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***In vitro* near-infrared imaging of natural secondary caries**

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Abstract

Secondary caries stands as the leading reason for the failure of composite restorations and dentists spend more time replacing existing restorations than placing new ones. Current clinical strategies, and even modern visible light methods designed to detect decay, lack the sensitivity to distinguish incipient lesions, are confounded by staining on the surface and within the tooth, or are limited to detecting decay on the tooth surface. Near-IR (NIR) imaging methods, such as NIR reflectance and transillumination imaging, and optical coherence tomography are promising strategies for imaging secondary caries. Wavelengths longer than 1300-nm avoid interference from stain and exploit the greater transparency of sound enamel and dental composites, to provide increased contrast with demineralized tissues and improved imaging depth. The purpose of this study was to determine whether NIR transillumination ($\lambda=1300\text{-nm}$) and NIR cross-polarized reflectance ($\lambda=1500\text{--}1700\text{-nm}$) images can serve as reliable indicators of demineralization surrounding composite restorations. Twelve composite margins ($n=12$) consisting of class I, II & V restorations were chosen from ten extracted teeth. The samples were imaged *in vitro* using NIR transillumination and reflectance, polarization sensitive optical coherence tomography (PS-OCT) and a high-magnification digital visible light microscope. Samples were serially sectioned into $200\text{-}\mu\text{m}$ slices for histological analysis using polarized light microscopy (PLM) and transverse microradiography (TMR). The results presented demonstrate the utility of NIR light for detecting recurrent decay and suggest that NIR images could be a reliable screening tool used in conjunction with PS-OCT for the detection and diagnosis of secondary caries.

Keywords

near-IR imaging; secondary caries; recurrent caries; detection; reflectance; transillumination

1. INTRODUCTION

Recurrent dental decay persists as the major etiologic factor in the annual failure rate of composite restorations and prompts dentists to spend more time replacing restorations than placing new ones [1, 2]. The most practiced clinical methods for evaluating demineralized tissue surrounding restorations are visual inspection for tissue discoloration and tactile examination with an explorer or periodontal probe at the restoration margins. Despite this common practice, extensive research by Kidd et al. and others suggests that neither

discoloration nor ditching (gaps distances $< 500\text{-}\mu\text{m}$ between restorative material and adjacent tissue) are reliable indicators of the integrity of the enamel or dentin, and a considerable number of restorations are unnecessarily replaced using such criteria [2–6]. Additionally, utilizing tactile examination to diagnose decay presents the risk of damaging vulnerable demineralized tissue that has the potential to remineralize its superficial region through fluoride treatment. In order to improve the accuracy of secondary caries diagnosis, new and reliable indicators of demineralized dental tissues adjacent to and along restorations accompanied with an assessment of the lesion activity are required [7–9].

NIR imaging modalities encompass a range of non-ionizing imaging techniques that may serve as reliable indicators of recurrent decay and facilitate more accurate and consistent diagnosis of secondary caries. Enamel manifests its greatest transparency from 1300–1700-nm in wavelength, and those wavelengths are most sensitive to changes in tooth mineralization based on changes in the scattering coefficient of the illuminated tissue. Compared to visible light, NIR light beyond 1300-nm exhibits a $\sim 20\times\text{--}30\times$ reduction in scattering in sound enamel, provides increased tissue transparency and optical penetration depth, and produces images of lesions with significantly higher contrast [10]. Imaging with NIR light also avoids the absorption bands of organic stain allowing direct imaging of decay beneath stained surfaces such as the occlusal grooves.

Recent studies have investigated the use of polarization sensitive optical coherence tomography (PS-OCT), at $\sim 1300\text{-nm}$, for the detection of demineralization beneath sealants and composites in addition to primary lesions [11–14]. These experiments demonstrated PS-OCT's ability to produce three-dimensional data sets that accurately indicate demineralized tissue surrounding restorations. Furthermore, PS-OCT measures the integrated reflectivity of the tissue, R , a physical quantity that directly correlates with Z , the percent mineral loss of the tissue. Although PS-OCT is a powerful imaging technique, the vast amounts of data produced from each scan and inherent difficulty of presenting and comprehending three-dimensional data sets make it poorly suited as a caries-screening tool. Another challenge is that the scanning area is relatively small compared to the size of the tooth such that you cannot 'see' the entire tooth anatomy, and increasing the scanning area increases the size of the data set and computational burden.

NIR two-dimensional imaging modalities have demonstrated high contrast identification of early surface demineralization using 1460-nm light in a reflectance geometry and sub-surface lesions using 1300-nm light in transillumination geometries [15–18]. Many composite restorative materials are translucent to NIR light and allow demineralization to be imaged directly through the restorations [19, 20]. A technique with these abilities should be ideally suited for the detection of recurrent caries in both locations; the surface enamel and the enamel of the cavity wall as defined by Kidd et al. These lesions are classified as either *outer lesions*, primary lesions that develop from the surface adjacent to the restoration, or *wall lesions*, decay along the enamel of the cavity as a result of microleakage or ditching.

NIR two-dimensional imaging exists as a promising clinical screening tool for the detection of demineralized tissue with the advantage of real-time video acquisition and presentation with simple interpretation. If proven as a reliable, sensitive and high contrast indicator of

recurrent decay, NIR imaging would be a viable rapid screening tool for secondary caries. This study used cross-polarization NIR reflectance and direct or indirect NIR transillumination geometries to capture NIR images of natural recurrent decay using an InGaAs camera. These images were compared with PS-OCT 2D ($\perp$) integrated reflectivity C-scan projections, PS-OCT ($\perp$) B-scans, and visible images. Polarized light and transverse microradiography images were obtained from thin sections produced by serial sectioning. Normalized NIR cross-polarized reflectance and NIR transillumination image line profiles were overlaid with TMR percent mineral line profiles across the region of interest for each sample.

2. MATERIALS and METHODS

2.1 Sample Preparation

Twelve composite margin segments (n=12) were selected from ten extracted teeth with existing restorations for participation in this study. Nine posterior teeth and one canine were collected from patients in the San Francisco Bay area with approval from the UCSF Committee on Human Research. The teeth were sterilized using gamma radiation and stored in 0.1% thymol solution to maintain tissue hydration and prevent bacterial growth.

A CO₂ laser (Impact 2500, GSI Lumonics Rugby, UK) was used to produce small incisions forming the outline of a 4×4-mm square around the region of interest (ROI) and serve as fiducial marks for PS-OCT scans. The laser was modified to produce a Gaussian output beam (single spatial mode) and pulse duration of between 10–15- μ s at a wavelength of 9.3- μ m. The laser was operated at a pulse repetition rate of ~ 220-Hz, with a spot size of ~750- μ m and a 150- μ m overlap between laser spots. The tooth was cooled with a low volume/low pressure air-actuated fluid spray delivery system that provided a uniform mist at a rate of 0.5-mL/min at room temperature.

After NIR images, PS-OCT scans and examiner analysis were performed, the samples were serially sectioned into ~200- μ m thick slices using a linear precision saw, Isomet 5000 (Buehler, Lake Buff, IL) perpendicular to the restoration margin. Thin sections were subjected to histological examination with polarized light microscopy and transverse microradiography.

2.2 High Resolution Digital Microscopy

Images were taken of the sample region of interest using a digital microscopy/3D surface profilometry system, the VHX-1000 from Keyence (Elmwood, NJ) outfitted with the VH-Z25 lens (magnification from 25–175 $\times$). Depth composition images were generated by translating the image plane of the objective along the vertical axis capturing a series of images containing all points of the tooth surface in optimum focus, followed by construction of an optimized 2D image using only in focus image regions from the series.

2.3 NIR Transillumination and NIR Cross-Polarized Reflectance

A high sensitivity, InGaAs, SWIR camera (SU320-KTSX-1.7RT/RS170) from Sensors Unlimited (Princeton, NJ), with a 320 $\times$ 256 pixel focal plane array and 25- μ m pixel pitch

was used to capture NIR cross-polarized reflectance, cross-polarized transverse transillumination and occlusal transillumination images. CP-reflectance images were acquired using polarized light from a tungsten halogen lamp (E Light) and capturing the reflected signal through an orthogonal linear polarizer, long pass 1500–1700-nm optical filter and lens system. CP-transverse transillumination images were acquired by focusing (40-mm NIR AC ThorLabs, Newton, New Jersey) polarized light from a tungsten halogen lamp laterally onto the sample and collecting the transmitted signal through an orthogonal linear polarizer, band pass 1300-nm optical filter (Spectragon, FWHM=80-nm) and lens system. Occlusal transillumination images utilized light from a tungsten halogen source and bifurcated fiber optic bundle equipped with two linear arrays to impart light angled apically at the cementoenamel junction from both sides of the tooth and collect transmitted signal through a band pass 1300-nm optical filter and lens system. Detailed schematics covering the geometry of each modality and theory of the resulting image contrast can be found in previous publications [21, 22].

NIR images were analyzed using the image analysis package provided by IgorPRO software (Wavemetrics, Lake Oswego, OR). Image line profiles 5 pixels in width were extracted from the sample ROI across the same sample plane for NIR reflectance and transillumination images. The line profiles were normalized from zero to 1, where 1 represents the maximum contrast value and zero the minimum across the ROI.

2.4 Polarization Sensitive Optical Coherence Tomography

An all-fiber-based optical coherence domain reflectometry (OCDR) system was used with polarization maintaining (PM) optical fibers, high-speed piezoelectric fiber-stretchers and two balanced InGaAs receivers that was designed and fabricated by Optiphase, Inc., Van Nuys, CA. The two-channel system was integrated with a broadband superluminescent diode (SLD) Denselight (Jessup, MD) and a high-speed XY-scanning system (ESP 300 controller and 850G-HS stages, National Instruments, Austin, TX) for *in vitro* optical coherence tomography. The high power (15-mW) polarized SLD source, emitted near-IR light at a center wavelength of 1317-nm with a spectral bandwidth full-width at half-maximum (FWHM) of 84-nm. The beam was focused onto the sample surface using a 20-mm focal length AR-coated planoconvex lens. This configuration provided lateral and axial resolutions of approximately 20- μ m and 10- μ m in air with a signal to noise ratio of greater than 40–50-dB. When imaging enamel tissues with refractive index $n \approx 1.63$, spatial resolution is increased relative to air due to the tissues greater refractive index [23]. The PS-OCT system is completely controlled using Labview software (National Instruments, Austin, TX).

2.5 CP-OCT Integrated Reflectivity Collapsed C-scan 2D Projections

Raw OCT data was analyzed using a custom written program in Labview (National Instruments, Austin, TX). Co-polarization and cross-polarization (CP-OCT) OCT images were acquired for each sample, however only the cross-polarization images were processed and analyzed. For speckle noise reduction, signals not exceeding four standard deviations from the mean background noise floor were reduced to the mean background value and a Gaussian blur smoothing algorithm was applied using a 5 $\times$ 5 pixel convolution kernel. In the

edge-detection approach, the enamel edge and the lower lesion boundary were determined by applying an edge locator. The program first locates the maximum of each a-scan, and differentiates the a-scan maximum as either demineralized or sound using the signal-to-noise ratio as a threshold. The lesion depth is calculated by locating the upper and lower lesion boundaries, calculated by determining the first pixel that does not satisfy the threshold of e^{-2} of the maximum value. The distance per pixel conversion factor was obtained experimentally by system calibration. A linear relationship was established between the OCT lesion depth and the histological depths measured using polarized light microscopy (PLM). Based on this relationship, a linear correction was applied to the lesion depth calculated from OCT (Corrected Data $1/4 \ 1.55 \text{ OCT pixel depth} - 66.2$).

Each A-scan of the CP-OCT images was reduced to single values representing the mean reflectivity per pixel integrated over the calculated lesion depth. The mean reflectivity per pixel was calculated by dividing the sum of each A-scan in linear intensity units (IU) by the number of pixels in each A-scan. The integrated reflectivity ($\bar{R}$) was calculated by integrating the reflectivity in IU units over lesion depth calculated as described above. 2D maps, referred to as collapsed C-scans were produced with each pixel representing the integrated reflectivity at each pixel.

2.6 Polarized Light Microscopy (PLM)

Polarized light microscopy (PLM) was used for histological examination using a Meiji Techno RZT microscope (Saitama, Japan) with an integrated digital camera, Canon EOS Digital Rebel XT (Tokyo, Japan). Sample sections 200- μm thick are imbibed in deionized water and examined in the brightfield mode with crossed polarizers and a red I plate with 550-nm retardation.

2.7 Digital Transverse Microradiography (TMR)

A custom-built digital TMR system was used to measure mineral loss in the different partitions of the sample. 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 $86.3 \pm 1.9 \text{ vol.\%}$ mineral varying from 50 to 300- μm in thickness using IgorPRO image analysis software. The calibration curve was validated via comparison with cross-sectional microhardness measurements, $r^2 = 0.99$ [24].

TMR images were analyzed using the image analysis package provided by IgorPRO software (Wavemetrics, Lake Oswego, OR). Image line profiles 100–150 pixels in width were extracted from the sample ROI representing the percent mineral at each pixel.

3. RESULTS AND DISCUSSION

Near-infrared images of composite restoration margin segments ($n=12$) revealed the presence of natural recurrent decay as high contrast optical signals from both wall and outer lesions. Polarization sensitive optical coherence tomography (PS-OCT) nondestructively

confirmed demineralized areas and validated the signals obtained in the NIR images through integrated reflectivity (R) collapsed C-scans that profiled the lesion's variation with depth from the surface with B-scans. Polarized light microscope (PLM) images of 200- μm cross-sections provided histological evidence of the presence of decay and were utilized to classify the lesion type. Transverse microradiography (TMR) images of the same cross-sections were used as the "gold standard" for confirming the presence of recurrent decay, reported as the volume percent mineral per pixel.

Figure 1 contains images obtained from a tooth with a class I composite restoration presenting a secondary outer lesion. This sample has a crack emanating from the margin, see image A of Fig. 1, that has peripheral demineralization. The mineral content in this area is reduced to 75%, see images E & G (sound enamel is ~83% mineral). The lesion penetrates from the surface of the enamel, through the dentinoenamel junction, into the dentin at the restoration margin and extends to the buccal surface, D. The appearance of the lesion in the NIR reflectance and transillumination B & C images and the PS-OCT F & H images was consistent with the PLM and TMR histology. The line profiles extracted from the NIR and TMR images are overlaid in G depicting how the change in NIR image contrast correlates with the decreased tissue mineral content.

The demineralized region defined by the TMR curve, labeled lesion in G, results in an increase in the reflectance signal (blue line) and a decrease in the transillumination signal (red line). This indicates that the lesion is both superficial, demonstrating a NIR reflectance signal, and deep, demonstrating a NIR transillumination signal, as confirmed by histology.

Figure 2 shows the diagnostic and histological images obtained from a sample with a class II composite restoration with a recurrent wall, or more accurately, a floor lesion. The decay exists at the proximal surface of the margin and penetrates into the dentin of the sample at approximately 50% mineral content, see D & E of Fig. 2. NIR reflectance and transillumination B & C images manifest areas of high contrast across the suspected margin coincident with the TMR profile G. Interestingly, the NIR transillumination signal does not present itself in the adjacent enamel or simply at the margin, but rather displays scattering information from beneath or behind the composite. This signal suggests that there may be residual decay left by the clinician, or as the PLM image in E indicates, results from the geometry of the wall lesion. Additionally, the NIR image B demonstrates the significant benefit of using NIR based detection when challenged with a NIR transparent composite.

The sample depicted in Fig. 3 presents as a class V composite restoration with superficial recurrent decay confined to the enamel of the tooth. Image E shows the profile of the lesion, which varies between 70–80% mineral content, E & G. The lesion is only visible in NIR reflectance C due to its very shallow profile and cervical location on the tooth. Line profiles extracted from the NIR images and TMR image are overlaid in G and indicate no change in the NIR transillumination signal but very high contrast in reflectance along the demineralized TMR curve. The recurrent decay is present in the 2D, R compressed image F and is shown to be beneath a small layer of composite in the PS-OCT image H which correlates well with the PLM and TMR histology.

This study shows the potential of two-dimensional near-infrared images as reliable indicators of recurrent decay from either wall or outer secondary caries lesions. The CP-OCT integrated reflectivity (R), collapsed C-scans are analogous to the NIR reflectance and transillumination images since they represent the overall reflectivity of the lesion. The similarities between the NIR reflectance images and collapsed C-scan OCT images in Figs. 1–3 is striking in terms of contrast and spatial features and the NIR transillumination images are similar yet reverse in contrast. Due to the promise of PS-OCT in dentistry, and previous demonstrations of the instruments utility in the diagnosis of secondary caries, the similarities in 2D NIR imaging and collapsed C-scan OCT data sets suggest that NIR images are suitable for fast screening of anterior and posterior teeth [11, 13]. Furthermore, the transparency of enamel and composite in the wavelength range of 1300–1700-nm will allow evaluation of dental tissue beneath or behind the restoration as demonstrated in Fig. 2.

Acknowledgments

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Fig. 1.

(A) High magnification digital image (25× magnification) (B) NIR transillumination image (C) NIR cross-polarized reflectance image (D) Polarized light microscope image (40× magnification) of 200 micron cross section (E) Transverse microradiography of cross section (F) 2D projection of the integrated reflectivity (G) Line profiles (NIR trans- Red, NIR ref-Blue, TMR- Black) normalized from 1-0 (% mineral for TMR, normalized image NIR pixel values) (H) PS-OCT B-scan.

Fig. 2.

(A) High magnification digital image (25× magnification) (B) NIR transillumination image (C) NIR cross-polarized reflectance image (D) Polarized light microscope image (40× magnification) of 200 micron cross section (E) Transverse microradiography of cross section (F) 2D projection of the integrated reflectivity (G) Line profiles (NIR trans- Red, NIR ref-Blue, TMR- Black) normalized from 1-0 (% mineral for TMR, normalized image NIR pixel values) (H) PS-OCT B-scan. NIR Line profiles, TMR and PLM cross-section, and PS-OCT B-scan locations are indicated by red arrows.

Fig. 3.

(A) High magnification digital image (25× magnification) (B) NIR transillumination image (C) NIR cross-polarized reflectance image (D) Polarized light microscope image (40× magnification) of 200 micron cross section (E) Transverse microradiography of cross section (F) 2D projection of the integrated reflectivity (G) Line profiles (NIR trans- Red, NIR ref-Blue, TMR- Black) normalized from 1-0 (% mineral for TMR, normalized image NIR pixel values) (H) PS-OCT B-scan. NIR Line profiles, TMR and PLM cross-section, and PS-OCT B-scan locations are indicated by red arrows.