Use of optical clearing and index matching agents to enhance the imaging of caries, lesions, and internal structures in teeth using optical coherence tomography and SWIR imaging
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Introduction
Studies have shown that various agents can be applied to tooth surfaces to enhance the imaging of caries lesions (tooth decay) and internal structures such as the dentinal enamel junction (DEJ), cracks, root canals and the pulp chamber. The refractive indices (RI) of enamel, dentin, and cementum are quite high at 1.63 and 1.54, and 1.58, respectively, and surface scattering and specular reflection can interfere with optical penetration into the tooth. Moreover, dental hard tissues are porous structures, and that porosity increases markedly with demineralization and hypomineralization. Filling the porous structure of subsurface caries lesions with a high RI fluid enables better optical penetration through such lesions to better view the underlying tooth structure for better assessment of lesion severity. For example, it has been demonstrated that optical clearing agents can be used to increase the visibility of deeply penetrating occlusal lesion images that have reached the underlying dentin and spread laterally under the enamel in optical coherence tomography (OCT) images that cannot be detected with dental radiographs. High refractive index fluids can also increase the contrast of caries lesions. In addition, the loss of the mobile water in sound enamel and dentin or displacement by another fluid profoundly changes light scattering in these tissues and optical penetration. The use of exogenous agents to enhance dental imaging is of increasing interest to the dental community with the introduction of new near-infrared (NIR) and short wavelength infrared (SWIR) transillumination and reflectance imaging methods along with OCT that exploit the high transparency of dental enamel at longer wavelengths beyond the visible range.

Optical properties of dental hard tissues from 400 to 2300 nm
Light attenuation in enamel and dentin are dominated by scattering in the visible and near-infrared (NIR) ranges [1]. The scattering coefficient of enamel decreases with increasing wavelength from 400 cm⁻¹ in the near-UV [1] to as low a value as 2–3 cm⁻¹ at 1310 nm in the short wavelength infrared (SWIR) ranges [2, 3] and continues to decrease beyond 1700 nm [4] (see Figure 26.1). The strong wavelength dependence of enamel suggests that scattering is dominated by the small submicron enamel crystals that act as Rayleigh type
scatters [1]. In contrast, light scattering in dentin remains high across the visible and NIR ranges due to the dentinal tubule structures a few microns in diameter that act as cylindrical Mie scatterers. The scattering of dentin is highly anisotropic and quite variable with position in the tooth and exceeds 100–200 cm$^{-1}$ across the visible and NIR [5] ranges. It has been suggested that the tubules can act as waveguides to guide visible light [6].

The absorption coefficient of enamel is too low to be measurable in the visible range while that of dentin is essentially wavelength independent with a value of approximately 4 cm$^{-1}$ above 400 nm [5]. At wavelengths beyond 1400 nm, water absorption increases [7] (see Figure 26.1) and becomes an important source of attenuation in enamel and dentin and contributes to the high contrast of caries lesions on both crown and root surfaces.

The demineralization of enamel and dentin occurs due to organic acids from cariogenic bacteria in the plaque or biofilm on the tooth surface. Highly porous demineralized areas of coronal caries appear whiter and are more opaque. Pores are formed in the tooth structure that increase the magnitude of light scattering by orders of magnitude. Demineralized tissues appear whiter than the surrounding sound tissues due to the increased diffuse reflectance, and clinicians refer to early caries lesions as white spot lesions [8, 9]. Hypomineralized areas of the tooth formed due to developmental defects and fluorosis are also highly porous and appear similar to demineralization due to caries. The earliest attempts to measure the optical properties of dental caries were limited to measurements of backscattered light from optically thick, multilayered sections of simulated caries lesions [10–12]. The microstructure of enamel and dentin caries lesions is complex, consisting of various turbid and transparent zones [13, 14]. During remineralization, pores and tubules are filled with mineral, and those areas are typically more transparent. There have been some attempts to measure and simulate light scattering in artificial and natural lesions [8, 12, 15]. Lesion areas are highly porous, allowing index matching fluids to imbibe into the lesion, effectively eliminating the lesion from an optical standpoint, i.e., the lesion becomes more transparent than the sound tissue. One approach is to assume that carious lesions will be filled with saliva, and that measurement in water replicates the conditions that will be encountered clinically. Darling et al. [16] measured the optical properties of lesion areas imbibed in water at 1300 nm and compared the increase in the magnitude of the scattering coefficient with mineral loss measured using microradiography. The attenuation in the lesion exceeds 100 cm$^{-1}$, a factor of 50 times higher than that of sound enamel at 2–3 cm$^{-1}$, and is a similar magnitude to that of the dentin. Moreover, it is hypothesized that water absorption is a major contributor to the contrast between sound and demineralized enamel beyond 1300 nm since deeply penetrating photons in sound enamel are likely absorbed by water. A comparison of three reflectance images of the occlusal surface of an extracted tooth with demineralization in the fissures are shown in the visible range, 1300 nm and 1450 nm in Figure 26.2 (top). Demineralization should appear whiter in reflectance, and the highest contrast of demineralization is at 1450 nm, while at visible wavelengths only the dark stains in the fissure can be seen. Multispectral comparisons of lesion contrast provide insight into the mechanism responsible for higher contrast at longer SWIR wavelengths. In a recent study [4], reflectance images of demineralization on tooth enamel surfaces were acquired at wavelengths near 1450, 1860, 1880, and 1950 nm. The magnitude of water absorption is similar at 1450 and 1880 nm but varies markedly between 1860, 1880, and 1950 nm. The highest contrast was at 1950 nm; however, the markedly higher contrast at 1880 compared to 1450 nm and similar contrast between 1860 and 1880 nm suggests that the enamel scattering coefficient continues to decrease beyond 1300 nm and that reduced light scattering in sound enamel is most responsible for the higher lesion contrast at longer SWIR wavelengths. Longer wavelength studies of the contrast of demineralization and calculus on root surfaces suggest that the water absorption is a greater contributor to the contrast between sound and demineralized dentin, since the contrast no longer increases beyond 1400 nm, unlike for enamel [17]. Figure 26.3 shows images of the root surface of an extracted tooth at visible, 850, 1300, and 1500 nm. Note how dark the tooth root appears due to the higher water absorption at 1500 nm, while images beyond 1400 nm all appear similar to the 1500 nm image in Figure 26.3 [17]. Tooth surfaces are often heavily stained, and stains accumulate within the porous structure of caries lesions where they can profoundly change the lesion’s optical appearance. The visual diagnosis of lesion activity is based on the lesion texture and color [18]. However, lesion color and texture are not reliable indicators of caries presence and activity, and clinicians often misdiagnose lesions based on the presence of stain, particularly in the pits and fissures of the occlusal surfaces. A major advantage in imaging in the SWIR is that the stains that are common on tooth occlusal surfaces do not interfere at wavelengths beyond 1150 nm since none of the known chromophores absorb light at longer wavelengths [19]. This can be seen in Figures 26.2 and 26.3, where in the visible images the lesion areas appear darker than the sound tooth structure due to absorption of stain, while the stain does not interfere at longer wavelengths. The photon energy is not sufficient for electronic excitation of the chromophores [20, 21].

**FIGURE 26.1** Light attenuation in enamel (red trace) due to light scattering and light attenuation due to absorption by water (blue trace) from [3, 7]. The visible, NIR (700–100 nm) and SWIR (1000–2500 nm) regions are also indicated by color shading.
FIGURE 26.2 (top) NIR reflectance images of a tooth with stained fissures and demineralization at visible, 1300 nm, and 1450 nm. Reprinted with permission from reference [19]. (bottom) In vivo images of a tooth with an approximal lesion and a stained fissure on the occlusal surface that was misdiagnosed as having a lesion. (A) Visible reflectance, (B) SWIR reflectance (1500–1750 nm), and (C) occlusal transillumination (1300 nm) images are shown. The SWIR images confirm that an approximal lesion is present but indicate that there is no demineralization present on the occlusal surface. Reprinted with permission from reference [72].

FIGURE 26.3 NIR Reflectance images of the root surface of a tooth showing dental calculus and root caries at visible, 850, 1300, and 1500–1750 nm wavelengths. Reprinted with permission from reference [17].
It is necessary to use SWIR wavelengths greater than 1150 nm to avoid significant interference from stains when measuring lesion contrast in reflectance and transillumination modalities [19]. Therefore, stains can be easily differentiated from actual demineralization in the SWIR range, which is not possible at visible and NIR wavelengths. Chung et al. [22] demonstrated that absorption due to stains contributed more to the lesion contrast than increased scattering due to demineralization at visible wavelengths [23]. Since it is impractical to remove stains from the deep grooves and fissures on tooth occlusal surfaces, lack of interference from stains at longer SWIR wavelengths is a significant advantage for transillumination and optical coherence tomography.

Influence of hydration

Teeth are highly porous with large fractions of mobile water, and the state of hydration of the tooth profoundly influences the lesion contrast and optical penetration in enamel, dentin, and cementum. The strong influence of hydration can be seen in transillumination measurements through thick tooth sections with lesions at 1300 nm; the contrast decreased markedly if the samples were left out of water overnight and allowed to dehydrate [22]. This effect can clearly be seen for a 5 mm-thick section in Figure 26.4. The mean contrast was 0.53 (0.097) fully hydrated and almost completely opaque 0.016(0.039) when dry for n = 6 samples, 5 mm thick [22]. The lesion contrast completely recovered after the samples were immersed in water overnight and rehydrated. Hydration studies involving the transillumination of the tooth sections indicated that loss of mobile water resulted in a significant reduction in sound enamel transparency and loss of lesion contrast. It is likely that the loss of mobile water leaves pores that act as scattering sites. In another study, it was found that rapid heating with a carbon dioxide laser can dehydrate dental hard tissues; the attenuation coefficient of enamel at 1310 nm increased significantly from 2.12 ± 0.82 to 5.08 ± 0.98 with loss of mobile water due to heating [24, 25]. These results indicate that it is extremely important to ensure that teeth are kept well hydrated for SWIR imaging studies, including optical coherence tomography [26]. It has been well established that a layer of water or saliva influences the contrast and visibility of early white spot lesions. Lesions are visible with higher contrast if the tooth surface is air-dried, and clinicians are taught that if the lesion is only visible when the tooth is dry, it is shallow and superficial. The rate of water loss from lesions has also been correlated with lesion activity; arrested lesions are less permeable to water due to the highly mineralized outer layer covering the lesion, and both optical and thermal imaging [27, 28] have been used to monitor optical changes associated with water loss. More recently, OCT and SWIR imaging have been used to assess lesion activity, and those methods are discussed in a following section.

Refractive index (RI), polarized light, and imbibing agents

Tooth enamel has a very high refractive index, resulting in high reflectivity from the enamel surface and high refraction and internal reflection that render photographing and imaging teeth more challenging. The refractive indices (RI) of enamel, dentin, and cementum are quite high at 1.63 and 1.54, and 1.58, respectively [29]. Optical coherence tomography has also been used to measure the refractive index in dental hard tissues [30]. The visibility of scattering structures on highly reflective surfaces such as teeth can be enhanced by the use of crossed polarizers to remove the glare from the surface [31, 32]. The contrast between sound and demineralized enamel can be further enhanced by depolarization of the scattered light in the area of demineralized enamel [33, 34].

Polarized light microscopy has been used to study teeth for more than 150 years [35]. Tooth enamel and dentin are birefringent due to both mineral and organic components in these tissues. Different zones of caries lesions have been identified and studied under polarized light [36–38]. Darling et al. [39] and others [40] developed quantitative methods to measure the mineral loss by imbibing different fluids of varying refractive index into tooth sections and measuring the birefringence. Quinoline has a refractive index of 1.63, the same as dental enamel, and it is common practice to immerse tooth sections in quinoline to study birefringence changes in the various zones of caries lesions.

Dental caries and demineralization and remineralization

During the past century, the nature of dental caries (tooth decay) in the US has changed markedly due to the introduction of fluoride to the drinking water, the advent of fluoride dentifrices and rinses, and improved dental hygiene. In spite of these advances, dental decay continues to be the leading cause of tooth loss in the US [41–43]. Root caries is an increasing problem with our aging population. Today the majority of newly discovered caries lesions are localized to the occlusal
pits and fissures of the posterior dentition (occlusal caries lesions) and the proximal contact sites between teeth (approximal or interproximal caries lesions). In the caries process, demineralization occurs as organic acids generated by bacterial plaque that diffuse through the porous enamel of the tooth dissolve the mineral. If the decay process is not arrested, the demineralization spreads through the enamel and reaches the dentin, where it rapidly accelerates due to the markedly higher solubility and permeability of dentin. The lesion spreads throughout the underlying dentin to encompass a large area, resulting in loss of integrity of the tissue and cavitation. When the mineral content falls below a certain level the tissue collapses forming an obvious cavity, and it is this process that led to use of “cavity” to describe a caries lesion. Caries lesions are usually not detected until after the lesions have progressed to the point at which surgical intervention and restoration are necessary, often resulting in the loss of healthy tissue structure and weakening of the tooth. Therefore, new diagnostic tools are needed for the detection and characterization of caries lesions in the early stages of development.

Caries detection and diagnostics

Conventional methods of caries detection and diagnostics

Caries lesions are routinely detected in the US using visual/tactile (explorer) methods coupled with radiography. These diagnostic and treatment paradigms were developed long ago and were adequate for large, cavitated lesions; however, they do not have sufficient sensitivity or specificity for the diagnosis of the early noncavitating caries lesions prevalent today. Radiographic methods do not have the sensitivity for early lesions, particularly occlusal lesions, and by the time the lesions are radiolucent, they have often spread extensively throughout the underlying dentin, at which point surgical intervention becomes necessary [44]. At that stage in the decay process, it is far too late for preventive and conservative intervention, and a large portion of carious and healthy tissue will need to be removed, often compromising the mechanical integrity of the tooth. If carious lesions are detected early enough, it is likely that they can be arrested/reversed by nonsurgical means through fluoride therapy, antibacterial therapy, or dietary changes [45].

Accurate determination of the degree of lesion activity and severity is of paramount importance for the effective employment of the treatment strategies mentioned above. Since optical diagnostic tools exploit changes in the light scattering of the lesion, they have great potential to assess whether or not the caries lesion is active and expanding, or whether the lesion has been arrested and is undergoing remineralization.

The most difficult to detect and the most common early enamel lesions are occlusal lesions. Occlusal lesions constitute 80% of the new lesions found today [46]. In the conventional method of occlusal caries detection, the clinician probes areas in the dentition that appear suspicious upon an initial visual inspection with the dental explorer [47]. If the probed area is soft and provides some resistance upon retraction of the instrument, the site is deemed to be carious. Studies suggest that the use of the dental explorer to probe for caries may actually promote or accelerate lesion formation [44, 48]. Thus, the use of a blunt explorer or none at all has been recommended by leading cariologists [49–51]. Clinicians base their diagnosis of occlusal lesions and treatment planning on the pit and fissure color and texture which is strongly influenced by the degree of staining. This can be misleading because lesion color does not provide sufficient information about the state of the lesion, i.e., whether it is progressing or arrested. Moreover, pigmentation can be due to staining from diet and other environmental factors and not from infection by microorganisms [52].

Optical transillumination

Optical transillumination was used extensively before the discovery of X-rays for detection of dental caries. The development of high-intensity fiberoptic light sources a few decades ago revived interest in optical transillumination for the detection of approximal lesions [53–58]. During fiberoptic transillumination, carious lesion appears dark because of decreased transmission due to increased scattering and absorption by the lesion; however operation in the visible range where light scattering in enamel is high greatly limits performance.

Almost 20 years ago, it was discovered that enamel was almost completely transparent at 1300 nm [2, 59].

The first NIR and SWIR transillumination studies involved proximal transillumination with a similar geometry to radiographs [60], but soon it was discovered the tooth can be imaged from the occlusal surface after shining light at and below the gum line, which we call occlusal transillumination [20, 61]. Approximal lesions can be imaged by occlusal transillumination of the proximal contact points between teeth by directing SWIR light below the crown while imaging the occlusal surface [59–61]. The first clinical study in 2009 demonstrated that approximal lesions appearing on radiographs could be detected in vivo with SWIR imaging proximal and occlusal transillumination with similar sensitivity [61].

SWIR transillumination from the occlusal surface can also be used to image occlusal lesions with high contrast, and multiple studies have shown that occlusal caries lesions can be imaged with high contrast in vivo and that SWIR occlusal transillumination is an excellent screening tool for occlusal lesions [20, 34, 61–65].

In a recent clinical study [66], SWIR transillumination and SWIR reflectance were used to screen premolar teeth scheduled for extraction for all coronal caries lesions, both approximal and occlusal, and the diagnostic performance of SWIR imaging was compared with radiography. SWIR imaging was shown to be significantly more sensitive than radiography for the detection of lesions on both occlusal and proximal tooth surfaces in vivo. In vivo SWIR transillumination (1300 nm) and SWIR reflectance (1600 nm) measurements of a tooth with caries are shown in Figure 26.2 (bottom) [65]. There are now several NIR imaging systems available for caries detection, and they employ both occlusal transillumination and reflectance; however, current commercial systems are only available at wavelengths less than 1000 nm, due to the high cost of sensors at longer wavelengths [67–69].
**Reflectance measurements**

Early enamel white spot lesions can be discriminated from sound enamel by visual observation or by visible-light diffuse reflectance imaging [9, 10]. However, color, in addition to the intensity of the reflected light, plays a large role in detecting those changes. Moreover, such changes are difficult to quantify, and the color of sound tooth structure varies markedly due to stains. In a recent study of natural lesions on 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 lesion contrast [22]. This renders the method useless in areas that are subject to heavy staining, namely the occlusal surfaces where most lesions are likely to develop. In fact, visible light reflectance was proposed three decades ago for use in monitoring early demineralization on tooth surfaces, but it has proven to be unsuccessful due to the problems indicated above [9]. In the early 1980s, ten Bosch et al. [8, 9] introduced an optical monitor that used optical fibers for visible light reflectance measurements on tooth surfaces.

In contrast to transillumination, which initially focused on approximal lesions, the first studies utilizing SWIR wavelengths for reflectance measurements targeted occlusal caries lesions. The contrast between sound and demineralized enamel is greatest in the SWIR due to the minimal scattering of sound enamel, and this can be exploited for reflectance imaging of early demineralization [16]. Wu et al. [70] reported that the contrast between early demineralization was significantly higher at 1310 nm than in the visible range. Zakian acquired hyperspectral reflectance images of occlusal caries lesions and demonstrated that multiwavelength images could be used to aid diagnosis [21]. The highest contrast is achieved at longer SWIR wavelengths coincident with higher water absorption [71]. Water in the underlying dentin and surrounding sound enamel absorbs the deeply penetrating light and reduces the reflectivity in sound areas. In turn, this results in higher contrast between sound and demineralized enamel. Hyperspectral reflectance measurements by Zakian show that the tooth appears darker with increasing wavelength [21]. The performance of SWIR reflectance is even more dramatic for natural lesions on the occlusal surfaces of extracted teeth that are typically stained, as can be seen in the images presented in Figure 26.2. The contrast is significantly higher for wavelengths greater than 1600 nm than at other wavelengths, and stains interfere significantly at wavelengths less than 1000 nm [19].

The first clinical study using SWIR reflectance utilized the wavelength range of 1500–1700 nm, and the diagnostic performance was higher than radiography and other NIR imaging modalities for the detection of proximal and occlusal lesions [66]. In vivo images of a tooth with an approximal lesion and a stained fissure on the occlusal surface that was misdiagnosed as having a lesion are also shown in Figure 26.2. The SWIR images confirm that an approximal lesion is present but indicate that there is no demineralization present on the tooth occlusal surface [72].

Recent imaging studies at wavelengths from 400 to 2350 nm show that high-contrast images of root caries can be acquired at wavelengths beyond 1400 nm [17]. Stains interfere significantly at wavelengths less than 1150 nm, yielding nondiagnostic contrast for root caries lesions. Significantly higher (P<0.05) lesion contrast was measured at wavelengths greater than 1400 nm, where water absorption is high. There was no further increase in contrast beyond 1400 nm as was observed for enamel, which suggests that the scattering coefficient of dentin does not decrease further with increasing wavelength. Therefore, it is likely that the high absorption of water at longer wavelengths reduced the light scattering from the surrounding and underlying normal dentin, thus increasing the lesion contrast. Reflectance images of the root surface of a tooth showing dental calculus and root caries at visible, 850, 1300, and 1500–1750 nm wavelengths are shown in Figure 26.3.

Recently, with the introduction of commercial NIR transillumination systems, contrast agents have been investigated for enhancing dental imaging. Li et al. have investigated the use of injecting indocyanine green (ICG) [73–76] in live rodents, and Abdelaziz et al. [77] have used externally applied ICG gels and liquids to determine if human approximal lesions are cavitated or not in NIR images.

### Reflectance measurements for lesion activity assessment

Optical changes due to the loss of water from porous lesions can be exploited to assess lesion severity with fluorescence, thermal, and SWIR imaging [27, 78, 79]. Since arrested lesions are less permeable to water due to the highly mineralized surface layer, changes in the rate of water loss can be related to changes in lesion structure, porosity, and activity. The highly mineralized surface layer can be detected with OCT and can serve as an indicator that the lesion has been repaired or remineralized [80–82]. When lesions become arrested by mineral deposition in the outer layers of the lesion, the diffusion of fluids into the lesion is inhibited. Therefore, the lesion permeability reflects the degree of lesion activity, and it can be indirectly measured via changes in SWIR reflectance during dehydration [83]. The lesion permeability monitored with SWIR reflectance measurements at 1450 nm is extremely sensitive to the transparent surface zone thickness [83].

### Optical coherence tomography

Optical coherence tomography (OCT) has great potential for imaging teeth in the SWIR due to the high transparency of enamel. OCT has been under development for dental applications for almost 25 years, although there are no dedicated OCT systems commercially available for dentists. Light scattering in sound enamel significantly decreases beyond 1300 nm, which may be advantageous for achieving greater imaging depths for optical coherence tomography. The first images of the soft and hard tissue structures of the oral cavity were acquired by Colston et al. [84, 85]. Feldchtein et al. [86] presented high-resolution dual wavelength 830- and 1280-nm images of dental hard tissues, enamel, and dentin caries and restorations in vivo. OCT can be used to measure the reflectivity within dental hard tissues to a depth of more than 5 mm in
enamel and 1–2 mm in dentin. OCT is valuable for obtaining high-resolution images of lesion structure and severity to aid diagnosis. The advantages of using cross-polarization OCT (CP-OCT) and polarization sensitive (PS-OCT) to monitor demineralization and remineralization have been demonstrated in several studies utilizing various lesion models and natural lesions [23, 33, 82, 87–89]. The ability of PS-OCT to monitor the formation of a distinct transparent surface zone due to remineralization has also been demonstrated [80–82]. Many clinicians are primarily interested in knowing how deep the occlusal lesions have actually penetrated into the tooth so that they can decide whether a restoration is necessary. The identification of occlusal lesions penetrating to dentin is poor, with an accuracy of ~50% [90, 91]. OCT is ideally suited for monitoring and improving the diagnosis of hidden subsurface lesions. Typically, lesions spread laterally under the enamel upon contacting the more soluble and softer dentin. Therefore, OCT can be used to determine if occlusal lesions have penetrated to the underlying dentin by detecting the lateral spread across the dentinal–enamel junction [65].

Since OCT can be used to image deep into the tooth, it can be potentially used to monitor erosion and wear by monitoring the remaining enamel thickness. Studies have shown that OCT can be used to measure the enamel thickness [92]. Multiple clinical [93] and in vitro studies have shown that [94–96] erosion and wear can be monitored using optical coherence tomography.

Transillumination and reflectance are better suited for rapid screening for the detection of caries, while OCT is better suited for acquiring tomographic images of the lesion showing structure and depth penetration that can aid in making an accurate diagnosis. Since the devices utilize InGaAs detectors and SWIR light sources, it is likely that the technology will merge in the future, leading to a single device that can be used for both screening and the acquisition of high-resolution images.

**Caries lesions and subsurface structures from tooth occlusal surfaces**

*In vitro* and *in vivo* studies have demonstrated that OCT can be used to determine if occlusal lesions have penetrated to the underlying dentin [65, 72] by detecting the lateral spread at the dentinal–enamel junction (DEJ). As mentioned earlier, most newly discovered lesions are in the occlusal surfaces, and radiographs do not perform well in detecting the penetration of such lesions through the enamel and into the dentin before surgical intervention is required; new methods are thus needed to detect these hidden occlusal lesions. Demineralization strongly scatters light and blocks optical penetration both into and out of the tooth. Even though the optical penetration of SWIR light can easily exceed 7 mm through sound enamel to image lesions on proximal surfaces with high contrast [59], the large increase in light scattering due to demineralization [16] typically limits optical penetration in highly scattering lesions (also dentin and bone) to 1–2 mm, thus cutting off the OCT signal before it reaches the dentinal–enamel junction (DEJ). The demineralization typically is highly localized to the pit and fissures and does not spread laterally until it reaches the DEJ, where the more soluble dentin is more easily demineralized. Therefore, most lesions extend laterally along the DEJ upon reaching the underlying dentin. OCT can detect that lateral spread since it can reach those lesion areas near the DEJ around the periphery of the lesion without having to penetrate through the demineralized, highly scattering enamel. Therefore the existence of very strong scattering/reflectivity from the surface of a pit and fissure that blocks optical penetration accompanied by strong reflectance peripheral to the fissure located below the surface at the position of the DEJ indicates the presence of an occlusal lesion that is severe enough to reach the dentin and spread significantly. The concept is illustrated in the diagram shown in Figure 26.5. Such an indication suggests that surgical intervention may be necessary, while the lack of spread suggests that the lesion is confined to the enamel and a small area of dentin and may be more successfully treated with remineralization therapy. A recent OCT image of an extracted tooth with an occlusal lesion taken using a new OCT prototype imaging system [97] that penetrates into the dentin and spreads laterally in the is shown in Figure 26.6 along with the matching microCT image dentin. The same approach can be used to image deep interproximal lesions from the tooth occlusal surface under the sound enamel [72, 98].

In the first clinical study using OCT to test this approach, 12 out of 14 lesions scheduled for surgical intervention examined *in vivo* using OCT exhibited increased reflectivity below
Handbook of Tissue Optical Clearing

FIGURE 26.6 (A) Color, (B) OCT, and (C) microCT images of an extracted tooth with a large occlusal lesion that has spread extensively in the underlying dentin. There is demineralization in all three fissures blocking optical penetration at the center of each fissure. Strong subsurface reflections adjacent to the left and center fissures in the OCT image indicate that the lesions have penetrated to the dentin in those areas but not for the fissure on the right, where the microCT confirms that the demineralization is localized to the surface.

the DEJ, which suggested that the lesions had spread to the dentin; considering that none of the lesions were visible on radiographs, this is a remarkable improvement in sensitivity over radiography and other existing technology [65]. In a subsequent clinical study using a high-speed swept source OCT, it was demonstrated that 3D images of hidden occlusal lesions showing the lateral spread at the DEJ in multiple directions could be rapidly acquired [72]. In contrast to fluorescence detection methods such as the Diagnodent, OCT has the added advantage of producing an image showing the spread of demineralization as opposed to a single reading that does not indicate the depth or area of demineralization, and stains do not absorb NIR light, so there is minimal interference from stain.

Optical clearing or index matching agents can be used to increase the optical penetration into the tooth and increase the contrast of the deeper demineralization near the DEJ and also allow better resolution of the lesion depth. A liquid on the tooth surface with a refractive index closer to that of tooth enamel (1.63) reduces the surface scattering and specular reflectance, increasing the quality of the OCT images. In addition, filling the pores of the lesion greatly reduces the scattering in the lesion, allowing better light penetration through the lesion body to better reach the true depth of the lesion. Even though such penetration is anticipated to lower the contrast of the lesion near the tooth surface, it is also expected to increase the optical penetration to deeper layers in the lesion.

The first study using high refractive index fluids to enhance the imaging of caries lesions with OCT was carried out 15 years ago by Jones et al. [26], and propylene glycol and glycerol were used to demonstrate that the application of high refractive index liquids increased penetration depth in sound enamel to a greater degree than dried or water-moistened samples. The results also demonstrated that image contrast between sound and carious enamel is dependent on the viscosity of the liquid and the degree of porosity of the carious lesion. In later studies by Kang et al. [99, 100] on extracted human teeth with occlusal lesions, OCT images were acquired after the application of water, glycerol, BABB (33% benzyl alcohol + 67% benzyl benzoate) and a Cargille liquid (Cedar Grove, NJ) (hydrogenated terphenyl 1-bromo-naphthalene) with a refractive index of 1.61. The purpose of these later studies focused on enhancing the detection of the penetration of occlusal lesions into the underlying dentin. The intensity ratio between the reflection at the surface and subsurface reflection from the lesion at the DEJ was measured at the same position for the different agents, and the contrast ratio was calculated and compared. Figure 26.7 shows OCT b-scans and a-scans at the indicated position (red arrows) acquired for one extracted tooth from that study for the four liquids; note how more and more of the body of the lesion at the center of the fissure becomes progressively visible along with the subsurface lesion areas in the dentin peripheral to the fissure. The intensity ratio is plotted in Figure 26.8 for the ten samples, and there was a significant increase in the integrated reflectivity and the contrast ratio of the subsurface lesion area for the higher refractive index liquids.

Those studies showed that optical clearing agents and image analysis methods (edge detection) can be used to increase the optical penetration and the visibility of subsurface lesions and the DEJ under sound and demineralized enamel in OCT images. The use of optical clearing agents significantly increases the visibility of subsurface lesions located under sound enamel peripheral to the pits and fissures in the occlusal surface. In addition, the visibility of the DEJ is also increased, which is potentially valuable for measuring the remaining enamel thickness for monitoring tooth wear and erosion. In a later study by Kang et al. [101, 102], it was demonstrated that a polysiloxane dental impression material (VPS) can be used to further improve the visibility of subsurface lesions and the DEJ in OCT images. OCT images are shown in Figures 26.9 and 26.10 of two extracted teeth from that study before and after application of VPS to the surfaces. The first tooth had a lesion that penetrated to the underlying dentin, while the demineralization was localized to the fissure surface for the second tooth. The DEJ is more visible after application of the VPS in Figure 26.10. Improving the visibility of the DEJ is important since the distance from the surface of the tooth to the DEJ is the remaining enamel thickness, which can be used to monitor tooth erosion and wear. The excellent performance of the VPS impression material is particularly exciting because these impression materials have been used clinically for many years and they are transparent, odorless, and
Use of optical clearing and index matching agents

Therefore, they can be used immediately as optical clearing agents for clinical OCT imaging. Moreover, since the impression material hardens in place it is easy to apply to upper (maxillary) teeth as well by holding in place for 10–15 seconds while it hardens as is currently done now with an impression tray. Polysiloxanes are used for intraocular lenses and dental impression materials, and the refractive index (RI) can be varied from 1.4 to 1.6 with RI increasing with the phenyl concentration [103, 104]. Kang used a common dental transparent vinyl polysiloxane (VPS) impression material (RI = 1.4) which showed a remarkable improvement in the visibility of subsurface structures around the fissure. The higher performance of the lower refractive index VPS impression material (RI = 1.4) suggests that index matching is not the most important criterion for increasing optical penetration. Viscosity and surface tension are also important factors because they influence the capillary penetration of the agent into the lesion pores and the narrow fissures. Vinyl polysiloxane impression materials are designed for intimate contact with tooth surfaces for accurate impressions; therefore they are hydrophilic and have a low enough viscosity to penetrate tooth fissures.

Carneiro et al. [105] investigated the use of silver nanoparticles to improve the contrast between sound and demineralized enamel in OCT images. The nanoparticles appeared to act as a contrast agent, increasing the visibility of lesion areas.

OCT Imaging of root surfaces

Optical clearing agents can also be used to increase the performance of OCT for the diagnosis of root caries and other defects on root surfaces due to the high permeability of the dentinal tubule network. The application of fluids with a higher refractive index than water greatly improves optical penetration of both sound and demineralized root surfaces. We suspect these agents act as optical clearing agents by filling the pores and tubules structures to reduce internal light scattering, and they act as index matching agents by reducing scattering and reflection at root surfaces. The diagnosis of root caries and root fractures is of increasing importance due to our aging population. In a recent study, 20 teeth with suspected root caries and dental calculus were imaged with optical coherence tomography (OCT) with and without the

**FIGURE 26.7** (Left) CP-OCT B-scans taken at the position of the dotted line in Figure 26.1 for the four liquids of varying refractive index (RI), water, glycerol, BABB, and Cargille liquid. CP-OCT A-scans extracted at the position of the upper and lower red arrows are shown on the left. The position of the two peaks analyzed is shown by the arrow for both the B-scan and A-scan images. Reprinted with permission from reference [100].

**FIGURE 26.8** Mean ± S.D. of the peak intensity ratios of each of the ten liquids for ten samples. The values of the refractive index are also listed for each liquid. Groups with the same color are statistically similar (P>0.05). Reprinted with permission from Reference [99].
addition of water (W), glycerol (G), and propylene glycol (PG) [106]. The reflectance was monitored in sound and demineralized areas before and after application of W, G, and PG. The mean values ± standard deviation for the attenuation coefficient, 1/e² optical penetration depth, and depth weighted reflectivity were calculated for a-scans positioned near the most severe area of each lesion for the twenty lesions. The optical penetration depth was significantly higher for PG and G ($p < 0.05$) compared to wet and dry surfaces. However, the optical penetration was statistically similar for PG and

**FIGURE 26.9** (Left) OCT b-scans taken at the same fixed position on a tooth in which no subsurface lesion was present, acquired without (A) and with (B) VPS. OCT a-scans extracted at the position of the upper and lower red arrows are shown on the right. Reprinted with permission from reference [102].

**FIGURE 26.10** (A) Polarized and non-polarized (B) light microscopy images acquired of tooth sections matching the position of the OCT scans shown in Figures 26.2 and 26.4. Reprinted with permission from [102].
G (p > 0.05) [106]. The visibility of subsurface root canals was also measured before and after application of the respective fluids. Propylene glycol and glycerol have similar refractive indices of 1.43 and 1.47 respectively, but very different viscosities. Glycerol has a much higher viscosity. The performance of glycerol was slightly higher than propylene glycol, but that difference was not significant. Glycerol is well suited for use in dental imaging due to its high biocompatibility. It is a common additive for food and pharmaceuticals and is used topically, orally, and intravenously for various medical applications. Its high viscosity, low vapor pressure, and miscibility in water are also important advantages for OCT imaging. The low vapor pressure is useful for in vitro OCT imaging of tooth samples over an extended period of time where it is important to avoid water loss due to evaporation. The optical penetration depth increases by almost a factor of two over wet lesions and by more than a factor of five over the dry lesion if glycerol is applied. The large increase in optical penetration or decrease in optical attenuation with the addition of optical clearing agents clearly indicates that they can be used to increase the visibility of deeper regions in root caries lesions.

The identification of root fractures is also a significant problem in dentistry, and OCT can potentially be used for imaging tooth roots using endoscopic scanning systems that can be inserted into the root canal [107]. The optical penetration through the root dentin can be greatly improved with an optical clearing agent, and one can easily envision filling the root with glycerol to improve imaging. The root surfaces of the same 20 teeth were also scanned at areas closer to the apical tip of the root where the root canal was visible well below the surface, and those are shown in Figure 26.11. The line profile used for comparison is shown in gray, and the position of the a-scan chosen for further analysis is indicated by the arrows. Note that on some regions of the scan, both sides of the root canal are visible to the left of the arrows, particularly in the image with glycerol. The mean integrated reflectivity of the root canal wall (weighted and unweighted with respect to the depth in the tooth) and the ratio of the reflectivity of the root surface and the wall of the root canal were calculated for the 20 samples. Since the distance from the root surface to the root canal varied markedly over the 20 teeth, it was necessary to factor in the depth or distance of the root canal from the surface in the comparison. The ratio of the root canal to surface intensity represents the visibility of the root canal wall, and it increases by a factor of three from wet to dry and by another factor of two from wet to G and PG. The bar chart in Figure 26.12 shows the mean ratios for the four conditions. The integrated reflectivity from the root canal wall also increased significantly progressing from dry to wet to G and PG [106].

Conclusions

One can easily envision how optical clearing agents can be utilized clinically for dental imaging, particularly an agent such as VPS that works well on both mandibular and maxillary occlusal surfaces. After an initial scan of a suspect fissure using OCT, those areas can be rescanned after application of the optical clearing agent to enhance the ability to detect the subsurface lesion either by revealing a subsurface reflection that was not visible without enhancement by the agent or by increasing the intensity of a very weak reflection for additional confirmation. It is also important to note how the speed and performance of OCT has improved over the past two decades. The OCT image shown in Figure 26.6B was acquired with a MEMS-based 3D SS-OCT system with a scanning range of 8 mm and scanning rate of 100 kHz, while the OCT images acquired in Figures 26.7, 26.9, and 26.10 were taken with a first-generation time domain OCT system with a scanning range of 6 mm and a scanning rate of only 100 Hz. Newer systems are 1000 times faster, with longer scanning range and higher sensitivity. Clinical OCT systems should soon be available for dentistry as manufacturing costs also decrease.
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