

Correlation of quantitative light-induced fluorescence and optical coherence tomography applied for detection and quantification of early dental caries

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Abstract. Fluorescence loss in enamel following demineralization has been correlated with the amount of mineral lost during the demineralization. The correlation between fluorescence loss measured by quantitative light-induced fluorescence (QLF) and the reflectivity loss measured by a versatile *en face* optical coherence tomography (OCT) system was investigated in a demineralization process to produce artificial dental caries. We used an OCT system that can collect A-scans (reflectivity versus depth), B-scans (longitudinal images), and C-scans (*en face* images). The power to the sample was 250 μW , the wavelength $\lambda = 850 \text{ nm}$, and the depth resolution in air 16 μm . A-scans, which show the profile of the reflectivity versus the depth of penetration into the tooth tissue, were used for quantitative analysis of the reflectivity loss. The results have shown that both the fluorescence radiance and reflectivity of the enamel decrease with increasing demineralization time. A linear correlation was observed between the percentage of fluorescence loss measured by QLF and the percentage of reflectivity loss measured by OCT. It was concluded that the decrease in reflectivity of the enamel during demineralization, measured by OCT, could be related to the amount of mineral lost during the demineralization process © 2003 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.1606685]

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1 Introduction

For many years radiography^{1,2} has been used for caries diagnosis and restorative decision-making in dentistry. However, besides subjecting patients to ionizing radiation, radiography is neither quantitative nor sensitive enough to detect very early dental caries lesions. Apart from quantitative light-induced fluorescence (QLF), which is currently used for detection and quantitative analysis of early caries,^{3,4} based on the established correlation between the mineral loss and the fluorescence loss in enamel following demineralization,⁵ limited quantitative methods are available for clinical detection and measurement of early demineralization of enamel that produces dental caries.

Quantitative analysis permits longitudinal assessment of the changes in the mineral status within the caries lesion, which would make it possible to determine the effect of advice and treatments tailored to inhibit demineralization and promote remineralization. Furthermore, detection of dental caries at the incipient stage enables the implementation of caries therapeutic regimes (such as fluoride application), which would permit an early lesion to be remineralized before the need for restorative intervention. It seems appropriate, therefore, to develop a system that can detect a dental caries lesion at its early stage and quantitatively monitor the mineral status of the lesion over a period of time.

Optical coherence tomography (OCT) has been used to produce longitudinal images of dental tissues^{6–9} of an orientation similar to that of the B-scan ultrasound images. In these images, a reduction in enamel reflectivity was observed in areas of demineralization.^{10,11} It is speculated that the decrease in reflectivity during demineralization might be related to the amount of mineral lost. The aim of the present study therefore was to investigate the correlation between the decrease in enamel reflectivity and the loss in enamel fluorescence following tooth demineralization. This was quantified using OCT and QLF to measure the reflectivity and fluorescence, respectively. A versatile *en face* OCT system described in the next section was used. The system was developed initially for retina imaging,^{12,13} and can collect A-scan, B-scan (longitudinal), and C-scan (transverse) images of a biological tissue. The same system was used for comparing OCT images with those from transverse microradiography (TMR).^{14,15}

2 OCT System

The apparatus (Fig. 1) consists of an interferometer excited by a pigtailed superluminescent diode (SLD), central wavelength $\lambda = 0.85 \mu\text{m}$, bandwidth $\Delta\lambda = 20 \text{ nm}$, which sends 250 μW power to the tooth. The OCT system was reported

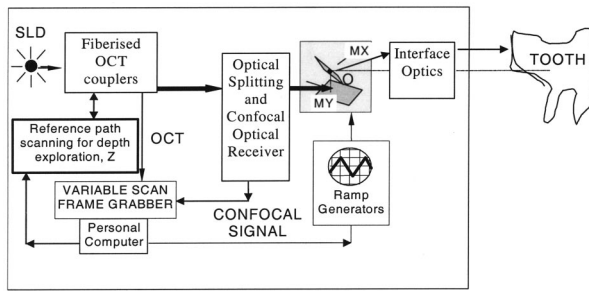


Fig. 1 Diagrammatic illustration of a stand-alone OCT-confocal instrument.

elsewhere,^{12,16} using a configuration of directional single-mode couplers. The system can operate in different regimes. In the longitudinal OCT regime, only one galvoscaner of the galvoscaner pair is driven with a ramp at 700 Hz and the translation stage is moved for the depth range required in 0.5 s. In this case, an OCT B-scan image is produced either in the x - z or y - z -plane. The depth range was 1 mm in air for the images presented in this paper. The generation of B-scan images differs from other reports on longitudinal OCT imaging¹⁷ where the B-scan image is constructed out of A-scans (depth profiles of the reflectivity).

In the transverse regime, one galvoscaner is driven with a ramp at 700 Hz and the other with a ramp at 2 Hz. In this way, a C-scan image in the x - y -plane is generated at a constant depth. Then the depth is changed by moving the translation stage and a new C-scan image is collected. The *en face* images of caries lesions in the present study were obtained in this way.

In addition to acquiring OCT images in rectangular directions, the system is also equipped with a second channel operating on the principles of confocal microscopy. In the sensing arm of the OCT, a splitting device redirects a part of the returned light from the tooth toward a photodetector behind a lens and a pinhole, which operate as a confocal receiver. The design is such that the two images, OCT and confocal, are in pixel-to-pixel correspondence.

The OCT and confocal signals carrying the information about reflectivity are applied to a dual-input frame grabber, which can display one or both of these signals under computer control. The frame grabber is synchronized with transistor-transistor-logic (TTL) signals delivered by the two ramp generators controlling the galvanometer pair. For an image size of 3×3 mm in the transversal section of the object, the pixel size is $\sim 10 \mu\text{m}$ and is determined by the numerical aperture of the interface optics. However, the depth resolution in the two channels is different. In the OCT channel, the depth resolution is governed by the spectral properties of the low coherence source, and is half of the coherence length of the source, i.e., $16 \mu\text{m}$. The depth resolution in the confocal channel is ~ 1 mm and is dictated by the interface optic and the optics of the confocal receiver (the numerical aperture of the focusing lens and the pinhole diameter). Both resolutions are the full width at half maximum of the signal profile measured by axially moving a mirror as an object through the focus of the interface optics. The depth resolution of the confocal channel can in principle be improved to a few microns by using a high

numerical aperture (NA) interface optics and a sufficiently small pinhole. However, as long as the OCT channel provides the depth resolution, the depth resolution in the confocal channel is conceived to be poorer than in the OCT channel. In this way, a very small percentage of the signal reflected by the tooth is sufficient for the confocal channel and most of the signal is used in the OCT channel. A large depth of focus allows collection of the OCT signal without the need for dynamic focus.¹⁸ If the depth of focus becomes similar to the coherence length, then the focus needs to be moved in synchrony with the translation stage in the OCT channel that determines the position in depth of the scatterer contributing to the OCT signal. This would have complicated the system used for this report.

The confocal image was useful for identifying the lesions, aligning the tooth, and evaluating the overall map of reflectivity along the x - or y -axis in the longitudinal regime or in the x , y -plane in the transversal regime. In the longitudinal regime, the confocal image shows little variation in depth owing to the large depth of focus used. However, the confocal channel may be very useful *in vivo* when lateral movement of the patient in the x or y scanning direction, respectively, can be picked up from the confocal image and used to correct the OCT image.

3 Methods

3.1 Caries Production and OCT-Confocal Imaging

Fifteen freshly extracted bovine incisor teeth free from caries, cracks, or enamel malformations were selected and polished with pumice slurry to remove organic contaminants from the labial surface of the teeth. The teeth were then painted with two coats of a nonfluorescent acid-resistant colorless nail varnish, except for three exposed windows (D1, D2, and D3), each measuring approximately 2 mm^2 in diameter, lying on the same horizontal level on the labial surface of the teeth. Carieslike lesions were then produced on each window by demineralization of the teeth in acidic buffer solutions containing 2.2 mM KH_2PO_4 , 50 mM acetic acid, 2.2 mM of 1 M CaCl_2 and 0.5 ppm fluoride, at a pH of 4.5 (Ref. 19). Prior to demineralization (0 h), the OCT (A-, B-, and C-scans) and fluorescent images of the windows on each tooth were recorded with the OCT and QLF systems, respectively. The OCT-confocal imaging was repeated on windows D1, D2, and D3 at 24, 48, and 72 h, respectively. After imaging was completed on each window, that window was sealed off with adhesive tape to prevent further demineralization until the last recording at 72 h on D3. The OCT-confocal images (Fig. 2) were acquired as described in previous publications.^{16,20} The A-scans were for quantitative assessment. These showed the depth (mm)-resolved reflectivity (dB) of the tooth tissue and therefore were used to calculate the degree of reflectivity (R) of the tissue at any depth in accordance with the method described by Amaechi et al.²¹

3.2 QLF Imaging and Analysis

Following the OCT imaging, the fluorescent image of each tooth was captured using the QLF clinical system, and the lesions [Figs. 3(a) to 3(d)] were analyzed quantitatively with QLF software, as described by van der Veen and de Josselin de Jong,²² to calculate the percentage of fluorescence loss (ΔQ). The QLF system consisted of a special intraoral cam-

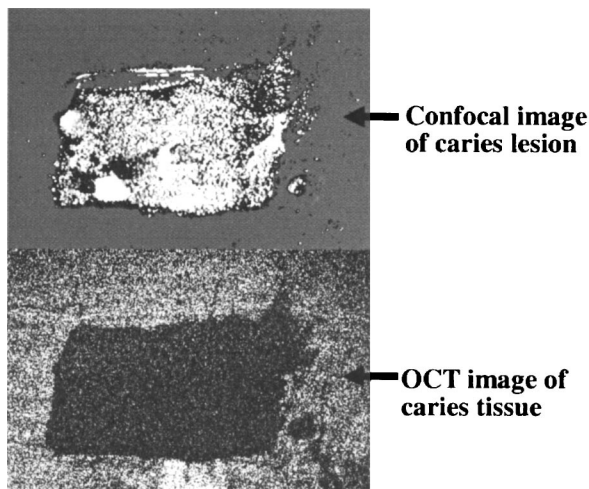


Fig. 2 Single frame of OCT at 0.5-mm depth and simultaneous confocal image. Lateral size: 5 × 5 mm.

era device connected to a computer fitted with a frame grabber (Comet, Matrox, Electronic Systems Ltd., Quebec, Canada) and to which the QLF software (QLF version 2000, Inspektor Research Systems BV, Amsterdam, the Netherlands) was installed. To visualize and capture the tooth image, white light from a special arc lamp (Philips bv, Eindhoven, the Netherlands) based on xenon technology was filtered through a blue-transmitting bandpass filter (Philips bv) with a peak intensity of $\lambda = 370$ nm and a bandwidth of 80 nm, to illuminate the tooth with a blue-violet light with an intensity of 13 mW/cm². A dental mirror provided uniform illumination, and with the aid of a color CCD sensor (Sony LS-1P, Tokyo, Japan), which had a yellow-transmitting ($\lambda \geq 520$ nm) filter (Philips bv) positioned in front of it (to filter out all reflected and backscattered light), the fluorescent image of the tooth was recorded and digitized by the frame grabber and was available for quantitative analysis with the QLF software. The fluorescence loss, ΔQ , is obtained by reconstructing the fluorescence of sound enamel at the site of the lesion from the fluorescence of the surrounding sound enamel (assumed to be 100%). The decrease in fluorescence was determined by calculating the percentage difference between the actual and reconstructed fluorescence surface. Any area with a fluorescence radiance drop of more than 5% was considered to be a lesion.

3.3 Statistical Analysis

The data obtained were analyzed statistically with the significance level (α) prechosen at 0.05. In order to assess the ability

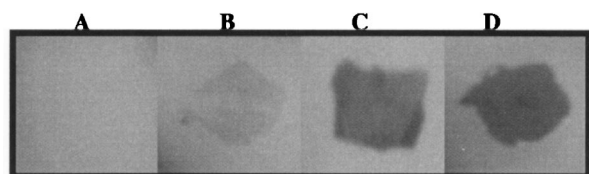


Fig. 3 QLF images of (a) sound tooth surfaces and demineralized (caries) surfaces at (b) 24 h, (c) 48 h, and (d) 72 h of demineralization.

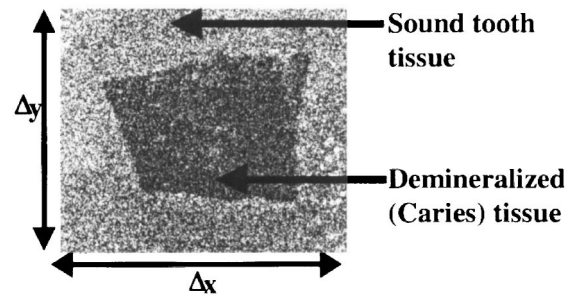


Fig. 4 Transversal OCT image of the lesion in Fig. 2(d) at a depth of 0.3 mm. $\Delta x = \Delta y = 3$ mm.

of OCT to quantitatively monitor the mineral changes in a tooth tissue during demineralization, the mean values of the degree of reflectivity, R (dB mm), of the tooth tissue at the four measurement intervals of 0, 24, 48, and 72 h were compared using analysis of variance (ANOVA) for repeated measures, and also correlated with time using the Pearson correlation coefficient (r). For comparison with the percentage of fluorescence loss (ΔQ) measured by QLF, the percentage of reflectivity loss ($R\%$) was determined as follows:

$$\begin{aligned} \% \text{ reflectivity loss (\% dB/mm)} \\ = \frac{(R_{\text{sound}} - R_{\text{demineralized}})}{R_{\text{sound}}} \times 100. \end{aligned}$$

The correlation between the mean values of $R\%$ and that of ΔQ was determined using the Pearson correlation coefficient.

4 Results

While both transversal (Fig. 4) and longitudinal (Fig. 5) OCT images showed the caries lesion as volumes of reduced reflectivity, the longitudinal images in addition showed the depth of the lesion into the tooth tissue. This was determined by correlating measurements obtained via both longitudinal and *en face* OCT imaging. The A-scan graphs (Fig. 6) showed the levels of reflectivity versus depth of penetration into the tooth tissue. Quantitative analysis²¹ of the degree of reflectivity of the tooth tissue evaluates an integral over the area below the reflectivity versus depth curve and results in a dB mm value. Such analysis showed that the reflectivity of the tissue decreased with increasing demineralization time (Fig. 6); 0 h = 31.86 ± 9.30 dB/mm, 24 = 14.45 ± 5.47, 48 = 8.66 ± 1.96,

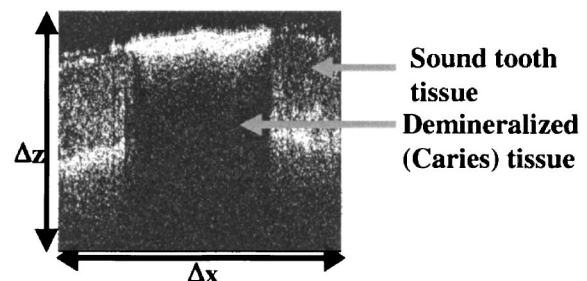


Fig. 5 Longitudinal OCT image of the caries lesion in Fig. 2(d) showing its depth in the tooth tissue. $\Delta x = 3$ mm, $\Delta z = 0.6$ mm.

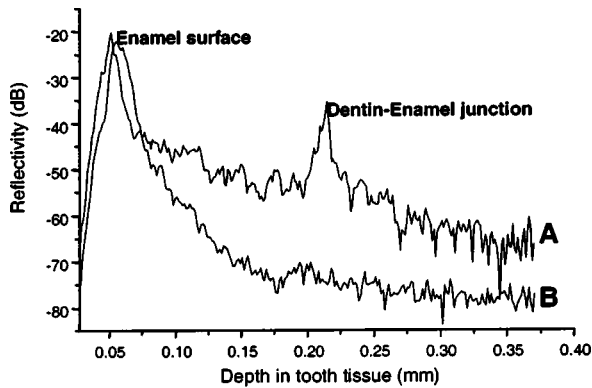


Fig. 6 A-scan showing the levels of reflectivity (dB) versus depth (mm) of penetration into the tooth tissue and illustrating the decrease in reflectivity with demineralization (A, B). A, sound tooth tissue; B, demineralized tooth.

$72 = 4.32 \pm 2.72$, and this was confirmed by the Pearson correlation coefficient (R versus time, $r = -0.944$). ANOVA indicated a significant difference ($n = 15, p < 0.001$) between the mean values of R at the four measurement intervals. The percentage of reflectivity loss ($R_{\%}$) increased with increasing demineralization time (Fig. 7), demonstrating a similarity with the percentage of fluorescence loss, ΔQ (Fig. 8). A linear correlation ($R_{\%} = 45.56 + \Delta Q, r = 0.963$) was observed between the percentage of fluorescence loss, ΔQ , and the percentage of reflectivity loss (Fig. 9).

5 Discussion

The utilization of an OCT system that can provide C-, B-, and A-scan images of tooth tissue to detect an incipient caries lesion as early as 24 h into its development was successfully demonstrated in this study. OCT was able to discriminate between sound and demineralized (cariou) tooth tissues by their degree of reflectivity, depicting cariou tissue as volumes of reduced reflectivity (Figs. 4 and 5). Reflectivity is only one of the other factors used by OCT to discriminate between sound and cariou tooth tissue. Other studies reported changes in polarization and birefringence as better markers than reflectivity for detecting demineralization, particularly for detecting

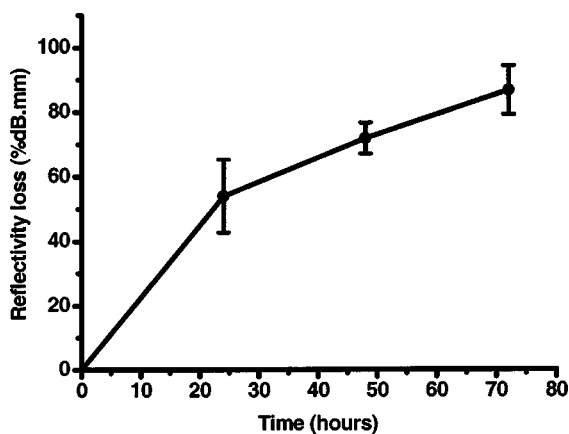


Fig. 7 Illustration of the increased percent of reflectivity loss with increasing demineralization time as quantified using OCT.

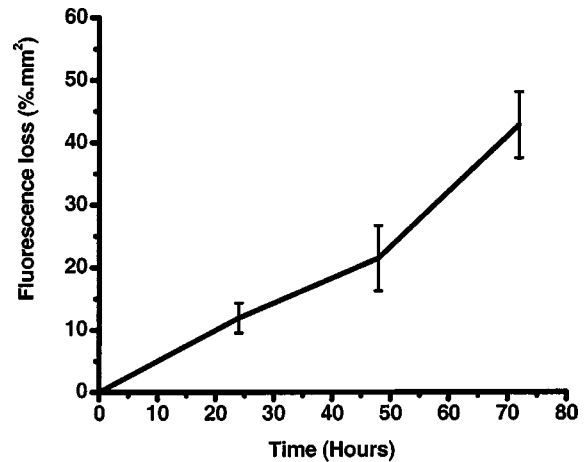


Fig. 8 Illustration of the increased fluorescence loss with increasing demineralization time as quantified using QLF.

early caries;^{10,23,24} hence the system used in the present study, which can only use reflectivity, is being developed to use polarization and birefringence in addition to reflectivity to discriminate between sound and demineralized tissue. The OCT technique applied to ophthalmology has evolved rapidly in the past few years.²⁵

Applications of OCT in dentistry have already been reported and cover *in vitro* images of dental and periodontal tissues^{6,7} as well as cariou lesions.⁸⁻¹⁰ However, all the reported methods delivered only longitudinal OCT images, which we believe restricts the interpretation of the high-resolution images. The system used in the study reported here incorporated a transversal mode. On examination of a tooth, the compounding of information in rectangular directions, transversal and axial, allows better diagnosis than when using longitudinal OCT imaging only. Successive displays of transversal and longitudinal cuts at different positions in the 3-D stack of *en face* OCT images give a direct view of the cariou volume.

The OCT system used by us incorporated a confocal imaging system that aids in identifying cariou lesions and align-

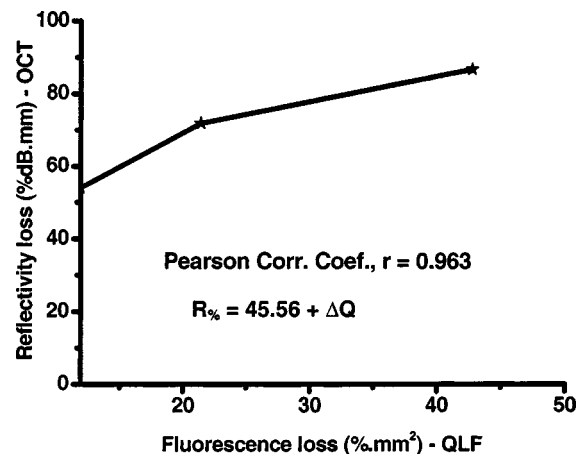


Fig. 9 Illustration of the relationship ($R_{\%} = 45.56 + \Delta Q$) between the percentage of fluorescence loss (ΔQ) and the percentage of reflectivity loss ($R_{\%}$).

ing the tooth. The early caries appears as a bright spot in the confocal image, which can also be displayed sideways, along with the *en face* OCT image at each depth (Fig. 2). The confocal system would be very useful in clinical (*in vivo*) application of the system, in that during clinical examination, the confocal image of the tooth is displayed on a computer screen, and on detection of a lesion on a tooth, the OCT imaging is initiated. Without the confocal system, the lesion has to be detected first by visual examination before the use of the OCT component to image and quantify the lesion. Therefore, the confocal system enhances the early detection of lesions and reduces the time spent in diagnosis. Although the confocal system was not absolutely necessary in the present study, it was used in order to demonstrate its usefulness, which would be particularly important during clinical application. The contribution of the confocal channel to the results of this study was discussed in Sec. 2.

In this study, different levels (1 to 3 days) of demineralization (and hence different levels of mineral loss) were shown by OCT as different degrees of reflectivity loss, and in this way it was possible to monitor the change in mineral status of the tooth tissue over a period of time. This ability was validated by QLF, which is a long-established method for detection and quantification of early demineralization of enamel. QLF is an optical technique that uses the natural fluorescence of teeth to discriminate between caries and sound enamel, based on the fact that the fluorescence radiance of a carious spot viewed with QLF is lower than that of the surrounding sound enamel.^{3,4,22} QLF measures the percentage of change in the fluorescence radiance of demineralized enamel with respect to the surrounding sound enamel and relates it directly to the amount of mineral lost during demineralization, although it does not show the depth of the lesion. However, the good correlation between the OCT results and an established method of detecting and quantifying demineralization indicated that the reflectivity lost in enamel during demineralization, measured using OCT, could be related to the amount of mineral lost during the demineralization process. The relationship between reflectivity loss ($R_{\%}$) and the actual amount of mineral lost (Δz) during demineralization ($R_{\%} = 41.47 + 0.009\Delta z$) was established in our previous studies,^{14,15} in which the reflectivity loss measured by OCT was correlated with the mineral loss measured by transverse microradiography, a method that has long been established as a gold standard for quantification of demineralization.

The decision to remineralize or restore a caries lesion depends on the depth of the lesion in the tooth. OCT was also able to show the depth of the demineralization (caries) inside the tooth tissue with the longitudinal images (Fig. 5), thereby proving to be a potential tool for decision-making in restorative dentistry. Amaechi et al.²¹ have demonstrated that OCT A-scans deliver quantitative data relating to the degree of change in reflectivity, and hence the degree of change in mineral level, of the tooth tissue following development of caries. This could be used to determine the effect of therapeutic agents (e.g., fluoride mouth rinses, fluoride dentifrice) or laboratory testing of a new oral health-care product. The provision of quantitative data as well as information on depth, all without dangerous ionizing radiation, gives OCT superiority over the conventional X-ray technology that has been the tool for caries diagnosis for many years.

6 Conclusion

It was concluded that as a technique for detection and analysis of early enamel caries, OCT correlated well with an established method of quantifying demineralization. The reflectivity lost in enamel during demineralization, measured using OCT, could be related to the amount of mineral lost during the demineralization process. OCT detected early enamel caries, showed the depth of the caries inside the tooth tissue, and quantitatively monitored tooth tissue demineralization over a period of time. This demonstrated that OCT would be a suitable tool for routine examination in the dental clinic and a useful device to quantitatively monitor, *in vivo*, the mineral changes over time in a caries lesion after application of a therapeutic agent. It would also be applicable in *in vivo*, *in situ*, or *in vitro* testing of the efficacy of products formulated to inhibit demineralization and/or promote remineralization. The potential of OCT as a caries diagnostic device that may possibly replace the conventional dental radiograph and eliminate the danger of hazardous ionizing radiation, is illustrated by the present study.

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