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Imaging Early Demineralization on Tooth Occlusal Surfaces with a High Definition InGaAs Camera

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Abstract

In vivo and *in vitro* studies have shown that high contrast images of tooth demineralization can be acquired in the near-IR due to the high transparency of dental enamel. The purpose of this study is to compare the lesion contrast in reflectance at near-IR wavelengths coincident with high water absorption with those in the visible, the near-IR at 1300-nm and with fluorescence measurements for early lesions in occlusal surfaces. Twenty-four human molars were used in this *in vitro* study. Teeth were painted with an acid-resistant varnish, leaving a 4×4 mm window in the occlusal surface of each tooth exposed for demineralization. Artificial lesions were produced in the exposed windows after 1 & 2-day exposure to a demineralizing solution at pH 4.5. Lesions were imaged using NIR reflectance at 3 wavelengths, 1310, 1460 and 1600-nm using a high definition InGaAs camera. Visible light reflectance, and fluorescence with 405-nm excitation and detection at wavelengths greater than 500-nm were also used to acquire images for comparison. Crossed polarizers were used for reflectance measurements to reduce interference from specular reflectance. The contrast of both the 24 hr and 48 hr lesions were significantly higher ($P<0.05$) for NIR reflectance imaging at 1460-nm and 1600-nm than it was for NIR reflectance imaging at 1300-nm, visible reflectance imaging, and fluorescence. The results of this study suggest that NIR reflectance measurements at longer near-IR wavelengths coincident with higher water absorption are better suited for imaging early caries lesions.

Keywords

Near-IR imaging; enamel; demineralization; dental caries; polarization; artificial lesions

1. INTRODUCTION

The caries process is potentially preventable and curable. If carious 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[1, 2]. Therefore, one cannot overstate the importance of detecting the decay in the early stage of development at which point non-invasive preventive measures can be taken to halt further decay. New imaging methods that provide improved contrast between sound and

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demineralized enamel will increase the clinician's ability to detect early caries lesions, assess the efficacy of intervention and identify high caries risk.

Optical imaging methods used for caries detection exploit either directly or indirectly (fluorescence) the increased light scattering in enamel caused by demineralization. Early enamel white spot lesions can be discriminated from sound enamel by visual observation or by visible-light diffuse reflectance imaging [3, 4]. Very early lesions can be detected visually, however color in addition to the intensity of the reflected light plays a large role in detecting those changes and such changes are difficult to quantify, moreover the color of sound tooth structure varies markedly. Specular reflectance is also a problem since enamel has a high refractive index. However, the visibility of scattering structures on highly reflective surfaces such as teeth can be enhanced by use of crossed polarizers to remove the glare from the surface [5, 6]. The contrast between sound and demineralized enamel can be further enhanced by depolarization of the scattered light in the area of demineralized enamel [7, 8]. A more difficult problem to overcome is visible light absorption due to stains. In a recent study of natural lesions in the occlusal surfaces of extracted teeth, the image contrast was actually negative as opposed to being positive in visible reflectance measurements indicating that absorption due to stains contributed more than increased scattering due to demineralization to the reflectivity in the visible[9]. This renders the method useless in areas that are subject to heavy staining, namely the areas where most lesions are likely to develop. In fact, visible light reflectance was proposed two decades ago for use in monitoring early demineralization on tooth surfaces but has proven to be unsuccessful due to the problems indicated above[4].

Laser induced fluorescence (LIF) or quantitative light fluorescence (QLF) has been used extensively to quantify the severity of incipient caries lesions [10-17] and aid in early detection. Major advantages of fluorescence include the removal of interference from specular refection; since the light detected is at a different wavelength to the incident light, and the interference of color variations in sound teeth would likely plays a minimal role. QLF measures the loss of the enamel native fluorescence as the lesion increases in severity [18]. The pores in the lesion formed due to demineralization, scatter and attenuate fluorescence that originates from the underlying sound enamel and dentin. The fluorescence loss (%) has been compared with the mineral loss integrated with depth, ΔZ (Vol.% mineral*μm) to give a measure of the lesion severity. However, stains prove to be a problem with fluorescence imaging methods since they both absorb visible light and contain fluorophores that fluoresce in the visible. Therefore reliable measurements require very clean surfaces and this may be very difficult to achieve in the pits and fissures of the occlusal surface.

Near-IR reflectance measurements yield high contrast for both artificial and natural caries lesions without interference from stains and color variations since none of the known chromophores absorb light in the near-IR beyond 1300-nm. In a previous study by Wu et al. [19], we compared the image contrast of artificial lesions produced on tooth buccal and occlusal surfaces between fluorescence, visible reflectance, near-IR reflectance and near-IR transillumination at 1310-nm. In that study near-IR reflectance (1310-nm) yielded the highest contrast, although it did not break statistically with fluorescence, it was significantly higher than visible reflectance. Near-IR transillumination did not work well because the highly scattering dentin masked the shallow enamel lesions. In that study, we had used 470-nm excitation and it has been suggested that better performance can be achieved with 405-nm excitation. More recent near-IR imaging studies suggest that near-IR wavelengths coincident with areas of higher water absorption are well suited for the detection of early demineralization on tooth surfaces. We hypothesize that higher water absorption in the underlying dentin and enamel reduces the reflectivity in sound areas and this in turn results in higher contrast between sound and demineralized enamel. Hyperspectral reflectance measurements by Zakian show that the tooth appears darker with increasing wavelength[20]. A recent study by Chung et al. [9] of natural occlusal lesions indicated that other near-IR wavelengths where water absorbs provided significantly higher contrast than at 1310-nm and the visible.

Light scattering in sound dental enamel decreases markedly in the near-infrared (NIR) region and studies have shown that enamel has the highest transparency near 1310-nm [21, 22]. At this wavelength, the attenuation coefficient is only 2 to 3 cm^{-1}, which is a factor of 20 to 30 times lower than in the visible region [22]. At longer wavelengths, water absorption increases significantly and reduces the penetration of the NIR light. Even though the light scattering for sound enamel is at a minimum in the NIR, the light scattering coefficient of enamel increases by 2-3 order of magnitudes upon demineralization due to the formation of pores on a similar size scale to the wavelength of the light that act as Mie scatterers [23]. Therefore, caries lesions can be imaged with optimal contrast at 1310 nm [8]. These seminal studies established the feasibility of acquiring high quality images of natural caries lesions using NIR transillumination. However, those imaging geometries are not well suited for imaging early lesions that are confined to the outer enamel surface and a previous study by Wu et al. [19] indicated that the contrast of early lesions was indeed low in transillumination.

The purpose of this study is to compare QLF, visible reflectance and reflectance at three NIR wavelengths 1300, 1460 and 1600-nm for imaging early lesions on occlusal surfaces. The study design is very similar to that carried out by Wu et al., however in this study the aim is to show that NIR reflectance measurements at wavelengths with high water absorption yielded higher contrast than conventional methods and NIR reflectance at 1300-nm. In addition, we employed 405-nm excitation for QLF, which is similar to the systems that are commercially available.

2. MATERIALS AND METHODS

2.1 Sample and Lesion Preparation

Twenty-four human teeth with non-carious occlusal surfaces were collected (CHR approved) and sterilized with gamma radiation. Tooth occlusal surfaces were bead blasted with 50-μm glass beads for twenty seconds to clean fissures and remove the outermost fluoride rich layers of enamel to ensure a homogeneous surface for demineralization. Next, teeth were mounted in black orthodontic acrylic blocks. Samples were stored in a moist environment of 0.1% thymol to maintain tissue hydration and prevent bacterial growth. The outlines of two 4×4 mm windows approximately 50-μm deep were cut on the occlusal surface of each tooth using a CO_2 laser (Impact 2500, GSI Lumonics Rugby, UK). The laser incisions also inhibit decay in the laser area due to thermal modification of the enamel and are therefore very effective in providing a separation between the sound and demineralized areas. The channels cut by the laser also serve as reference points for serial sectioning and are sufficiently narrow that they do not interfere with calculations of the image contrast. The enamel surrounding the 4×4 windows created by the laser was covered with a red acid-resistant varnish (Revlon, New York). The varnish was removed using acetone after the lesions were generated. We chose not to use a clear varnish as was done in an earlier study[19] because it is difficult to ensure complete coverage over irregular tooth surfaces. Artificial lesions were created within the 4×4 windows by immersing each tooth into a 50 ml aliquot of a Ca/PO_4/acetate solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 0.075 mol/L acetate maintained at pH 4.5 and a temperature of 37 °C for either 24 and 48 hours. This method produces two groups with 12 teeth in each group with 1-day and 2-day lesions, respectively. In Figure 1, depth composition 2-D images taken with a Keyence VHX-1000 digital microscope are shown for both artificial lesion groups.

2.2 Visible and NIR Cross Polarization Images

In Figure 2, the schematic for the NIR reflectance imaging is shown. In order to acquire reflected light images, NIR light was directed towards the occlusal surface through a broadband fused silica beamsplitter (1200-1600-nm) Model BSW12 (Thorlabs, Newton, NJ) and the reflected light from the tooth was transmitted by the beamsplitter to the imaging camera. Crossed polarizers were placed after the light source and before the detector and used to remove specular reflection (glare) that interferes with measurements of the lesion contrast. The NIR reflectance images were captured using a high resolution, high sensitivity InGaAs NIR/SWIR Camera (Model GA1280JS) with a 1280 × 1024 pixel format, and a 12.5 μm pixel pitch.

In this method, the demineralized region appears lighter than the sound enamel because the demineralized region scatters the light increasing the amount of light scattered/reflected back towards the camera. In order to acquire visible light images, an Ocean Optics fiber-coupled tungsten-halogen lamp (Model HL-2000-FHSA) with the same DFK 31AF03 FireWire camera equipped with an Infinimite lens was used and the images were captured using a CCD camera. A beamsplitter was not used and illumination was directed at the occlusal surface with a 5° angle from the surface normal, A and B of Fig. 2 rotated by 85 ° towards the camera D.

2.3 Fluorescence Loss Measurements

To collect fluorescence images, a GaN laser diode module “Blu-Ray” (λ=405-nm) operating with 60-mW (Photonic Products, UK) was used as an excitation source. A 500-nm long-pass filter #C47-616 (Edmund Scientific, Barrington, NJ) and a DFK 31AF03 FireWire camera (resolution 1024 × 768) from the Imaging Source (Charlotte, NC) equipped an Infinimite lens (Infinity Photo-optical, Boulder, CO), were used to image the fluorescence from the surface at wavelengths longer than 500-nm. Imaging was carried out in the dark to avoid the interference of ambient light. The same illumination scheme used for visible reflectance was used without a beamsplitter. QLF systems have been employed using several excitation wavelengths ranging from 370-488-nm. The fluorescence emission spectra and quantum yields are expected to be independent of excitation wavelength, according to Kasha's law as long as there is sufficient energy to populate the excited state [24]. A study by Endo et al.[25] was consistent with Kasha's law; they measured demineralization using two QLF systems operating at 488-nm and 370-nm and reported similar performance. However, more recent studies by Zhang et al. [26] suggest that performance differs with varying excitation wavelengths. Therefore, we chose the excitation wavelength of 405-nm to be consistent with clinical systems and the best-reported diagnostic performance. QLF-fluorescence loss measurements are typically reported as a ratio in intensity (fluorescence radiance) of the lesion area compared with an equivalent sound area on the tooth.

3. RESULTS AND DISCUSSION

The higher resolution and improved size of the new InGaAs sensor provides more detailed images of the occlusal images and aids in visualizing variations in the lesion severity and the complex topography of the occlusal surface. Examples of each image for two samples are shown in Figs. 3 & 4. In reflectance, the lesions appear lighter than the surrounding sound enamel and in fluorescence the lesions appear darker. The reflectivity from the sound tooth areas in the NIR infrared reflectance images are extremely low due to the weak scattering by the sound enamel. Staining and discoloration are visible in tooth fissures from the visible-light reflectance images. Staining and discoloration can interfere with reflectance imaging in the visible region, however the chromophores responsible for stains in the visible region, do not absorb NIR light and therefore do not interfere at NIR wavelengths. Stains are a major limitation for visible light reflectance imaging and fluorescence. In a recent study of natural lesions in the occlusal surface, the contrast of the lesions at visible wavelengths was actually negative since absorption by stains contributed more to the lesion contrast than the increased reflectivity due to increased scattering from the demineralized lesion areas making it impractical for use in the pits and fissures of the occlusal surfaces[9]. Since the windows in the occlusal areas were bead blasted to remove all stains and the outermost layers of enamel layers, stained enamel was not a factor in this study.

NIR reflectance measurements at 1460 nm and 1600 nm yielded the highest contrast and it was significantly higher than for 1300-nm reflectance measurements, fluorescence and the visible reflectance measurements with polarized light. A more complete paper will be submitted for publication including an extensive statistical comparison of the lesions along with measurements of the lesion depth and severity measured with polarization sensitive optical coherence tomography.

It is highly desirable that the image contrast increases markedly with lesion severity so that one can gauge the depth and severity of the lesion from a single image. Since shallow lesions manifest low contrast in NIR transillumination, one approach is to acquire both reflectance and transillumination images and use both to gauge severity. For example, superficial hypomineralization does not appear darker on NIR transillumination images while the more severe caries lesions do [27]. Zakian et al.[20] has taken the approach of using three NIR wavelengths to estimate lesion severity. Although they have focused on more severe lesion types. Zhang et al. [28] showed using reflectance measurements and Monte Carlo simulations of photon propagation, that shorter wavelengths yielded higher contrast for very shallow demineralization and that longer wavelengths yield higher contrast for deeper lesions. The lesion contrast across each 4 × 4 mm window varies markedly within the window and with the different imaging wavelengths. The lesions appear much more uniform in the visible light reflectance images then they do in the near-IR reflectance images. Examination of the lesions with optical coherence tomography and polarized light histology indicate that the lesion severity exhibits considerable variation. The fluorescence images show more variation across the lesion area than the visible reflectance images but do not show as much variation as the near-IR images. More extensive studies over a greater range of lesion severity are required to determine the optimum performance ranges for each of these imaging methods.

The lesion contrast was significantly higher for fluorescence than it was for 1300-nm reflectance and visible light reflectance. This is better performance vs. these other modalities than in the prior study of Wu et al. .[19] in which 470-nm excitation was employed as opposed to 405-nm excitation. Linear image contrast was compared in this study, while in most QLF studies the fluorescence radiance from a specified area is reported as the relative fluorescence radiance loss from a particular area. However, the results should be similar since one is comparing a linear array of values (line-profile) for image contrast and a 2D matrix of values (area) for the fluorescence radiance calculation.

The lesion contrast for 1300-nm was much lower than it was in the prior study Wu et al.[19]. If the image contrast in the near-IR is particularly sensitive to the lesion severity than we would expect large differences in performance. Another possibility is that superluminescent diode light sources were used for the NIR reflectance measurements as opposed to filtered light from a tungsten halogen lamp. Speckle and other factors may have lowered the contrast. The performance of both the visible reflectance and the fluorescence appeared better in this study. The approach of bead blasting the occlusal surface to aid lesion development and remove stains may have enhanced the contrast for the shorter wavelengths. This was not done for the study of Wu et al. [19].

This study clearly demonstrates that a NIR imaging system has considerable potential for the imaging of early surface demineralization with high contrast on both occlusal surfaces. The NIR wavelengths coincident with higher water absorption yielded significantly higher contrast than other methods. The high contrast between sound enamel and early enamel demineralization suggests that reflective NIR imaging can be effective for routine monitoring white spot lesions during chemical intervention. Since NIR wavelengths are safe the dentist, they can acquire multiple NIR images of the lesions during subsequent visits to determine if fluoride therapy is effective in arresting the lesion or whether the lesion has expanded, requiring more aggressive intervention. Such an approach is not practical with radiographic methods due to repeated x-ray exposure. In addition to the high contrast in this study, another potential advantage NIR imaging has over visible imaging methods and fluorescence-based methods is the lack of interference from stains and discoloration, since stains are not visible in the NIR.

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REFERENCES

1. NIH. Diagnosis and Management of Dental Caries throughout Life. NIH Consensus Statement. 2001:1–24. [PubMed: 11699634]
2. Featherstone JDB. Prevention and reversal of dental caries:role of low level fluoride. Community Dent Oral Epidemiol. 1999; 27:31–40. [PubMed: 10086924]
3. Angmar-Mansson B, ten Bosch JJ. Optical methods for the detection and quantification of caries. Adv. Dent. Res. 1987; 1(1):14–20. [PubMed: 3481546]
4. ten Bosch JJ, van der Mei HC, Borsboom PCF. Optical monitor of in vitro caries. Caries Res. 1984; 18:540–547. [PubMed: 6593126]
5. Benson PE, Ali Shah A, Robert Willmot D. Polarized versus nonpolarized digital images for the measurement of demineralization surrounding orthodontic brackets. Angle Orthod. 2008; 78(2): 288–293. [PubMed: 18251618]
6. Everett MJ, Colston BW, Sathyam US, Silva LBD, Fried D, Featherstone JDB. Noninvasive diagnosis of early caries with polarization sensitive optical coherence tomography (PS-OCT). SPIE Proceeding. 1999; 3593:177–183.
7. Fried D, Xie J, Shafi S, Featherstone JDB, Breunig T, Lee CQ. Early detection of dental caries and lesion progression with polarization sensitive optical coherence tomography. J. Biomed. Optics. 2002; 7(4):618–627.
8. Fried, D.; Featherstone, JDB.; Darling, CL.; Jones, RS.; Ngaotheppitak, P.; Buehler, CM. Early Caries Imaging and Monitoring with Near-IR Light. W. B Saunders Company; Philadelphia: 2005.
9. Chung S, Fried D, Staninec M, Darling CL. Multispectral near-IR reflectance and transillumination imaging of teeth. Biomed. Opt. Express. 2011; 2(10):2804–2814. [PubMed: 22025986]
10. Ando M, Hall AF, Eckert GJ, Schemehorn BR, Analoui M, Stookey GK. Relative ability of laser fluorescence techniques to quantitate early mineral loss in vitro. Caries Res. 1997; 31(2):125–131. [PubMed: 9118184]
11. Hafstroem-Bjoerkman U, de Josselin de Jong E, Oliveby A, Angmar-Mansson B. Comparison of laser fluorescence and longitudinal microradiography for quantitative assessment of *in vitro* enamel caries. Caries. Res. 1992; 26:241–247. [PubMed: 1423438]
12. Angmar-Masson BA, Al-Khateeb S, Tranaeus S. Intraoral use of quantitative light-induced fluorescence detection method. Indiana Conference on the Early Detection of Dental Caries I. 1996:39–50.
13. Lagerweij MD, van der Veen MH, Ando M, Lukantsova L. The validity and repeatability of three light-induced fluorescence systems: An in vitro study. Caries Res. 1999; 33:220–226. [PubMed: 10207198]
14. Eggertsson H, Analoui M, Veen M. H. v. d. Gonzalez-Cabezas C, Eckert GJ, Stookey GK. Detection of early interproximal caries in vitro using laser fluorescence, dye-enhanced laser fluorescence and direct visual examination. Caries Res. 1999; 33:227–233. [PubMed: 10207199]
15. de Josselin de Jong E, Sundstrom F, Westerling H, Tranaeus S, ten Bosch JJ, Angmar-Mansson B. A New Method for in vivo Quantification of Changes in Initial Enamel Caries with laser Fluorescence. Caries Res. 1995; 29(1):2–7. [PubMed: 7867045]
16. van der Veen MH, de Josselin de Jong E, Al-Kateeb S. Caries Activity Detection by Dehydration with Qualitative Light Fluorescence. S Indiana Conference on the Early Detection of Dental Caries II. 1999:251–260.
17. Stookey, GK. Quantitative Light Fluorescence: A Technology for Early Monitoring of the Caries Process. W. B Saunders Company; Philadelphia: 2005.
18. ten Bosch JJ. Summary of Research of Quanitative Light Fluorescence. Indiana Conference on the Early Detection of Dental Caries II. 1999:261–278.
19. Wu J, Fried D. High contrast near-infrared polarized reflectance images of demineralization on tooth buccal and occlusal surfaces at λ=1310-nm. Lasers in Surgery and Medicine. 2009; 41(3): 208–213. [PubMed: 19291753]
20. Zakian C, Pretty I, Ellwood R. Near-infrared hyperspectral imaging of teeth for dental caries detection. Journal of Biomedical Optics. 2009; 14(6):064047. [PubMed: 20059285]

21. Fried D, Featherstone JDB, Glena RE, Seka W. The nature of light scattering in dental enamel and dentin at visible and near-IR wavelengths. Appl. Optics. 1995; 34(7):1278–1285.
22. Jones RS, Fried D. Attenuation of 1310-nm and 1550-nm Laser Light through Sound Dental Enamel. SPIE Proceeding. 2002; 4610:187–190.
23. Darling CL, Huynh GD, Fried D. Light Scattering Properties of Natural and Artificially Demineralized Dental Enamel at 1310-nm. J. Biomed. Optics. 2006; 11(3):034023.
24. Lakowicz, JR. Principles of Fluorecence Spectroscopy. Kluwer Academic; New York: 1999.
25. Ando M, Analoui M, Schemehorn BR, Stookey GK. Comparison of light-induced and laser-induced fluorescence methods for the detection and quantification of enamel demineralization. SPIE Proceeding. 1999; 3593:148–153.
26. Zhang L, Nelson LY, Seibel EJ. Red-shifted fluorescence of sound dental hard tissue. Journal of Biomedical Optics. 2011; 16(7):071411. [PubMed: 21806257]
27. Hirasuna K, Fried D, Darling CL. Near-IR imaging of developmental defects in dental enamel. J. Biomed. Opt. 2008; 13(4):044011. [PubMed: 19021339]
28. Zhang L, Nelson LY, Seibel EJ. Spectrally enhanced imaging of occlusal surfaces and artificial shallow enamel erosions with a scanning fiber endoscope. Journal of Biomedical Optics. 2012; 17(7):076019. [PubMed: 22894502]

Fig. 1.

Depth composition 2-D images of 1-day (top) and 2-day (bottom) lesions using Keyence VHX-1000E digital microscope (Itasca, IL)

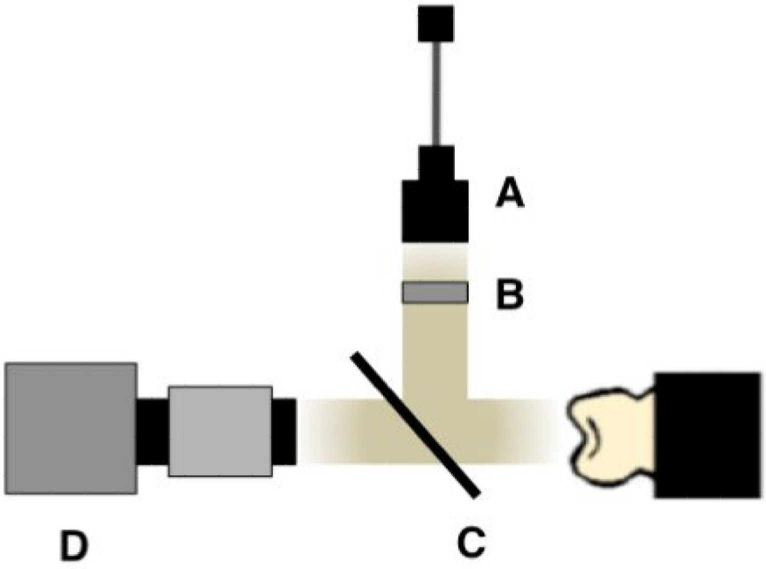

Fig. 2.

Schematic diagram for NIR reflectance imaging: (A) 1300, 1460 and 1600 μm SLD light source, (B) NIR linear polarizer, (C) beamsplitter, and (D) UTC Aerospace Systems (GA1280JS) High-resolution InGaAS SWIR Camera (1280 × 1024 pixel format, 12.5 μm pitch) (Princeton, NJ).

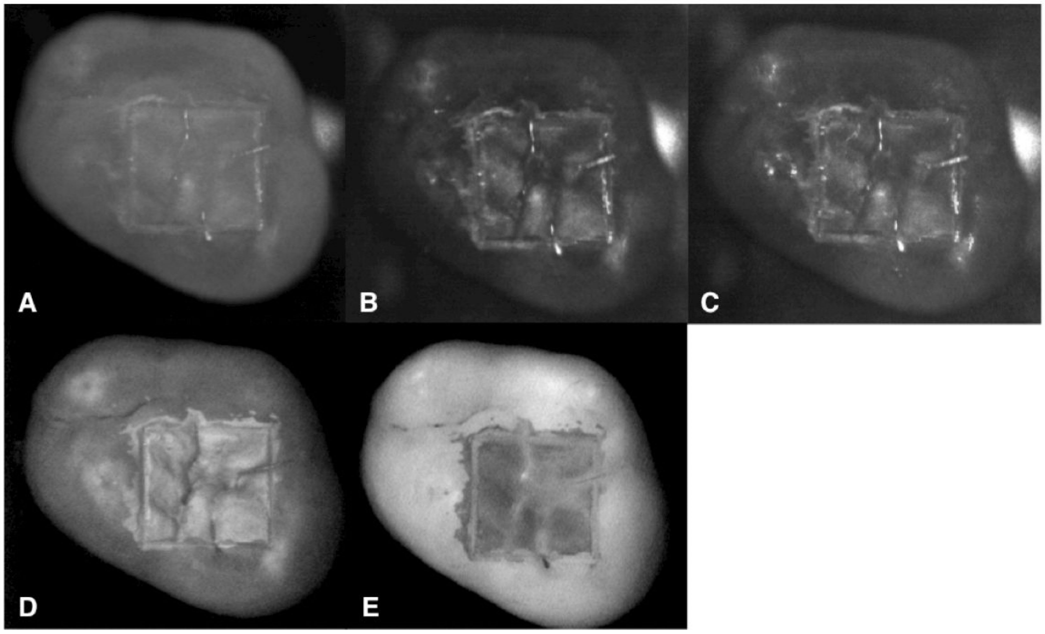

Fig. 3.
Occlusal images of 1-day lesions are shown for one sample: NIR reflectance images w/ crossed polarizers: (A) 1300 nm, (B) 1460 nm, (C) 1600 nm, (D) visible reflectance image w/crossed polarizers and (E) fluorescence image.

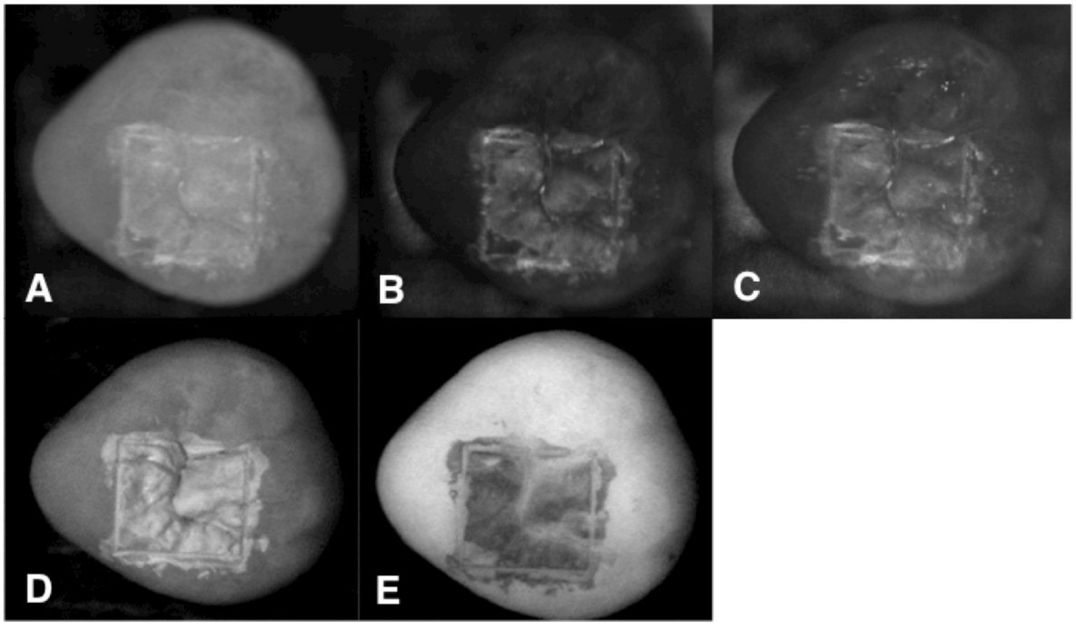

Fig. 4.

Occlusal images of 2-day lesions are shown for one sample: NIR reflectance images w/ crossed polarizer's (A) 1300 nm, (B) 1460 nm, (C) 1600 nm, (D) visible reflectance image w/crossed polarizers and (E) fluorescence image.