Detection and analyzing plane of non-cavitated approximal caries by cross-polarized optical coherence tomography (CP-OCT)

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Abstract

Objective: The objective was to assess the detection ability and the effect of analyzing plane of CP-OCT for non-cavitated approximal caries. Methods: Thirty human extracted premolars were selected based on micro-computed tomography (\(\mu\)-CT): \(\mu\)-CT=0: sound (n=12), \(\mu\)-CT=1/2: caries into outer-/inner-half of enamel (n=6 each), \(\mu\)-CT=3: caries into outer one-third of dentine (n=6). Teeth were mounted in a custom-made device to simulate approximal contact, and scanned from the marginal ridge above the contact area. CP-OCT images were analyzed by deepest caries extension from horizontal and coronal planes, and repeated 48 hrs later. Sensitivity, specificity, percent correct, area under the ROC curve (Az), intra-examiner repeatability and correlation with \(\mu\)-CT were determined. Results: Sensitivity/specificity/Az for Horizontal-plane, Coronal-plane, and Deepest from both planes were 94%/58%/0.76, 81%/100%/0.90, and 94%/58%/0.82. Coronal-plane had significantly higher specificity than Horizontal-plane and Deepest (p=0.004) but Horizontal-plane and Deepest were not different (p=1.00). Horizontal-plane had significantly lower Az than Deepest (p=0.048), but Coronal-plane was not different than Horizontal-plane (p=0.07) or Deepest (p=0.20). Correlation coefficients were Horizontal-plane (0.53, p<0.001), Coronal-plane (0.84, p<0.001), and Deepest (0.66, p<0.001). Conclusion: Within the limitations of this study, CP-OCT could be used to detect non-cavitated approximal caries. Analysis using the Coronal-plane is superior to the Horizontal-plane. Clinical Significance: It is challenging to detect non-cavitated approximal caries clinically due to the adjacent tooth. CP-OCT is a nondestructive, no ionized-radiation caries detection method. CP-OCT seems suitable to detect non-cavitated approximal caries and observing the Coronal-plane appears better than Horizontal-plane.

Key words (6 words):
Cross-polarized optical coherence tomography, approximal (interproximal), dental caries, detection, analyzing plane
1. Introduction

Optical coherence tomography (OCT) is a non-invasive technique to quickly cross-sectionally image the internal biological structure, which does not require ionizing radiation [1]. The principal of OCT is that the light splits into two arms, sample and reference arms. The sample light shines on the tissue surface, while the reference light shines on the reference mirror. After light resorption, reflection and scattering, the reflected backlight from the sample arm and reference arm interfere within a Michelson or Mach-Zehnder interferometer. Then the interference light signal is recorded by a photodiode (PD) or charge-coupled device (CCD) which is dependent on the type of OCT [2]. After Fourier transform of the signal, A-scan (depth) and B-scan (cross-sectional images) are generated and 3-Dimensional (3D) images can be created to show the internal structure.

Dental hard tissues are comprised of three solid structures: enamel, dentin, and cementum. For dental crowns, enamel comprises the outer shell and internally contiguous with the inner shell dentin at an interface known as the dento-enamel junction (DEJ). The refractive indices of enamel and dentin were 1.631±0.007, 1.540±0.013 respectively [3]. In 1998, OCT images of human stomatological hard and soft tissues were presented for the first time [4]. The imaging depth of OCT is highly influenced by the translucency of the medium. Because sound enamel is almost transparent, OCT at wavelength 1300 nm can capture the whole thickness of enamel and 1-2 mm in dentin [5],[6]. Therefore, it is reasonable to assume OCT would be a useful technique for detection of dental caries. As a multifactorial disease, dental caries is the localized demineralization of dental hard tissues, which is initiated within the bacterial biofilm [7]. During enamel caries formation, the demineralization in non-cavitated caries causes dissolved organic matrix between the enamel crystals and leaves irregularly shaped enamel nanorods and numerous pores, which also result in the decrease in the refractive indices [8]. When scanning a tooth surface with OCT, dental caries appears as a homogeneous, highly backscattering area contrasted with the sound tissue [4]. The decreased reflectivity of enamel and dentin during the demineralization
process is related to the increasing amount of mineral loss [9].

There are many reports on the dental application of OCT in the past decades. For example, Fourier Domain OCT (FD-OCT) has recently gained popularity in dental hard tissue disorders: to detect non-cavitated and cavitated occlusal dental caries [10]; to evaluate restoration quality [11]; and to assess erosive tooth wear [12]. Previous studies have demonstrated that polarization-sensitive optical coherence tomography (PS-OCT) can also be used in many aspects of cariology [13], [14], [15], [16]. For caries detection, PS-OCT can be used not only to detect the demineralization directly on tooth surfaces [17], but also to detect artificial/simulated approximal caries from the occlusal direction in vitro [18]. However, there are no clinical studies using OCT (neither FD-OCT nor PS-OCT) to detect non-cavitated, early stage, approximal caries of posterior teeth, especially with the adjacent teeth existing.

With light propagation from air to enamel, strong reflection on the tooth surface occurs. This phenomenon can prevent the differentiation between the strong reflectance surface and the underlying caries lesion, especially for conventional OCT systems [19]. In order to reduce surface reflection, another measurement mode cross-polarization mode was applied on swept source OCT (SS-OCT). Cross-polarization swept source optical coherence tomography (CP-OCT), which does not strongly depolarize the incident polarized light, can reduce the surface reflection by 20–30 dB and increase the contrast between sound and demineralized enamel [20]. CP-OCT was able to quantify the mass of the biofilm/dental plaque [21]; quantify existing remineralization of occlusal caries [19]; measure the integrated reflectivity of remineralized natural root caries [22]; and examine the interface integrity between dental composites, which is a material to restore cavities; and dental hard tissue in vivo [19] and in vitro [23, 24]. CP-OCT even had the potential to image dental hard tissue and longitudinally monitor demineralization peripheral to orthodontic brackets, which are used for orthodontic treatment [25], [26].

Compared to smooth and occlusal surface caries, the visual detection of approximal
caries is difficult due to the presence of adjacent teeth [27]. For non-cavitated caries, the outer enamel surface was remineralized, masking an underlying extensive dentinal caries that is difficult to detect by conventional techniques [28]. Radiography has been an important approximal caries detection technique [29]. When detecting non-cavitated approximal caries (extension to the inner half of the dentin), the sensitivity for bitewing radiography ranged from 45% to 55% [29]. SS-OCT, on the other hand, showed significantly higher sensitivity (92%) than radiographs [30]. To prevent, maintain and improve oral health, it is important to detect non-cavitated caries lesions (at early stage) and monitor frequently, especially for high caries risk cases. Since it is difficult to detect and follow non-cavitated approximal caries lesions with conventional techniques (visual and radiography), searching for alternative techniques is necessary. One disadvantage of radiography is that it cannot be repeated many times in a short period because of the ionizing radiation. Therefore, non-invasive OCT, which doesn’t require ionizing radiation, was considered as a potential technique to detect approximal caries. To the best of the authors’ knowledge, although some new OCT systems were proved to be capable of scanning an entire tooth and monitoring occlusal and approximal lesions from the occlusal surface [31], there is no report to demonstrate the ability of SS-OCT to detect non-cavitated approximal caries, nor Cross-polarization SS-OCT (CP-OCT).

The B-scan image (cross-sectional image, longitudinal reflected light intensity) shows not only position and depth, but also the magnitude of the back-scattered light intensity. A collection of B-scans at different depths are subsequently used to reconstruct 3D volumes of the tissue [10]. When analyzing the images, one/two of three Cross-sectional planes were chosen according to the objective [22],[30],[32]. Ueno et al applied SS-OCT to the enamel and dentin artificial demineralization from two directions, the lesion surface and parallel to the lesion surface. Interestingly, scanning parallel to the lesion surface detected a deeper demineralized lesion body than scanning from the lesion surface [33]. Their finding demonstrated that different analyzing/scanning planes might influence demineralization measurements on the OCT.
In a recent review manuscript about the OCT detection and diagnosis of dental caries, the authors concluded the importance of a commercially viable OCT device to be easily deployed and interpreted by the clinician [34]. To further transition CP-OCT into clinical practice, validation studies are essential to determine the clinical utility. As validation research, the purposes of this study were to assess: 1) the detection ability and 2) the effect of analyzing plane of CP-OCT images for non-cavitated approximal caries on premolars in vitro. We hypothesized that CP-OCT could detect non-cavitated approximal caries and analyzing planes would affect detection results.

2. Materials and methods

2.1. Specimen Preparation

2.1.1. Teeth selection

The collection of human teeth for use in dental laboratory studies is approved by the Institutional Review Board. Extracted human teeth were collected from dental practitioners across the State of Indiana and transported in 0.1% thymol solution. The inclusion criteria for this study was sound and non-cavitated caries on approximal surfaces of premolars. Teeth that had the following conditions were excluded: visible defects, stains, obvious fluorosis or cracks on approximal or other surfaces. All selected teeth were cleaned with Robinson’s brush on a slow speed rotary handpiece under water, and then stored in a 4°C refrigerator in an air-tight humid container with 0.1% thymol solution.

2.1.2. Micro-computed tomography (μ-CT) image acquisition

The teeth were mounted and secured on Lego® bricks (The LEGO Group, Billund, Denmark) using utility wax (Heraeus Kulzer Inc., Lafayette, IN, USA). The micro-computed tomography (μ-CT) images were acquired by μ-CT instrument (Skyscan 1172, Kontich, Belgium) at 80 kV, 134 μe, 8.99 μm pixel size resolution with an Al + Cu filter. Two-dimensional (2D) images were reconstructed using NRecon version 1.6.6
software (Bruker microCT, Kontich, Belgium). Two examiners observed and scored the 2D images using image display software (CT-Analyser, Bruker microCT, Kontich, Belgium). Premolars (N=44) were selected based on caries extension, which was determined by the deepest caries extension. The caries extension distribution was as follows: for the main study, thirty study teeth [12 teeth with sound surfaces (μ-CT=0), 6 teeth with caries extended into the outer half of the enamel (μ-CT=1), 6 teeth with caries extended into the inner half of the enamel (μ-CT=2), and 6 teeth with caries extended into the outer one-third of the dentin (μ-CT=3)]; for training, twelve teeth with or without non-cavitated caries (μ-CT=0: 3 teeth, μ-CT=1: 3 teeth, μ-CT=2: 3 teeth, and μ-CT=3: 3 teeth); and two additional sound premolars as adjacent teeth in the study model.

2.1.3. Model assembly
We assembled adjacent sound premolars as reference. Triad® visible light cure resin (DENTSPLY International, Inc., York, USA) was applied around the root to resemble the thickness and anatomy of the gingiva and the cervical part of the teeth at the level of the cementoenamel junction. At the same time, to simulate approximal contact, the study tooth was mounted on Lego® bricks and placed next to an adjacent sound premolar. Triad resin was applied in the same manner described above. Dental floss was used to confirm that an approximal contact existed. All models were kept separately in an air-tight humid container with 0.1% thymol solution in a 4°C refrigerator.

2.2. CP-OCT examination
2.2.1. CP-OCT Setting
The CP-OCT dental imaging instrument (Santec Inner Vision IVS-300-S-L-C, Santec Corporation, Komaki, Japan) was used in this study. It utilizes a swept laser source (Santec Model HSL-200-30, operating with a 30 kHz sweep rate), with the center wavelength 1,310 nm (bandwidth 30 nm), axial resolution ≤ 12 µm in air. The long neck cross-polarization probe, which is suitable for dental examination, was equipped with lateral scan area 5×5mm. The parameters of this OCT setting were focus depth
3mm, working distance 1mm, lateral resolution 30 µm. The probe was mounted in a custom-built holder, while the teeth were mounted on a custom-built platform (Figure 1).

2.2.2 CP-OCT image acquisition
The study tooth and adjacent sound tooth were settled on the platform as shown in Figure 1. The platform was adjusted perpendicularly and horizontally to make sure the contact area was in the center of the scanning image and teeth were 1 mm from the probe. Since hydration significantly affects the OCT signal intensity [35], before scanning, we used a cotton pellet soaked with deionized water to keep the surface moist for 1 min to control the hydration condition, making sure no visible water droplets remained while scanning. The scanning was performed from the occlusal direction, and then 3D OCT volume data were configured from the 512 B scans, and saved as Data.bi and DataInfo.ini format on Inner Vision software (Santec corporation, rev2.4.0, Komaki Aichi, Japan).

2.2.3 CP-OCT image processing
3D OCT data were displayed on Inner Vision software as 2D images. To reduce speckle noise, the noise reducing filters (both median filter and gaussian filter) were applied to the data. According to a previous study, the refractive indices of enamel, dentin, and cementum were 1.631±0.007, 1.540±0.013, and 1.582±0.010, respectively [3]. The refractive indices of caries vary widely depending on the degree of demineralization [36]. When the enamel mineral content decreased from 87% to 50%, the refractive indices changed from 1.63 to 1.52 [37]. In this study, the average refractive index of demineralized enamel 1.550 was chosen instead of the default value of 1 for air, as in other OCT studies [20], [38]. The information of the light intensity was automatically calculated by Inner Vision, and then outputted 1D wave images, 2D grayscale images and 3D tomographic images.

On the original 2D grayscale images, each pixel is displayed in grayscale with white
indicating high light intensity and black representing low light intensity. In order to obtain the optimal contrast between caries and sound tissue, 2D images were processed with color scale images instead of original grayscale images. The brightness and contrast were configured to cover an intensity range between 50dB and 0dB. On color scale images, black represented light intensity 0, green represented light intensity 0-15, sky blue represented light intensity 15, red represented light intensity 25-30, and white represented light intensity 50.

2. 2. 4. Interpretation criteria of CP-OCT images and training

The reliability of visual assessment of OCT images can be enhanced by training in usage of visual interpretation criteria [39], [40]. Prior to the main CP-OCT image analysis, standardized training was performed with 2D CP-OCT color scale images on 12 training teeth. Dental caries, as increased signal intensity, was shown as an elevated wave on 1D wave images. On 2D images, caries was shown as a highlighted area on grayscale images and as a deep colorful area on color scale images. There were three planes of 3D volumes, which were the horizontal, coronal and sagittal planes (Figure 2). In the current study, analysis was based on the Horizontal-plane and Coronal-plane 2D color scale images from dynamic slicing of 3D OCT volumes. The visual border between the colorful area and surrounding green and black area was observed as the boundary of dental caries. The interpretation criteria were developed based upon the location, lesion depth and pattern of the light intensity range on 1D files. The dental caries extension criteria of μ-CT and CP-OCT images (Horizontal-plane and Coronal-plane) images is shown in Table 1.

2. 2. 5. Main CP-OCT image analysis

Two days after training, using processed 2D CP-OCT color scale images from 30 study teeth, maximum caries extension on the Horizontal-plane and Coronal-plane were determined by the trained examiner, who was blinded for caries extension, based on the criteria mentioned in Table 1. The maximum caries extension from the Horizontal-plane and Coronal-plane was recorded as Deepest caries extension.
2. 2. 6. Repeat CP-OCT examination

One week after the main CP-OCT image analysis, using the same 2D CP-OCT color scale images, the examiner repeated image analysis in the same manner described previously (in 2. 2. 5 Main CP-OCT image analysis).

2. 3. Statistical analysis

Intra-examiner repeatability was evaluated using kappa and weighted kappa statistics. Caries was defined as $\mu$-CT score $> 0$. Sensitivity was further evaluated based on caries extension. Comparisons between the percent correct, specificity, sensitivity, and area under the ROC curve (Az) for the OCT horizontal and coronal analyzing planes and deepest extension were performed using bootstrap analyses. The correlations of the measurements with the $\mu$-CT were also calculated using bootstrap methods. The bootstrap methodology uses resampling techniques to estimate statistics and perform comparisons for values that are not normally distributed. In this case, the bootstrap also provides a way to properly account for the correlations between repeats and between methods. A 5% significance level was used for all tests. Analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

3. Results

The overall sensitivity, sensitivity by caries extension specificity, percent correct, and Az are presented in Table 2.

3. 1 Sensitivity and Specificity

For sensitivity, there were no significant differences among the three methods, overall or by caries extension (overall / $\mu$-CT=1 / $\mu$-CT=2 / $\mu$-CT=3, $p \geq 0.09$ / $p \geq 0.06$ / $p = 1.00$ / $p = 1.00$, respectively). For specificity, the Coronal-plane (100%) was significantly higher than the Horizontal-plane (58%) and Deepest extension (58%) ($p = 0.004$); but Horizontal-plane and Deepest extension were not significantly different from each other ($p = 1.00$).
3. 2 Percent correct and Az
The percent correct ranged from 80 to 88%, with no significant differences (p≥0.26). For Az, the Horizontal-plane had significantly lower Az (0.76) than the Deepest extension (0.82, p=0.048), but the Coronal-plane (0.90) was not significantly different than the Horizontal-plane (p=0.07) or the Deepest extension (p=0.20).

3. 3 Intra-examiner repeatability and correlation with "μ-CT"
Intra-examiner repeatability was high. Weighted kappas were 0.90, 0.95, and 0.95 for the Horizontal-plane, Coronal-plane, and Deepest extension, respectively. CP-OCT measurements showed weak to moderate correlations with μ-CT. Correlation coefficients with μ-CT were 0.53, 0.84, and 0.66 (all p<0.001) for the Horizontal-plane, Coronal-plane, and Deepest extension.

4. Discussion
The OCT technique of visualization is being tested in different fields of dentistry [41]. In this in vitro validation study, the CP-OCT was evaluated for detection of approximal non-cavitated caries lesions from the occlusal direction. Our results showed CP-OCT images could be used as a potential technique for the detection of non-cavitated approximal caries, and analyzing planes might have some influence on the results.

4. 1. Ability for detection
4. 1. 1. Sensitivity and specificity
Bitewing radiography is the most widely used clinical technique with sensitivity 38%-66% when detecting approximal enamel and dentinal caries in vivo [42]. When caries are radiographically evident, mineral loss is already 30%-40%. The actual depth of clinical caries is hence deeper than that on a radiograph [43]. Therefore, when detecting non-cavitated approximal caries (extension to the inner half of the dentin), the sensitivity for bitewing further decreased to 45%-55% [29]. For CP-OCT in the current study, the overall sensitivity was 81%-94%, which was comparable to another clinical
study that found the sensitivity of SS-OCT was 92% when detecting non-cavitated approximal caries [30]. The high sensitivity across these two studies indicates that non-cavitated approximal caries would be detected by CP-OCT.

The specificity in the current study (58%-100%) is also higher than SS-OCT for detecting non-cavitated approximal caries (47%) [30]. The higher specificity observed in the current study might be due to the cross-polarization mode. In the case of non-cavitated caries, the visual caries boundary was created due to high reflectivity from the superficial enamel and low backscatter signals of the underlying tissue [32]. Compared to SS-OCT, cross-polarization reflectance has high contrast of demineralization, and could detect caries depth 50μm under intact surfaces enamel [44]. Additionally, interpretation criteria were used by a trained examiner to differentiate the increased backscattered intensity due to specular reflection from demineralization [39]. Specificity was also improved by the use of color scale images that enhanced the contrast of the range of intensity of demineralization [24].

4.1.2. Percent correct and Az
The percent correct of CP-OCT to detect non-cavitated approximal caries was 80% to 88% in the current study. Diagnostic test performance is typically measured by an ROC curve. The area below that fitted ROC curve (Az) is an index of accuracy for the detection performance [45]. Az value 0.5 represents random decision and 1.0 represents a perfect diagnostic justification [46]. The Az values enable comparisons of detection accuracy among methods or procedures. With the Az 0.76-0.90 in the current study, CP-OCT showed the ability to detect demineralization comparable to PS-OCT (Az 0.796) [47]. The current study also revealed that the detection accuracy of CP-OCT was similar to digital bitewing (Az 0.797) and conventional panoramic (Az 0.768) for detecting approximal caries [48], and also comparable with CP-OCT for detecting non-cavitated occlusal caries (Az 0.55-0.73) [49]. The current study demonstrated the accuracy of CP-OCT in non-cavitated approximal caries detection.
4.1.3. Intra-examiner repeatability and correlation with μ-CT

For intra-examiner repeatability, weighted kappa statistics were calculated to determine the repeatability of the examiner between the first and second examinations. Weighted kappas ranging from 0.90 to 0.95 indicated that CP-OCT is repeatable, which was similar to Vaswani et al study, in which weighted kappas were 0.71-0.86 when using PS-OCT to detect smooth surface non-cavitated caries [47]. Interestingly, it was contrary to Hilsen et al. study, who found that there was high variability in assessing the images of non-cavitated occlusal caries *ex vivo* [49]. Since the visual interpretation of OCT images requires appropriate calibration [50], the difference in repeatability might be explained by the usage of visual interpretation criteria. [39]. This was also demonstrated by Holtzman et al, where the examiners were trained 90 minutes then improved their accuracy on detecting caries on OCT images [40]. For Hilsen et al. study, although CP-OCT images were evaluated by two experienced examiners, visual criteria training before examination was not mentioned. In fact, one of the two examiners showed comparable repeatability (weighted kappa 0.72) to the current study. Therefore, training using interpretation criteria could enhance the differentiation of increasing backscattered intensity due to specular reflection from demineralization enamel and/or dentin.

The correlation results from the current study showed that CP-OCT presented weak to moderate correlations with the gold standard. It is important to note that even OCT is a subjective technique, and the proper algorithm for the determination of the caries boundary is still not available. Since light intensity was the original data captured by OCT, and it is linearly related to the square root of the degree of demineralization [23, 51, 52], many researchers attempted to develop mathematical algorithms to rapidly and accurately measure the edge of caries automatically based on the light intensity [53]. Lenton et al. chose light intensity of 12.3 dB as the cut-off value. Values above 12.3 dB were assessed as caries and those below were assessed as sound [24]. However, they didn’t explain how 12.3 dB was chosen as the cut-off value. Fried et al. chose $1/e^2$ decrease in intensity as cut-off intensity values on PS-OCT images [16] and CP-OCT
images [54]. However, this method does not always work effectively [55]. In the current study, the boundary of the caries was a visually observed line between the highly-scattering colorful area with the darker region with dento-enamel junction (DEJ) as a landmark. However, although the DEJ was clear in sound tissue, it was not quite clear in caries tissue on OCT images either CP-OCT or SS-OCT in other studies [8], because the enhancement of light scattering within caries was over an extent that the light photons could effectively probe the underlying DEJ [56]. Nonetheless, it brings hope to develop CP-OCT as a probe for approximal caries detection/quantification.

4.1.4. Image contrast by the Color Scale

In grayscale OCT images, caries was recognized as increased highlighted signal intensity, which was induced by the amount of light scattering and depolarization from demineralized micro porous enamel and/or dentin [51]. This is a common phenomenon in CP-OCT imaging of enamel due to the birefringence properties of the enamel, the parallel polarization state of the incident beam, and the low backscattering of sound enamel [52]. The boundary of the caries is the observed line between the highly-scattering area with the darker region, which was not very clear on original grayscale images. The caries area, however, was more visible with higher image contrast, and the color scale images augment the differentiation of areas of higher backscattering and depolarization within caries [24],[55]. Therefore, the color scale images were overlaid to analyze caries depth in the current study. With different colors representing different arrangements of light intensity, the boundary of caries was easier to determine by the examiner.

4.2. Effect of analyzing planes

Displays of horizontal, coronal and sagittal planes at different positions in the 3D stack of CP-OCT images would directly show caries extension [57]. In the current study, the Horizontal-plane, Coronal-plane and Deepest extension had similarly high sensitivities. The application of cross-polarization might contribute to this result. The lateral resolution (Horizontal-pane view) of the cross-polarization probe is 30 µm combining
12 µm axial resolution (Coronal-plane view). With this resolution, CP-OCT could detect non-cavitated caries in any analyzing plane. In other words, even with reflections at the caries/sound tissue interface as small as half a micrometer in depth, it could be detected by CP-OCT because of small image pixel size.

However, there was a large disparity for the specificity among the Horizontal-plane (58%), Coronal-plane (100%), and Deepest extension (58%). This disparity could be explained by the non-parallel alignment of enamel mineral crystals causing different intrinsic birefringence, and also the form-birefringence caused by the anisotropy of the architecture of crystals and liquid-filled pores [58]. Ueno et al demonstrated detection from parallel to the lesion surface could detect lesion body deeper than detection from perpendicular to the lesion surface using SS-OCT [33]. The light intensity and attenuation coefficient from parallel to the lesion surface were lower than the values from perpendicular to the lesion surface [33]. Therefore, the specificity maybe different for different analyzing planes. On the other hand, enamel subsurface specular reflections on the coronal plane were more than on the horizontal plane [15]. With the capture of high light intensity from surface tissue, once tissues on the coronal plane were recognized as sound tissue, it was highly possible to be sound tissue. This would be the potential reason why the Coronal-plane has such high specificity (100%).

Sensitivity, specificity and Az values are useful for the evaluation of diagnostic ability. Generally speaking, the higher Az value (diagnostic accuracy) represents better power of distinguishing positive and negative cases [59]. The International Caries Detection and Assessment System (ICDAS) was recommended for the visual detection and classification of dental caries because many researches demonstrated the Az were all substantial ($\geq 0.75$) [60]. In the current study, CP-OCT is an optical technique, and visual interpretation criteria was applied to analyze the images. For the analyzing plane, the Horizontal-plane, Coronal-plane and Deepest extension showed substantial Az (0.76, 0.90, 0.82). It has been suggested that caries diagnostic methods should have value of at least 0.75 for sensitivity and over 0.85 for specificity, and SS-OCT met these
criteria for evaluation of occlusal caries compared with X-ray radiography [50]. For the current study, the coronal plane CP-OCT imaging seems to meet these criteria, with overall sensitivity 0.81, specificity 1.0. Therefore, the coronal plane would be the better analyzing plane.

CP-OCT system employs near-infrared (NIR) laser as a light source with the center wavelength near 1310 nm, because enamel manifests its highest transparency near 1300-nm with weak scattering and absorption [41]. Based on our results, CP-OCT has potential for non-cavitated approximal caries detection. It is important to recognize that, at NIR wavelength (from 780 to 1550 nm), not only OCT, but NIR and short-wave infrared (SWIR) imaging systems also had superior diagnostic performance than radiograph for the detection of approximal caries *in vitro* [61],[62] and *in vivo* [63]. However, as an adjunct technique, either OCT or NIR/SWIR, should be used to support the conventional oral examination to confirm the caries borderlines in case of clinical uncertainty [34].

5. Conclusion
Within the limitations of this *in vitro* study, it is suggested that, as a non-ionizing radiation technique, CP-OCT has high sensitivity and high specificity, and it is a reliable and accurate technique for the detection and estimation of the depth of non-cavitated approximal caries. Regarding the analyzing plane, the coronal plane would be the better analyzing plane based on demonstrating balanced detection performance, higher sensitivity, specificity and Az compared with other planes. The reliability of visual assessment of CP-OCT images to detect non-cavitated approximal caries may be enhanced by the use of interpretation criteria and color scale.

**Competing interests**
The authors declare no conflict of interest.
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Table 1: the criteria of μ-CT and CP-OCT (Horizontal-plane and Coronal-plane) images. Red line showed location of dental caries. White arrow pointed to DEJ in CP-OCT images.

<table>
<thead>
<tr>
<th>Score</th>
<th>μ-CT images</th>
<th>CP-OCT images Horizontal-plane</th>
<th>CP-OCT images Coronal-plane</th>
</tr>
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<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
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<td><img src="image6.png" alt="Image" /></td>
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<tr>
<td>2:</td>
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<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>3:</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Score 0: Sound, no caries.
Score 1: Dental caries extends to the outer half of the enamel.
Score 2: Dental caries extends to the inner half of the enamel and does not extend beyond the dentino-enamel junction.
Score 3: Dental caries extends to the outer one-third of the dentine.
Table 2. Sensitivity overall and based on caries extension, specificity, percent correct, and area under ROC curve (Az).

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>% Correct</th>
<th>Az</th>
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<td>Overall</td>
<td>94(^a)</td>
<td>58(^a)</td>
<td>80(^a)</td>
<td>0.76(^a)</td>
</tr>
<tr>
<td>(\mu)-CT=1</td>
<td>100(^a)</td>
<td>100(^a)</td>
<td>83(^a)</td>
<td></td>
</tr>
<tr>
<td>(\mu)-CT=2</td>
<td>83(^a)</td>
<td>100(^a)</td>
<td>80(^a)</td>
<td></td>
</tr>
<tr>
<td>(\mu)-CT=3</td>
<td>100(^a)</td>
<td>100(^b)</td>
<td>88(^a)</td>
<td>0.90(^a,b)</td>
</tr>
</tbody>
</table>

H-Plane indicates Horizontal plane and C-Plane indicates Coronal plane. Deepest indicates deepest of H-Plane and C-Plane. Caries extension is as follow: \(\mu\)-CT=1: caries extended the outer half of the enamel, \(\mu\)-CT=2: caries extended the inner half of the enamel, and \(\mu\)-CT=3: caries extended the outer one third of the dentin.

Same superscript letter indicates there is no significant difference between methods in each outcome (comparisons within a column).
Figure 1. This diagram shows setting for CP-OCT. The teeth were mounted on a custom-built platform simulating approximal contact.
A: both study and adjacent sound teeth simulating approximal contact, B: probe scanning window and measurement point, C: CP-OCT probe, D: custom-built adjustable platform.
Figure 2. This diagram shows analyzing planes. Solid lines indicate Coronal-plane and dot lines indicate Horizontal-plane.