination

If an interproximal lesion is present it appears as a dark area due to the attenuation of the light that is scattered from the underlying lesion (Fig. 8-A). The enamel is illuminated by the scattered light from the inner core of highly scattering dentin and the gingival tissues as the light propagates up from below. Interproximal lesions are visible from below the contact point on adjacent teeth even though the light has to propagate through several mm of enamel. The circumferential ring of transparent enamel surrounding the dark dentin core is broken by the interproximal lesion, making it easily discernible.

Occlusal lesions are also visible as dark areas in the occlusal pits and fissures as seen in Fig. 8-B. Occlusal demineralization, which shows up as a darkened area with NIR transillumination, is presumed to be deeper than demineralization that shows up only with reflectance imaging.

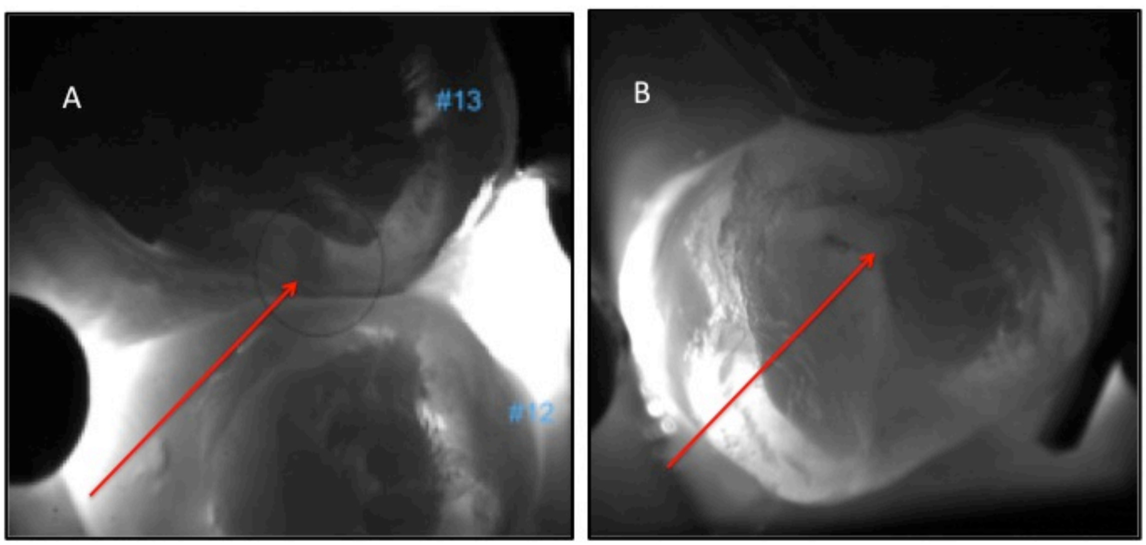

Figure 8. Occlusal NIR-transillumination of (**A**) interproximal lesion of maxillary premolar, and (**B**) occlusal lesion in central groove of maxillary premolar.

Interproximal-Transillumination

The interproximal or facial-lingual probe is shown in Fig. 9. An optical fiber delivers NIR light to the large Teflon diffuser placed on either the facial or lingual side of the tooth to provide uniform illumination of the contact point. The 1300 nm light transmitted from the light source passes through the tooth and is directed to the imaging camera with a mirror positioned on the opposite side of the tooth (see Fig. 10). The light source can be rotated for positioning on either the facial or lingual side of the tooth.

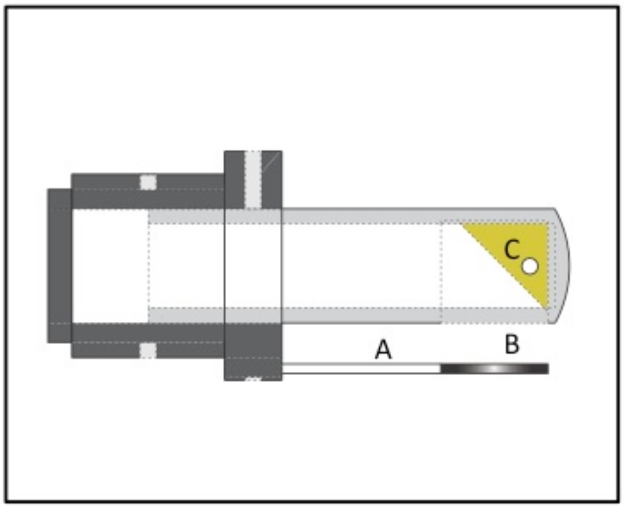

Figure 9. Interproximal transillumination probe with (A) optical fiber guide (clear tubing), (B) Teflon diffuser and (C) right-angle gold plated mirror.

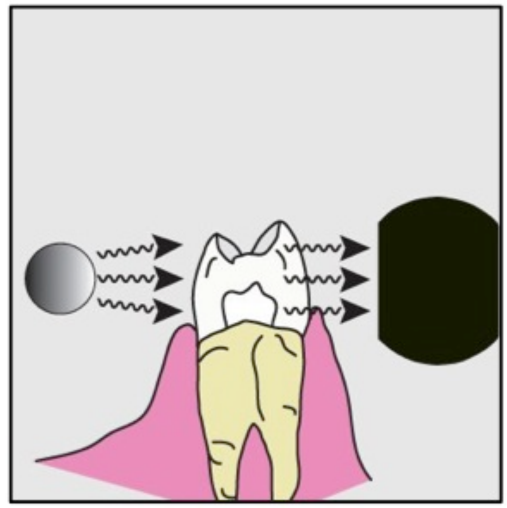

Figure 10. NIR imaging geometry of interproximal transillumination

The interproximal probe provides uniform illumination of the contact point between adjoining teeth for optimal lesion contrast and avoids saturation of the imaging camera. Fig. 11 shows NIR images of maxillary and mandibular premolars with a interproximal lesions. The lesions are clearly visible in the NIR with high contrast. Most interproximal lesions can be resolved by imaging the lesion from the occlusal surface with the occlusal probe. However, the redundancy of having two different probes, which show the lesion from different views, may afford greater accuracy in identifying lesions; some lesions are only visible in one of the configurations and not the other.

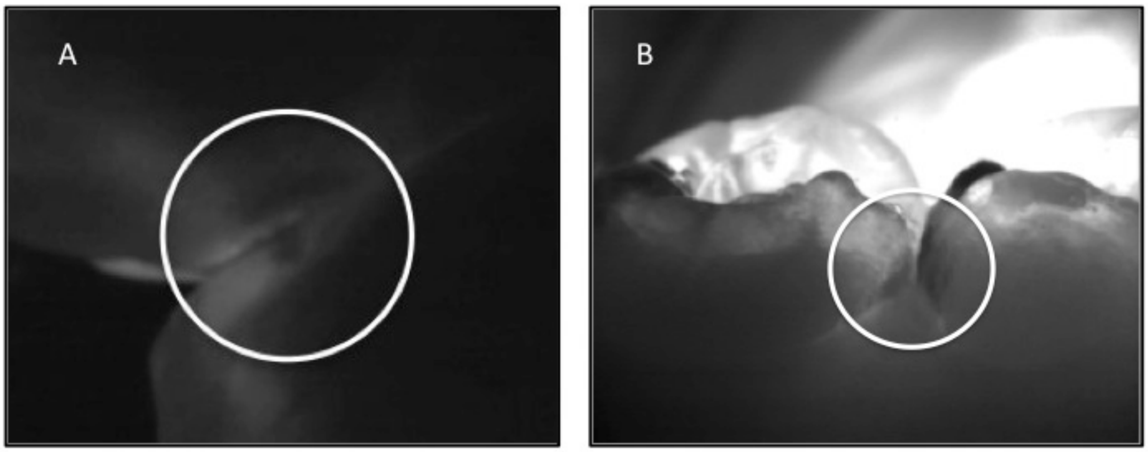

Figure 11. Proximal NIR-transillumination of (**A**) interproximal lesions of two maxillary premolars, and (**B**) interproximal lesion of mandibular premolar. The interproximal lesion appears as a darkened area in the enamel near the contact point. darkened area in the enamel near the contact point.

Light centered at 1300-nm is generated using a super-luminescent laser diode (SLD), SLD72 (COVEGA Corporation, Jessup, MD) with 50-nm bandwidth and Newport modular controller model 8008. The power was determined empirically by experimenting with various settings and broad bandwidth was chosen to avoid speckle and the related image degradation that is common with narrow bandwidth light sources.

After imaging, the severity of each proximal lesion was scored by review of the recorded video. The scoring system was as follows: (0) No lesion visible, (1) Lesion less than halfway through the enamel, (2) Lesion more than halfway through enamel, but not involving dentin, (3) Lesion involving dentin, but less than halfway to the pulp, (4) Cavitated lesions involving dentin, more than halfway to the pulp.

2.4 Radiographic Examination

All of the extracted premolars were imaged in a conventional bitewing configuration through the use of a digital x-ray system. Both mesial and distal interproximal contacts were visible on each tooth in the radiograph. The same settings were used for each tooth. Digital images were taken on a Kodak 2200 Intraoral x-ray system with settings of 70 kvp, 7 mA and 6 f (+/-). Interproximal lesions were evaluated from the corresponding bitewing radiograph. Examiners were blinded to the results from the NIR interproximal transillumination exam.

After imaging, the severity of each proximal lesion was scored by review of the bitewing radiograph. The scoring system was as follows: (0) No lesion visible, (1) Lesion less than halfway through the enamel, (2) Lesion more than halfway through enamel, but not involving dentin, (3) Lesion involving dentin, but less than halfway to the pulp, (4) Lesions involving dentin, more than halfway to the pulp. This scoring system corresponded exactly to that which was used in the NIR interproximal transillumination exam.

2.5 Sample Preparation for Histological Analysis using PLM and TMR

Extracted teeth from patients involved in the study were collected with approval from the UCSF Committee on Human Research, cleaned, sterilized with gamma radiation and stored in 0.1% thymol solution to preserve tissue hydration and prevent bacterial growth. The teeth were mounted on orthodontic resin blocks for consistency purposes, which were then imaged for radiographic evaluation and histological sectioning. After imaging for radiographic examination, teeth were cut into serial sections 200-µm thick using a linear precision saw, the IsoMet 5000 (Buehler, Lake Buff, IL).

2.6 PLM and TMR Analysis

In order to confirm our results from the conventional (visual & x-ray) and NIR examinations, a gold standard was needed for comparison. Polarized light microscopy and transverse microradiography have both been shown to be highly sensitive in determining enamel lesion depth and are acceptable gold standards.

Polarized Light Microscopy

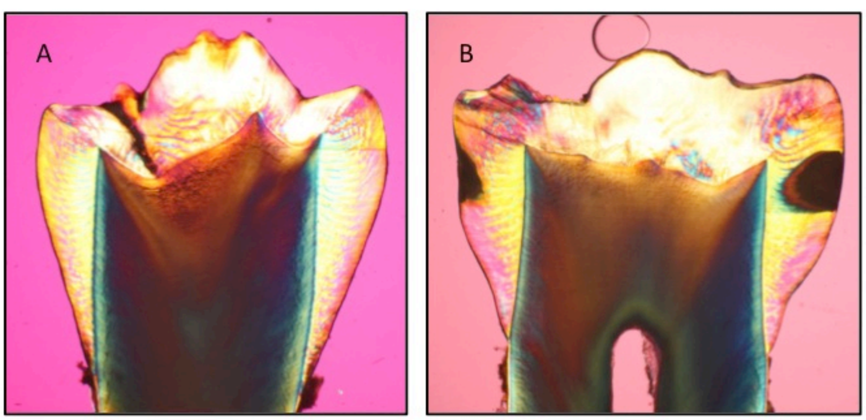

Figure 12. PLM histology slices of premolars with easily discernible (**A**) occlusal lesion extending nearly into the dentin and **(B**) bilateral interproximal lesions of different severity. One lesion is less than halfway to the DEJ and the other is more than halfway to the dentin.

PLM can be used to calculate the depth of a lesion. Opaque regions in PLM images of enamel indicate areas of demineralization and it is easy to distinguish transparent sound regions (Fig. 12).

Polarized light microscopy was carried out using a Meiji Techno RZT microscope (Saitama, Japan) with an integrated digital camera, Canon EOS Digital Rebel XT (Tokyo, Japan). The sample sections were imbibed in water and examined in the brightfield mode with crossed polarizers and a red I plate with 550-nm retardation. The histological sections were scored according to the maximum penetration of caries in each particular section matching the line-profiles used for the contrast measurements.

It is sometimes difficult to differentiate dentin lesions using PLM alone. For samples difficult to classify using PLM, TMR was used to verify.

Transverse Microradiography

TMR is a highly sensitive method used to measure change in mineral content in both enamel and dentin samples. It can be used to measure the volume percent mineral content in areas of demineralization in the different teeth sections. The mineral content can be automatically calculated from the gray levels of the images of each section.

A custom-built digital microradiography (DM) system was used to measure mineral loss in the natural caries lesions (Fig. 13). High-resolution microradiographs were taken using Cu K_a radiation from a Philips 3100 x-ray generator and a Photonics Science FDI x-ray digital imager (Microphotonics, Allentown, PA). The x-ray digital imager consists of a 1392 x 1040-pixel interline charge-coupled device (CCD) directly bonded to the coherent micro-fiber optic coupler that transfers the light from an optimized gadolinium oxysulphide scintillator to the CCD sensor. The pixel resolution is 2.1 um and images can be acquired in real time at a frame rate of 10fps. The image size is 2.99 X 2.24 mm with a pixel resolution of 2.15 um. A high-speed motion control system with Newport 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 (Fig. 14). [19] The experimental setup is shown in reference 19.

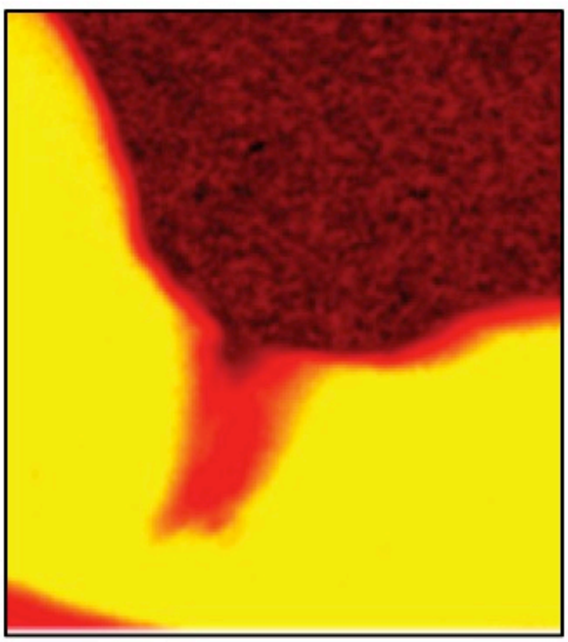

Figure 13. TMR image of premolar section showing occlusal lesion

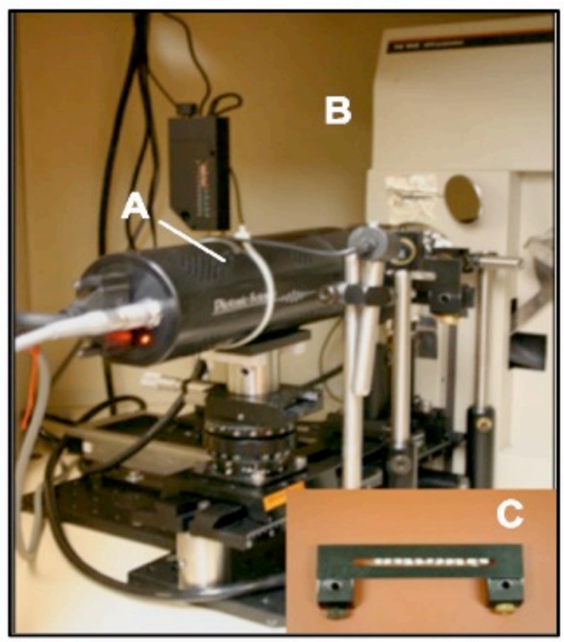

Figure 14. High-resolution digital x-ray microradiography system (TMR). (A) FDI x-ray imager with motion control system, (B) x-ray diffractometer, and (C) sample mount.

The combination of PLM and TMR examinations serve as a gold standard for lesion severity. By calculating sensitivity and specificity for each method used, we are able to compare the results from both the conventional (visual & radiographic) examinations and our NIR (reflectance and transillumination) examinations.

3. RESULTS

High-quality images were obtained for each subject for each premolar that was planned for extraction. In total there were 31 patients recruited to participate in the study. A total of 87 teeth were imaged according to the protocol outlined in the methods section.

Due to the limitations placed by time constraints, only the first 10 consecutive patients were used in the tabulation of raw data for calculating true positive rate (TPR) and false positive rate (FPR) for the ROC space plots. A total of 36 teeth were used for these calculations.

3.1 Occlusal Lesions

In the visual examination, a total of 32 teeth were identified as having occlusal demineralization according to the protocol outlined by the ICDAS criteria. Most of these lesions were small and were classified as having an ICDAS of only 1. In fact only 9 lesions were classified as having an ICDAS of >2.

In the NIR examination, we were able to detect a total of 47 occlusal lesions with the reflectance probe, giving an incidence of 54% of teeth with occlusal demineralization. This was heavily underestimated in our sample size calculation of 10-20% of teeth having lesions. Of these lesions, 30 were identifiable with the occlusal transillumination as well, suggesting that those lesions that were only seen in the reflectance and not the transillumination were shallow lesions.

Both conventional visual examination techniques as well as NIR examination techniques were compared against the gold standards of PLM and TMR to calculate sensitivity and specificity for both methods using the first 10 patients in our study.

$$\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}}$$

$$\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}}$$

Figure15. Sensitivity and Specificity calculations.

After calculating sensitivity and specificity for each technique, a receiver operating characteristic (ROC) space plot was calculated and graphed in Microsoft Excel. These ROC space plots depict the relative trade-offs between the two methods. The y-axis illustrates the sensitivity of each of the methods, whereas, the x-axis illustrates the inverse of the specificity of each method. The diagonal line represents a "random guess" and points plotted above and to the left of the line were considered better than a random guess. The further a plotted point was from the diagonal line in the appropriate direction, the more effective the diagnostic modality was at accurately detecting lesions.

For the visual examination, occlusal lesions were detected with a true positive rate (sensitivity) of only TPR= 0.4. The visual examination also only yielded a specificity of 0.39. This indicates that the accuracy of our visual examination was virtually no better than a random guess. NIR on the other hand, had a true positive rate of TPR= 0.71 and a specificity of 0.77 (see Fig. 16).

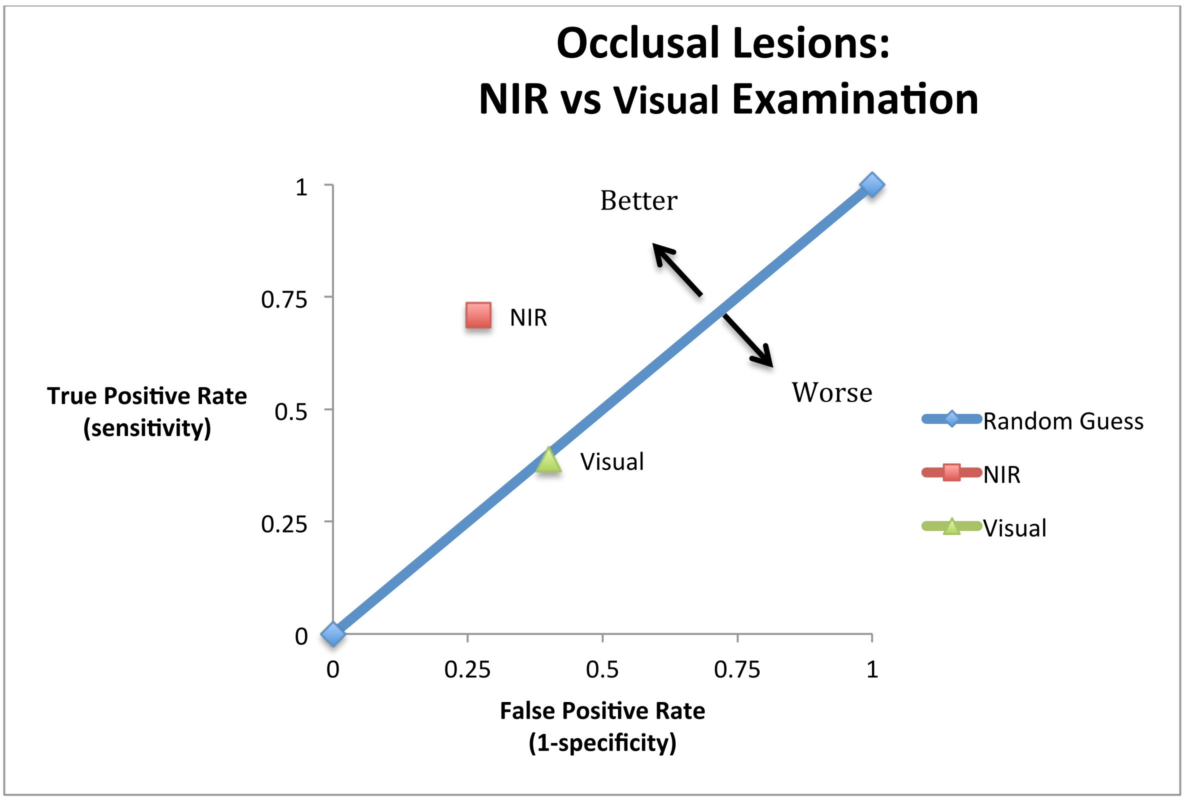

Figure 16. ROC space plot comparing NIR and visual examination in detection of occlusal lesions.

3.2 Proximal Lesions

There were much fewer interproximal lesions identified in both the conventional radiographic methods as well as NIR methods. 12 proximal lesions were identified in the NIR using either the Occlusal or Interproximal transillumination probe configurations. Once again, only the first 10 consecutive patients were used in the calculation of sensitivity and specificity to be used in the ROC space plots.

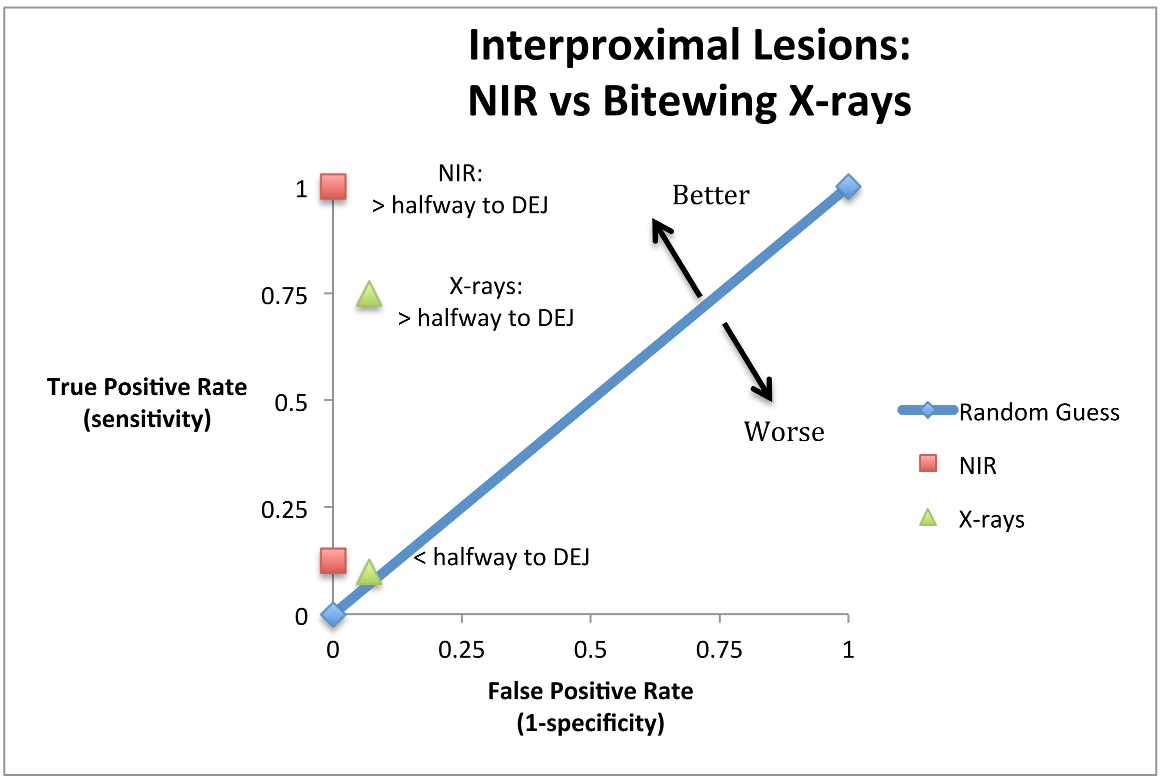

Figure 17. ROC space plot comparing NIR and radiographic examination in detection of interproximal lesions.

For the radiographic examination, proximal lesions that extended less than halfway through the enamel had a true positive rate (sensitivity) of only TPR=0.10. However, for lesions that extended more than halfway through the enamel, the true positive rate improved to TPR=0.5. Overall, the bitewing radiographs had a specificity of .75, indicating that they were fairly good at identifying lesions if they were actually present and without many false positives.

For the NIR examination, proximal lesions that extended less than halfway through the enamel had a true positive rate of only TPR=0.13. However, for lesions that extended more than halfway through the enamel, the NIR techniques were able to identify all of them, which yielded a sensitivity of 100%. NIR techniques also had very good specificity of 1.0. No lesions were identified that were not also present in the PLM gold standard. Both methods appeared to improve in both sensitivity and specificity as the proximal lesion got progressively deeper (see Fig. 17).

The results of this study demonstrate that the NIR techniques employed in this study appear to be just as effective in detecting demineralization lesions both interproximally and occlusally. In fact, NIR was more sensitive and specific than conventional methods in the detection of both interproximal lesions as well as occlusal lesions.

4. DISCUSSION

4.1 Explanation of results and findings

A major benefit of having several NIR imaging modalities is that it allows the clinician many different views of the same lesion. This redundancy can help reinforce a clinical diagnosis of a suspected lesion. The combination of reflectance, interproximal transillumination, and occlusal transillumination give the examiner ample tools to successfully detect a lesion (see Fig. 18).

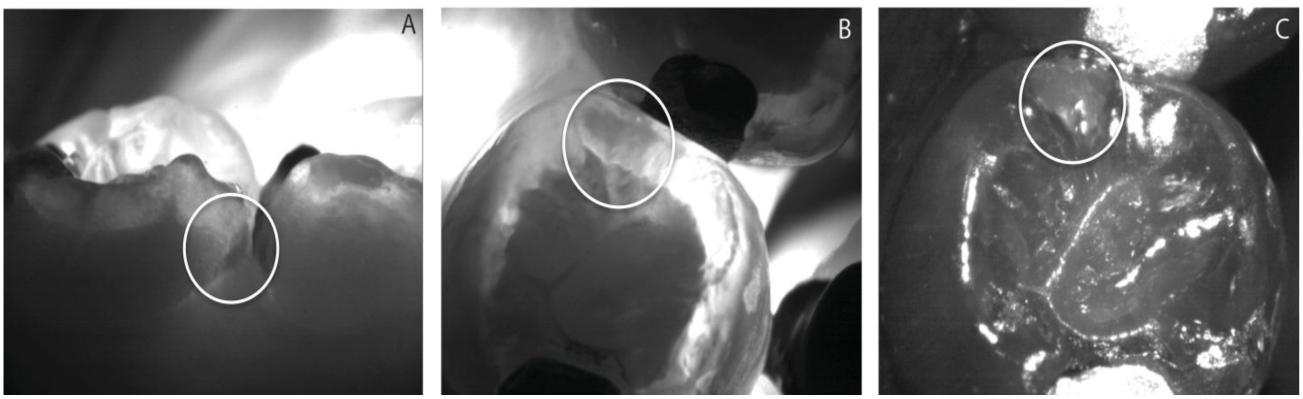

Figure 18. Interproximal lesion on a single tooth in 3 different NIR imaging modalities:(**A**) interproximal transillumination, (**B**) occlusal transillumination., and (**C**) reflectance.

A major drawback of solely using visual examination to detect occlusal demineralization is that staining in the occlusal pits and fissures can be misleading. The ROC plot in Fig. 16 demonstrates that simple visual examination was inferior in both detecting occlusal lesions as well as discriminating between true demineralization and simply staining. The fact that staining can be confounding could help explain the increase in the false positive rate in visual examination. Occlusal stains did not show up in the NIR (see Fig. 19).

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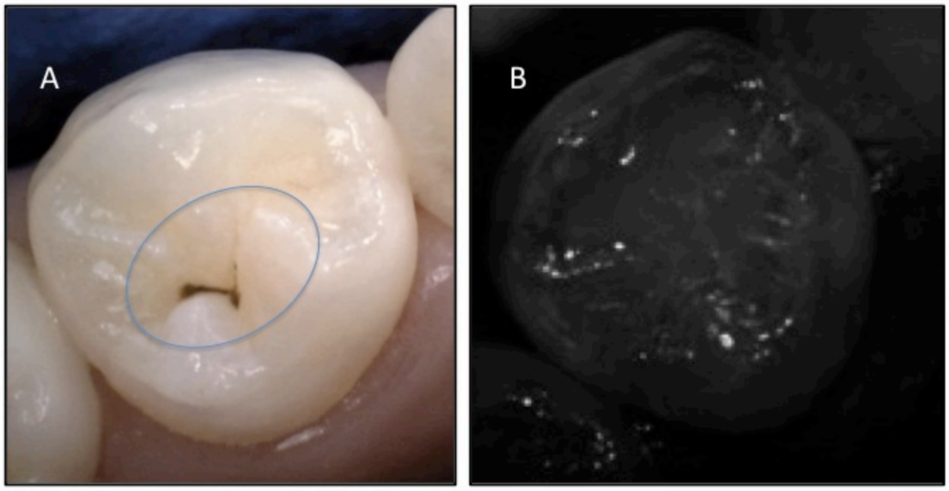

Figure 19. Confounding occlusal pit and fissure staining does not show up in the near-infrared. (**A**) Dark staining presumed to be carious lesion. (**B**) NIR shows that there is no lesion present.

The NIR-reflectance probe was especially beneficial for identifying early occlusal demineralization. The ROC plot demonstrates that visual examination was only fairly sensitive when it came to identifying occlusal lesions and had several more false negatives.

4.2 Complications of study

There were several unanticipated complications in imaging our subjects in this study. The primary complication involved imaging interproximal lesions with our interproximal NIR transillumination probe, and may have had a significant impact on our results. Because our subject population was mostly adolescent to young adult, the patients' gingival heights were very healthy and very few patients presented with reduced periodontium. This made it more challenging to transilluminate the NIR light through the contact points to image interproximal lesions. In previous *in vitro* studies, this was not a concern because teeth were mounted in resin blocks to be imaged and there was no confounding gingival collar to obstruct the view. This was also less of a concern in older patients who had reduced periodontium or gingival recession (see Fig. 20). Since most proximal lesions are usually below the interproximal contact point, this could account for why we were not able to able to view some of the suspected proximal lesions with the proximal configuration of the NIR-transillumination probe (see Fig. 21-B). However, those proximal lesions that appeared in both the occlusal and proximal configurations had a good likelihood of being true positives. We think that the added redundancy of having two different NIR-transillumination configurations is of great value.

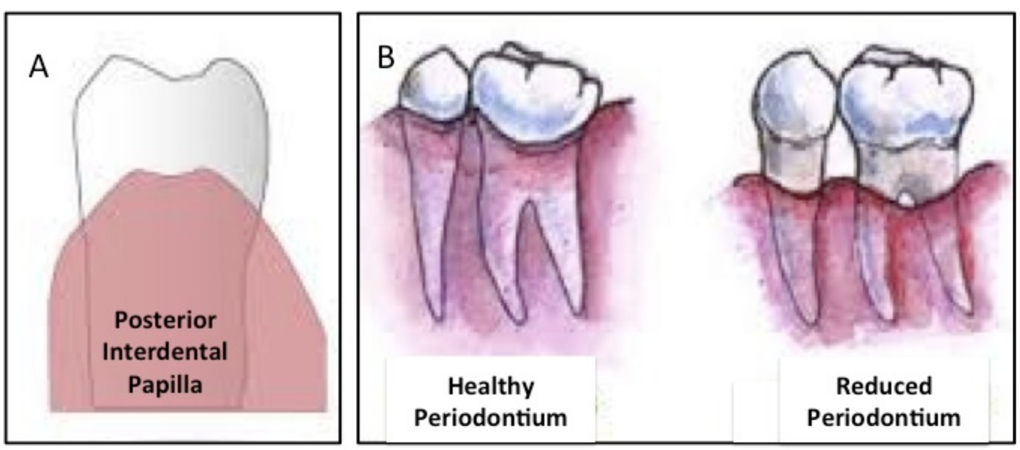

Figure 20. (**A**) Illustration of posterior interdental papilla present in between gingiva of healthy adolescents. (**B**) Illustration depicting changes in posterior gingival height patients due to disease or physiological aging.

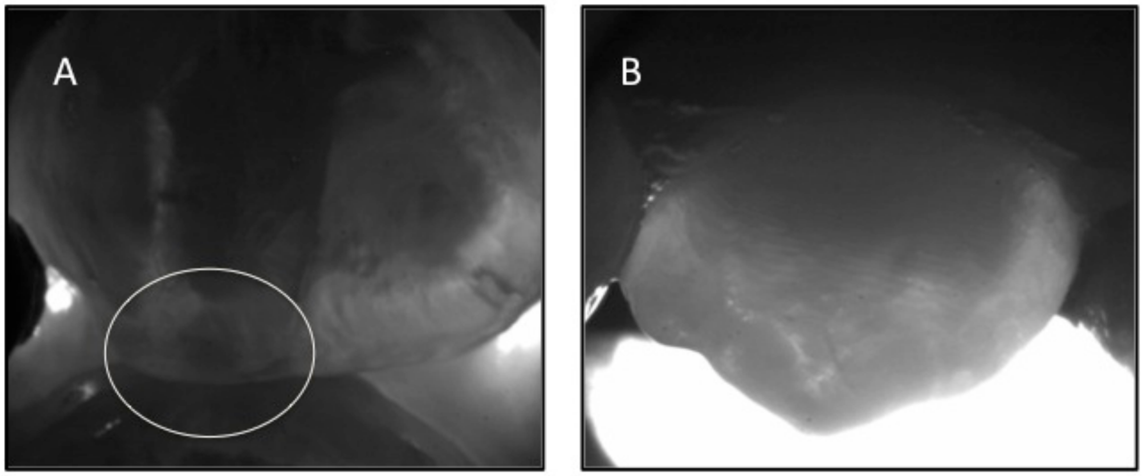

Figure 21. (**A**) Interproximal lesion of maxillary premolar can be seen in occlusal transillumination configuration. (**B**) The same lesion cannot be seen in the proximal configuration due to the high interdental papilla obstructing the view.

Other complications of our study involved the pool of patients we drew from to recruit our subjects. It is well understood that many orthodontic patients receiving prescribed extractions for orthodontic purposes have severe crowding of the dental arches. NIR in-vitro studies performed before this study did not encounter this problem because teeth were not imaged to simulate the conditions of a crowded dental arch. Our subjects were all orthodontic patients at the UCSF orthodontic clinic and, therefore, many patients had very crowded dental arches with overlapping teeth (see Fig. 22). This made it extremely difficult to image

Figure 22. Many patients had crowded dental arches that made it difficult to image. In most cases we were still able to capture high quality images using the NIR probes.

certain teeth with our probes. All in all, the NIR probes were still quite effective in capturing high-quality images even in the most crowded dentitions.

We recruited our subjects from a population of patients in the Bay Area, where water is fluoridated to a level of up 0.7 ppm. Although we view public water fluoridation as generally beneficial in most cases, many young patients at UCSF present with hypomineralized areas on the lobes, cusps, and smooth surfaces of the teeth that is presumed to be fluorosis. These fluorosed areas can be confounding because they appear very similar to true demineralization.[20] Fluorosis typically does not appear in the same location on a tooth as true enamel demineralization, which is usually in the occlusal grooves, interproximal surfaces and cervical margins of teeth (see Fig. 23). We used our clinical judgment to not include areas of fluorosis when evaluating premolars for demineralization. Occasionally we imaged a premolar with so much hypomineralization that it made it difficult to distinguish between fluorosis or actual demineralization.

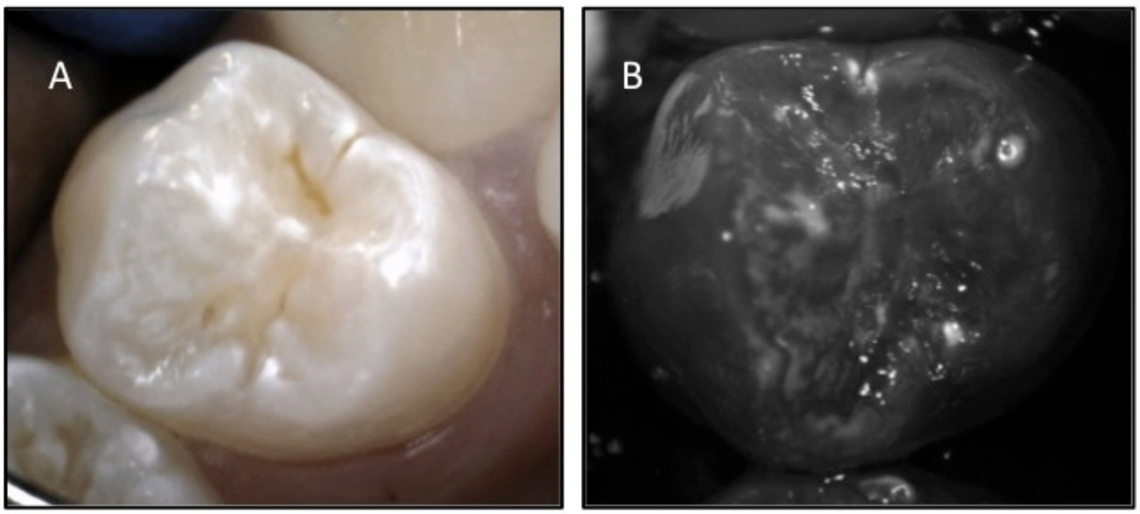

Figure 23. Areas that are hypomineralized due to fluorosis (**A**) appear similar to demineralization in the NIR but in different areas (**B**).

4.3 Points of Interest

During the process of imaging the premolars of all of our subjects, there were several notable findings we came across that were not included as part of the study protocol.

Although the NIR-reflectance probe was used to evaluate demineralization on the occlusal surfaces of teeth, it was surprising how effective it was at imaging areas with calcified plaque or calculus. These areas showed up as bright white spots contrasted by the dark black appearance of the enamel (see Fig. 24).

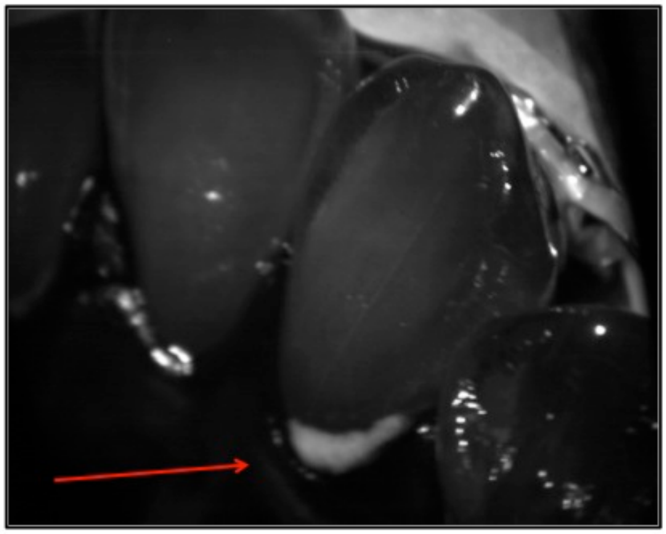

Figure 24. Calculus shows up as bright white area in NIR

Some composite restorations are so well color-matched and with such good margins & shape that they can even be difficult to distinguish from the adjacent natural tooth structure. During our clinical visual examination there were some premolars in which we were unable to recognize that a composite restoration was present. It was not until we imaged the same tooth with our NIR protocol that it became clearly evident that a composite restoration was present (see Fig. 25).

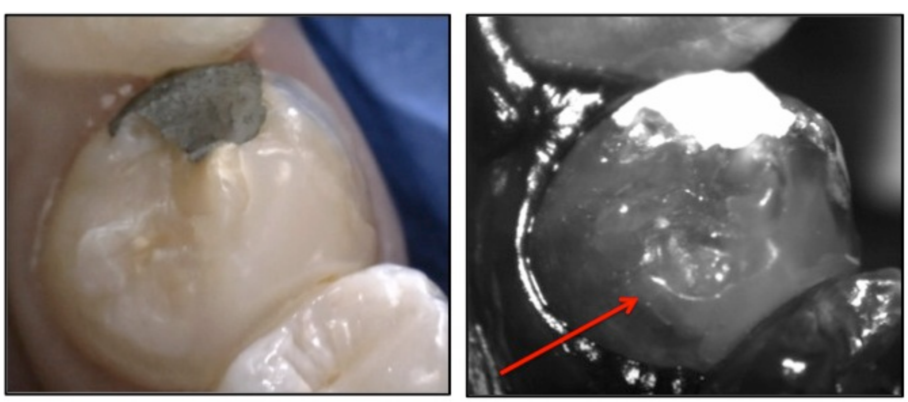

Figure 25. Composite may not show up in the visible (**A**), but is evident with the NIR (**B**)

In one of the premolars from the study there was an interesting lesion in the central groove that presented with an ICDAS score of 2 or 3, which was one of the more notable lesions we identified in our visual clinical examination. However, in the NIR-reflectance the lesion, the lesion had a peculiar appearance similar to that of calculus. When this same tooth was imaged with NIR-transillumination with the occlusal probe it did not appear, indicating that it was either a very shallow lesion (despite what the visual examination showed) or that it was in fact calculus (see Fig. 26).

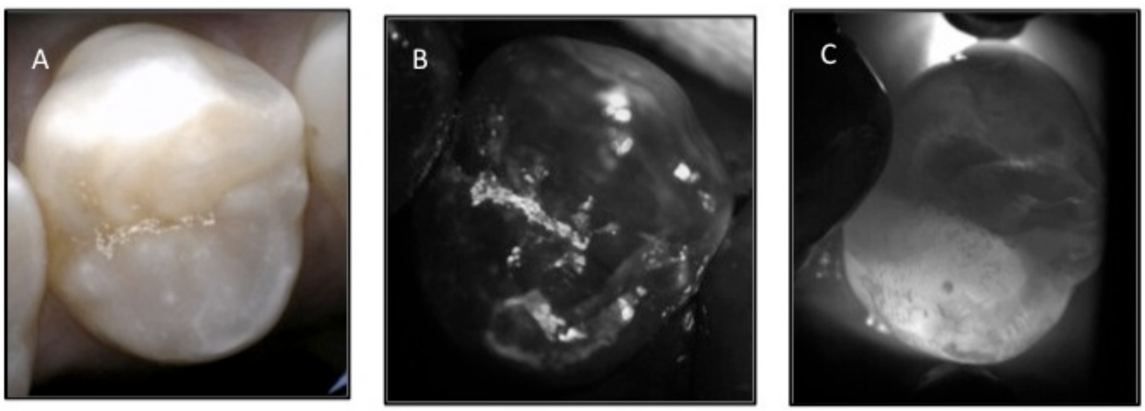

Figure 26. Visible (**A**) and NIR-reflectance (**B**) showed what appeared to be demineralization in the central groove. NIR transillumination (**C**) did not show presence of a lesion, indicating that it was either very shallow or actually calcified

We came across several teeth with suspected lesions that were not included as part of the study. Although these lesions could clearly be seen with the NIR probes, they were not extracted and therefore no PLM or TMR could be performed on them to confirm our observations. For example, an obvious interproximal lesion could be seen on the mesial of the maxillary premolar shown in Fig. 27 with both the occlusal and interproximal configurations. Once the adjacent tooth was extracted, an intraoral photograph was taken of the mesial surface to help confirm the actual presence of a lesion. A definite white spot lesion was present on the mesial approximal surface of the tooth, thereby, supporting our observation (see Fig. 27-B).

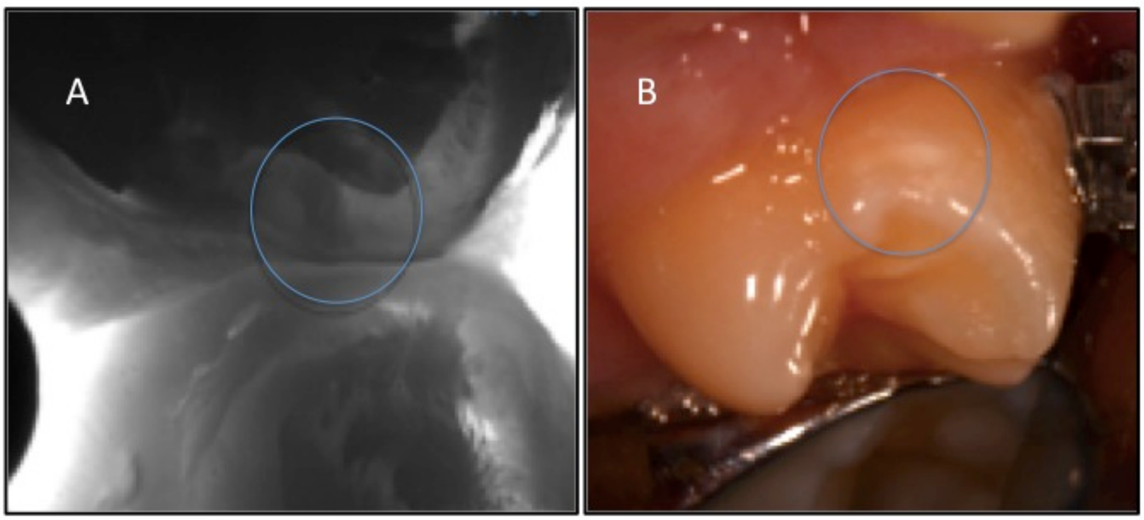

Figure 27. Interproximal lesion detected on non-extracted premolar (**A**) confirmed when adjacent tooth was removed (**B**).

One of our subjects had bilateral enamel defects on the mesiobuccal cusps of both maxillary 1st molars. These areas appeared as suspicious carious lesions upon visual examination but appeared with much less contrast than anticipated in the NIR, suggesting that these areas may be non-carious enamel defects; possibly congenital defects (see Fig. 28). NIR may serve as a useful tool in evaluating many other congenital defects and dental anomalies; an area not yet heavily explored with this technology.

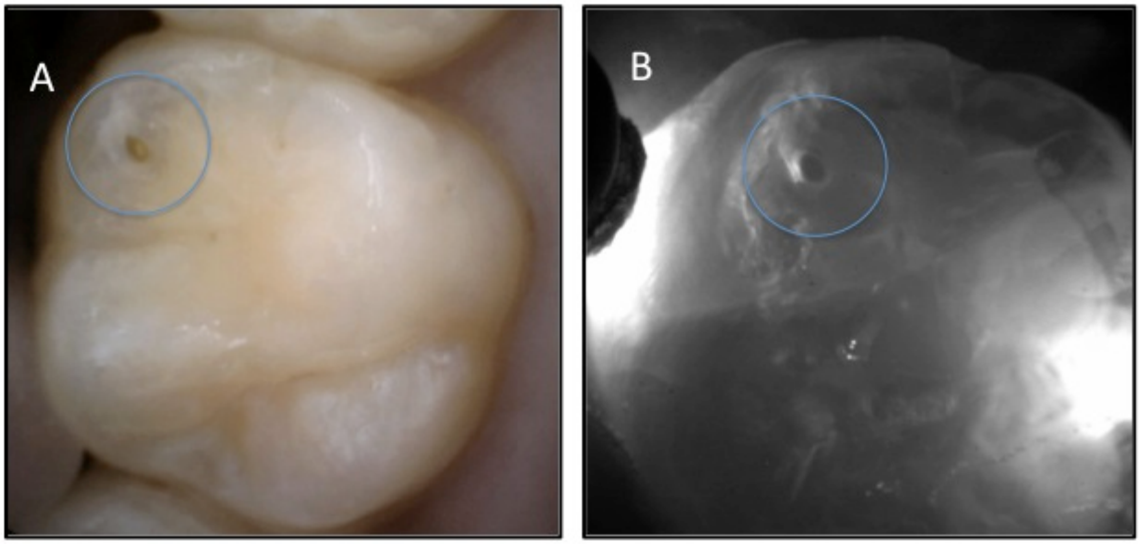

Figure 28. Congenital defect present on the mesiobuccal cusp of the maxillary first molar. Shown in the visual examination (**A**), and in the NIR (**B**).

5. CONCLUSIONS

In this *in vivo* study we evaluated the accuracy of near-infrared as an imaging modality for detecting occlusal and proximal lesions. We compared NIR detection methods of transillumination and reflectance with that of more conventional methods that dentists have used for decades, including bitewing x-rays and visual examination.

Results from our study showed that NIR was more sensitive and specific than their conventional counterparts in detecting both occlusal and proximal lesions.

NIR shows great promise as a diagnostic tool for detecting early demineralization and carious lesions. There are many other dental applications using NIR that should be explored further.

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