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Near Infrared Optical Imaging of Early Dental Caries

by

Robert Simon Jones, DDS

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Oral and Craniofacial Sciences

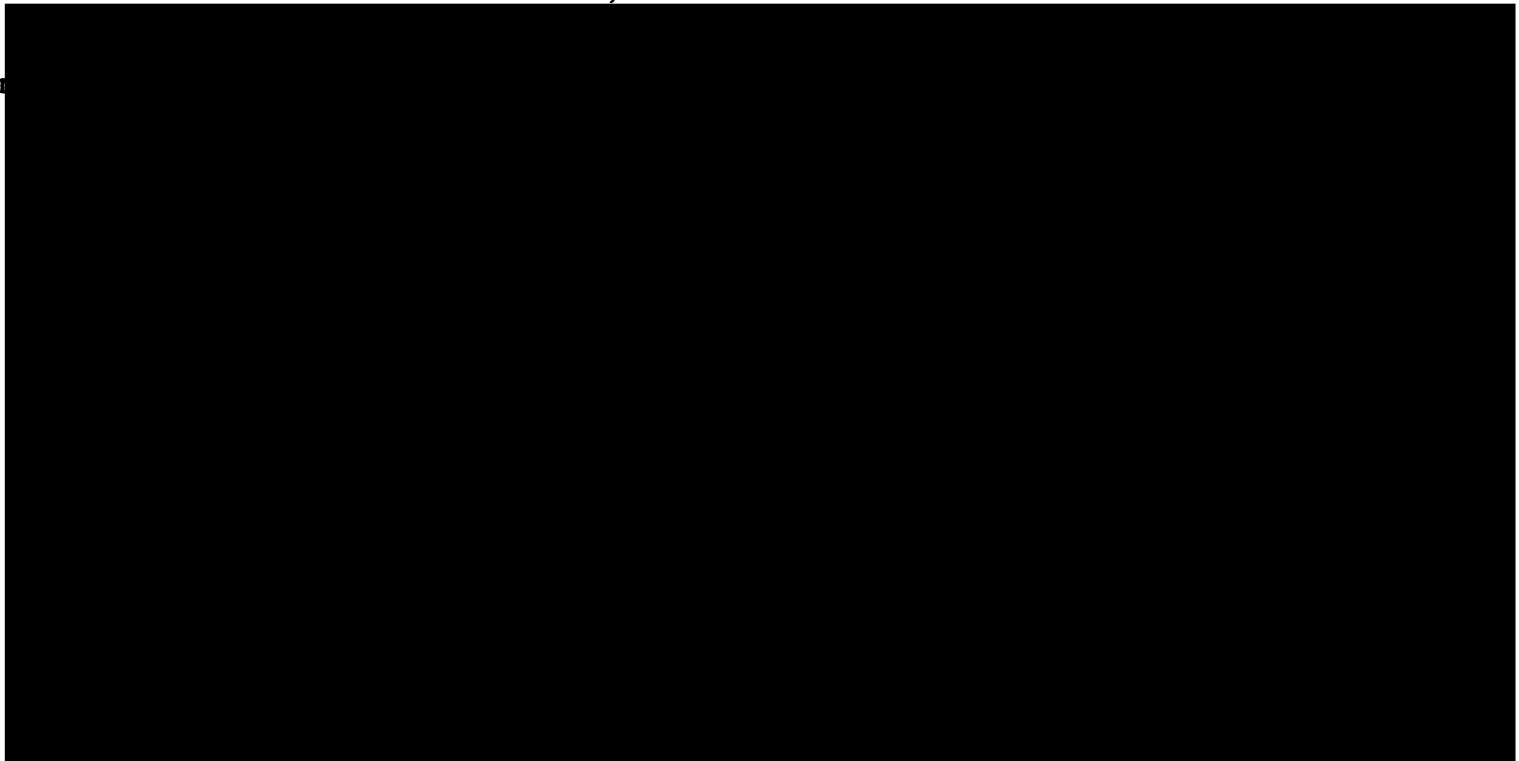
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Abstract

New imaging technologies are needed for the early detection of dental caries, especially in high risk regions such as the interproximal and occlusal surfaces. The objective of this thesis work was to investigate the potential of near infrared (NIR) imaging methods for the detection and assessment of early enamel caries. If detected in the early stages, enamel caries can often be treated by non-surgical methods that promote the tissue remineralization and repair without the need for restorative intervention. This work demonstrated that NIR wavelengths, especially 1310 nm, are well suited for imaging enamel dental caries. NIR transillumination and Polarization Sensitive Optical Coherence Tomography (PS-OCT) are two promising optical imaging systems that were studied in this work and addressed the needed improvements to measure depth resolved changes in enamel demineralization and remineralization.

A NIR transillumination system was employed to acquire high contrast images of simulated interproximal lesions showing significant advantages over visible light and x-ray imaging for caries detection. The potential of Polarization Sensitive Optical Coherence Tomography (PS-OCT), an imaging system that has found broad applications in medicine and biology, was examined for occlusal caries detection. PS-OCT was useful for enhancing the image contrast of caries, minimizing the interference of surface reflections, and quantifying the severity of caries lesions through a straightforward integration method. This work found that the method to quantify the lesion severity had a strong correlation to the overall lesion mineral volume loss. PS-OCT had the ability to monitor the

progression of artificial enamel caries lesions on occlusal surfaces and image lesions underneath composite sealants. PS-OCT was used to image the remineralization of several different artificial lesion models. One such model demonstrated that PS-OCT can image the repair and restoration of the surface zone enamel after remineralization. NIR transillumination and PS-OCT have promising potential for *in vivo* applications to non-destructively detect and monitor early enamel caries with greater success than conventional methods.

The future role of NIR transillumination is likely to be a detection system, capable of screening the dentition for early decay, with PS-OCT providing more valuable diagnostic information on lesion assessment, especially with occlusal lesions.

TABLE OF CONTENTS

TITLE PAGE	Page i
DEDICATION	iii
PREFACE	vi
ABSTRACT	vii
TABLE OF CONTENTS	ix
LIST OF TABLES	xiii
LIST OF FIGURE	xiv

Chapter I: **INTRODUCTION**

1. INTRODUCTION	2
Caries Treatment and Management	3
Conventional Caries Detection	6
Existing Optical Caries Detection	9
Fluorescence based Caries Detection	9
Optical Transillumination	12
2. SIGNIFICANCE	14
3. BACKGROUND	15
NIR Transillumination	15
Optical Coherence Tomography: Introduction	15
Optical Coherence Tomography: Axial Resolution	20
Optical Coherence Tomography: Lateral	23
Polarization Sensitive OCT: Introduction	24
Polarization Sensitive OCT System	27
Optical Coherence Tomography: Dentistry	29
4. FIGURES AND FIGURE LEGENDS	32
5. REFERENCES	35

Chapter II: **NEAR INFRARED TRANSILLUMINATION FOR IMAGING EARLY ENAMEL CARIES**

1. INTRODUCTION	44
2. MATERIALS AND METHODS	46
Attenuation Measurements at 1310 nm and 1550-nm	46
Sample preparation for NIR Imaging at 1310-nm	48
NIR Imaging at 1310 nm	48
NIR Imaging at 830 nm	50
Visible and X-ray Imaging	51
Image Analysis	51
3. RESULTS	52

Attenuation Coefficients at 1310 nm and 1550 nm	52
Near-IR Transillumination	53
4. DISCUSSION	55
5. FIGURES AND FIGURE LEGENDS	59
6. REFERENCES	74

Chapter III: IMAGING ARTIFICIAL CARIES UNDER COMPOSITE SEALANTS AND RESTORATIONS

1. INTRODUCTION	77
2. MATERIALS AND METHODS	78
Artificial Occlusal Caries	79
Sealant Restorations	79
Polarization Sensitive Optical Coherence	
Tomographic Imaging	80
3. RESULTS	82
Imaging Artificial Caries Progression on Occlusal	
Surfaces	82
Imaging Artificial Caries Underneath Dental	
Sealants	83
4. DISCUSSION	85
5. FIGURES AND FIGURE LEGENDS	89
6. REFERENCES	97

Chapter IV: IMAGING ARTIFICIAL CARIES ON THE OCCLUSAL SURFACES WITH POLARIZATION SENSITIVE OPTICAL COHERENCE TOMOGRAPHY

1. INTRODUCTION	101
2. MATERIALS AND METHODS	102
Artificial Occlusal Caries	102
Polarization Sensitive Optical Coherence	
Tomographic Imaging	103
Histological Evaluation	104
3. RESULTS	106
Artificial Occlusal Caries Progression	106
Assessing Lesion Severity and Depth	107
4. DISCUSSION	108
5. FIGURES AND FIGURE LEGENDS	115
6. REFERENCES	120

Chapter V: THE EFFECT OF HIGH REFRACTIVE INDEX LIQUIDS ON PS-OCT IMAGING OF DENTAL CARIES

1. INTRODUCTION	125
2. MATERIALS AND METHODS	126
Artificial and Natural Occlusal Caries	126
Polarization Sensitive Optical Coherence	
Tomographic Imaging	127
Index Matched Liquids and Image Analysis	127
3. RESULTS	129
Artificial Occlusal Lesions	129
Natural Occlusal Caries	130
4. DISCUSSION	131
5. FIGURES AND FIGURE LEGENDS	135
6. REFERENCES	143

Chapter VI: IMAGING ENAMEL REMINERALIZATION OF pH CYCLED ARTIFICIAL LESIONS WITH PS-OCT

1. INTRODUCTION	146
2. MATERIALS AND METHODS	148
Artificial Smooth Surface Caries	148
Polarization Sensitive Optical Coherence	
Tomographic Imaging	150
Histological Analysis (Digital Microradiography)	151
Synchrotron Radiation Fourier Transform	
Infrared Spectroscopy	152
3. RESULTS	152
Remineralization of Severe Early Artificial Caries	152
Remineralization of Mild Early Artificial Caries	154
4. DISCUSSION	155
5. FIGURES AND FIGURE LEGENDS	161
6. REFERENCES	168

Chapter VII: IMAGING ENAMEL REMINERALIZATION OF SURFACE SOFTENED ARTIFICIAL LESIONS WITH PS-OCT

1. INTRODUCTION	174
2. MATERIALS AND METHODS	175
Artificial Smooth Surface Caries	175
Polarization Sensitive Optical Coherence	
Tomographic Imaging	176
Histological Analysis	177
3. RESULTS	177
4. DISCUSSION	179
5. FIGURES AND FIGURE LEGENDS	183
6. REFERENCES	187

Chapter VIII: **SUMMARY AND CONCLUSION**

1. SUMMARY	190
NIR Transillumination	191
Polarization Sensitive Optical Coherence Tomography (PS-OCT)	194
2. CONCLUSION	199
3. REFERENCES	200

11/01/2011 11:01:11

LIST OF TABLES

Chapter	Table	Page
Chapter V	Table 1: Treatments before each PS-OCT scans	135
	Table 2: Imaging Depth and Lesion Contrast	135
Chapter VI	Table 1: Lesion Assessment with PS-OCT of the 10 day lesions	161
	Table 2: Lesion Assessment with Digital Microradiography of the 10 day lesions	161
Chapter VII	Table 1: Lesion Severity	183
	Table 2: Lesion Depth and Surface Zone	183

LIST OF FIGURES

Chapter	Figure	Page
Chapter I	Figure 1: A low coherence Michelson interferometer	32
	Figure 2: Autocorrelation function and axial resolution.	33
	Figure 3: PS-OCT system	34
Chapter II	Figure 1: The experimental setup, which measures the optical attenuation of a section of enamel placed in the sample cuvette.	59
	Figure 2: A diamond grit finished plano-parallel tooth section of 1.0mm thickness	60
	Figure 3: 1310-nm Near-Infrared Transillumination	61
	Figure 4: sensitivity versus wavelength of the Alpha NIR TM	62
	Figure 5: 830-nm Near-Infrared Transillumination	63
	Figure 6: sensitivity versus wavelength of the DMK-3002-IR	64
	Figure 7: Beer-Lambert plot for 1310-nm	65
	Figure 8: Beer-Lambert plot for 1550-nm	66
	Figure 9: Index Matching Enamel	67
	Figure 10: 1310 nm NIR transillumination, visible, x-ray	68
	Figure 11: NIR transillumination images of tooth sections different thicknesses	69
	Figure 12: 830 nm versus 1310 nm NIR transillumination	70
	Figure 13: Contrast ratio between sound enamel and	71

the simulated lesion for the three imaging systems for varying section thickness

Figure 14: 1310-nm NIR transillumination image of a whole tooth sample 72

Figure 15: Plot of the attenuation coefficient of dental enamel 73

Chapter III	Figure 1: PS-OCT images of lesion progression	89
	Figure 2: The mean reflectivity was plotted versus the square root of time	90
	Figure 3: PS-OCT controls: Concise™ sealant	91
	Figure 4: PS-OCT controls: Aeliteflo™ sealant	92
	Figure 5: PS-OCT lesions: Concise™ sealant	93
	Figure 6: PS-OCT lesions: Aeliteflo™ sealant	94
	Figure 7: The mean reflectivity of the artificial lesions and the controls under different optical depths of the sealants	95
	Figure 8 : PS-OCT images underneath the composites	96
		97

Chapter IV	Figure 1: Artificial caries on a select area	113
	Figure 2: The mean reflectivity was plotted versus the square root of time	114
	Figure 3: Depth profiles from the PS-OCT images of the sound and carious tooth	115
	Figure 4: Artificial occlusal caries images: PS-OCT, Digital Microradiography, and Polarized Light	116
	Figure 5: A mineral content profile of sound and artificial occlusal carious enamel	117
	Figure 6: Lesion Severity: ΔZ versus the integrated	118

	reflectivity	
	Figure 7: Lesion Depth: PS-OCT versus Digital Microradiography	119
Chapter V	Figure 1: Mounting Jig	136
	Figure 2: PS-OCT images under dry, wet, propylene glycol, glycerol, and index matched liquid	137
	Figure 3: PS-OCT images with glycerol: initial and 5 minutes after application	138
	Figure 4: PS-OCT images of an advanced natural lesion	139
	Figure 5: Digital Microradiography of an advanced natural lesion sectioned	140
	Figure 6: PS-OCT images of a natural lesion	141
	Figure 7: Digital Microradiography of a natural lesion sectioned	142
Chapter VI	Figure 1: Polarized Light Microscopy images of 10 day pH cycled lesions before and after fluoride enhanced remineralization	162
	Figure 2: PS-OCT images of 10 day pH cycled lesion before and after fluoride enhanced remineralization	163
	Figure 3: Digital Microradiography images of 10 day pH cycled lesion before and after fluoride enhanced remineralization	164
	Figure 4: PS-OCT images of 3 day pH cycled lesion before and after fluoride enhanced remineralization	165
	Figure 5: Digital Microradiography images of 3 day pH cycled lesion before and after fluoride enhanced remineralization	166
	Figure 6: FTIR spectra of remineralized lesion zones	167

Chapter VII	Figure 1: Visual images of the artificial surface softening caries	184
	Figure 2: PS-OCT images of 9 day surface softening lesions before and after fluoride remineralization	185
	Figure 3: Polarized Light Microscopy and Digital Microradiography images of 9 day surface softening lesions before and after fluoride remineralization	186

CHAPTER I

INTRODUCTION

The objective of this thesis work is to investigate the potential of near infrared imaging methods for the detection and assessment of early enamel caries. Research in near infrared dental imaging has been prompted by the necessity in current and future caries management for new diagnostic technologies. New technologies need to improve upon the diagnostic abilities of existing methods by: (1) detecting enamel caries in earlier stages of development with high sensitivity and specificity, (2) allowing repeated longitudinal monitoring without an increase in health risk to a patient, (3) having minimal restrictions on surface type and morphology during imaging, (4) the ability to detect early recurrent decay under restorations, (5) imaging the depth resolved changes of the structure of a lesion, (6) quantifying lesion severity or overall mineral loss from caries demineralization and mineral gain after lesion remineralization, and (7) assessing the lesion activity, whether arrested or active, of early enamel caries.

Near infrared (NIR) transillumination and Polarization Sensitive Optical Coherence Tomography (PS-OCT) are two promising optical imaging systems that were studied in this work and address the desired improvements in early enamel caries detection and lesion assessment. As will be discussed throughout this work, both technologies have unique and specific potential roles in diagnostic imaging of dental caries. In the second chapter of this work, the first NIR dental transillumination system was used to detect *in vitro* artificial and natural enamel interproximal caries and study the benefits of using non-ionizing NIR light in optical imaging of dental enamel. In subsequent chapters, *in vitro* caries models were used to test the ability of PS-OCT to provide depth resolved images of occlusal surface caries, to longitudinally

monitor lesion progression, to quantitate lesion severity and correlate optical changes with changes in mineral volume, to image enamel caries under resin restorations, and to image the remineralization and repair of early enamel caries. These *in vitro* studies demonstrate the potential of NIR transillumination and PS-OCT to improve upon the deficiencies of current diagnostic technologies and methods.

1.2 Caries Treatment and Management

The treatment of dental caries has evolved from extraction toward restorative based treatment, with a contemporary practice of minimally invasive restorative techniques. Minimally invasive restorative dentistry has become a standard in dentistry because of the advances in resin based materials and dentin bonding agents that allow more conservative restoration of dental hard tissue than traditional G.V. Black based amalgam and gold restorations (Bowen, 1963; Bowen *et al.*, 1982; Buonocore, 1955; Murdoch-Kinch and McLean, 2003; Tyas *et al.*, 2000). Although there are important material properties that warrant larger and more aggressive techniques in traditional dentistry, it is important to recognize that the result of G.V. Black's traditional restorative dentistry to remove tooth structure that is predisposed to caries reduces the complexity of later caries diagnosis, in regions such as the interproximal contact areas and occlusal pits and fissures. The widespread use of fluoride has greatly reduced the overall prevalence of caries, especially on smooth surfaces (Dirks, 1974; McDonald and Sheiham, 1992), but the predisposed and diagnostically challenging regions of the tooth, particularly the occlusal pit and fissures, remain a continued problem to

this day. Occlusal caries, which represent around 80% of new caries detected (Dodds, 1996), continue to be difficult to identify and monitor, particularly in the early stages, using conventional methodologies. The nature of dental decay, especially pits and fissure caries, has changed dramatically with the use of fluoride, where lesions are often *hidden* underneath an intact veneer of surface enamel (Pitts, 1997). Therefore, in many cases, occlusal lesions develop slowly and do not cavitate, making lesion detection with conventional methods even more challenging than in the past. The hidden nature of occlusal caries makes diagnosis very subjective with visual inspection, where staining of the fissures can increase false positives (Lussi, 1993) and lead to unnecessary treatment. Although the effect of this unnecessary treatment is a small preventive resin filling, the conservative restoration can affect the long term prognosis of the tooth, since the adhesion of the resin based restoration to the tooth enamel can eventually fail and dental caries can develop underneath these restorations (Kidd and Beighton, 1996). Unfortunately, no current diagnostic method is capable of evaluating the structural integrity of resin composites and can detect caries under these materials. The diagnostic difficulties are exacerbated in conservative interproximal slot based preparations, since the margins of these conservative restorations are not able to be inspected with conventional methods. These conservative preparations are believed to not only preserve tooth structure but to increase the longevity of a resin composite by reducing the occlusal load and wear (Leinfelder, 1996). Tooth preservation and material longevity outweigh the inherent diagnostic difficulties in conservative surgical preparations. In many

situations, recurrent caries under resin based restorations lead to substantial tooth loss and in some cases root canal treatment and extractions. Therefore, even the current goal of minimally invasive restorative dentistry that aims to preserve tooth structure during the long term remains an elusive goal in today's clinical practice. The current practice of minimally invasive dentistry requires new advances in caries detection to identify early enamel decay in diagnostically challenging regions of the teeth, in order to preserve tissue structure. Minimally invasive dentistry also requires new diagnostic systems that can detect recurrent or secondary caries around resin based composite materials, whose material properties can fail over time and occlusal load (Abell *et al.*, 1983).

Recently, there has been much interest in shifting the management of early caries from conservative restorative dentistry toward a non-surgical or 'medical' model (Featherstone, 2000; Stewart and Hale, 2003; Thompson and Kaim, 2005). Both NIDCR sponsored and international consensus conferences have identified the research in non-surgical methods that promote tissue remineralization and repair as an important area in future caries treatment and cited the need for improved early caries diagnostic systems (NIH, 2001; Pitts and Stamm, 2004). Improved caries diagnostic systems will be essential in not only the practice of non-surgical caries management but helping validate various non-surgical therapies in clinical trials. There is currently a solid foundation for non-surgical caries management that involves dietary changes, prescription fluoride applications, and antimicrobial agents (for review see (Featherstone, 2000)); however, extensive future research is sorely needed in both the development of

novel compounds and the management of their delivery. Current diagnostic techniques can not provide the necessary diagnosis and monitoring needed for the successful implementation of non-surgical therapy. In the following sections, conventional methods and newer commercially available caries detection methods will be reviewed to discuss their contributions and shortcomings in aiding both minimally invasive restorative dentistry and the new era of non-surgical therapy.

1.3 Conventional Caries Detection

Tactile and visual inspection is the primary method for a dentist to clinically diagnosis caries. In clinical practice, tactile inspection involves, in most cases, a sharp explorer, where a dentist uses moderate pressure to affirm if the surface or area is 'soft or leathery' and resists removal of the instrument. The use of a sharp explorer can detect cavitated or near cavitated lesions; early enamel lesions that are far from cavitation are difficult to detect. Furthermore, occlusal lesions are often 'hidden' under a veneer of intact enamel are also difficult to detect. For occlusal lesions, tactile and visual inspection have been shown to have low sensitivity, ~ 0.3 , because of the hidden nature of the lesions (ten Cate and van Amerongen, 1996). Tactile and visual inspection offer high specificity ~ 0.95 for advanced occlusal lesions that extend into the dentin, but the specificity is below 0.80 for lesions confined to the enamel that can be treated with non-surgical therapy (Bader 2001). The ability to detect near cavitated lesions is in itself problematic because the insertion of a sharp explorer can advance the

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progression of a lesion by damaging the outer surface of the lesion (Ekstrand *et al.*, 1987) and cause an ingress of bacteria. The explorer has also been implicated in possibly transferring cariogenic bacteria from site to site (Loesche *et al.*, 1973). Several countries outside the United States have eliminated or reduced the use a sharp explorer for the above reasons, as recommended by many cariologists (Kidd *et al.*, 1993; Stookey, 2005a).

Visual inspection, supplemented with a *blunt* explorer, is strongly recommended as the best compromise method for clinical practice and research. Clinical research has attempted to improve the diagnostic ability of visual examination by creating an ordinal scale of diagnostic criteria (ICDASII, 2005). The diagnostic criteria involved in this visual examination of a tooth's color, loss of translucency, opacity, presence of shadowing under wet and dry conditions can provide valuable information about early enamel caries. However, differentiating early enamel caries with extrinsic and intrinsic staining and development defects, such as fluorosis and enamel hypoplasia, is difficult by visual and tactile inspection because these simple methods do not provide depth resolved information about the lesion structure. Although visual examination aided by tactile inspection of the surface of the lesion, whether smooth or rough, with a blunt explorer, has been suggested to determine the activity of a lesion (Ekstrand *et al.*, 1997; Nyvad *et al.*, 1999), there is not a universally accepted measure of whether a lesion is remineralized and arrested or progressing and active. These drawbacks limit the ability of visual and tactile methods to detect,

assess, and monitor early enamel lesions, especially in the interproximal and occlusal areas of the tooth.

Bitewing radiographs remain the gold standard for detecting interproximal caries and later stage occlusal dentinal lesions. A majority of interproximal molar caries are incapable of being detected by visual and tactile methods and require radiographic examination (Hennon *et al.*, 1969). However, early interproximal enamel caries are difficult to detect and assess by bitewing x-rays, since early decay is confined to a small region of enamel within the broad proximal dimension of the tooth (Gratt, 1994). Digital subtraction radiographic methods can improve caries detection, particularly for interproximal caries (Wenzel, 2004), and can be helpful in implementing conservative restorative preparations. But even digital bitewing x-rays do not provide the sensitivity needed in the non-surgical management of caries, where early lesions need to be identified and imaged more frequently than is currently recommended for ionizing x-ray imaging (Lireka, 1987). Although the risks of diagnostic x-rays are considered low, there is still a considerable need to fully understand the health risk of low level diagnostic radiation (BRER, 2005). Since there is no absolute safe radiation dose, even future optimized low level radiation digital x-ray systems should not be considered appropriate for caries monitoring. Another confounding element in early caries assessment with both conventional and digital bitewing radiographs is the difficulty in avoiding interproximal tooth overlap and the inability to safely re-take x-rays. Also, because of the overlapping topography of the tooth crown, bitewing x-rays are not able to detect early occlusal enamel lesions or recurrent

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decay beneath pit and fissure enamel restorations. Radiographs do provide universal diagnostic information in the oral cavity, being able to image various tissues, and can image decay under resin based and alloy materials. The advantages of radiographs in dentistry are numerous; however, their use for early caries detection and monitoring, especially the occlusal region, is rather limited.

1.4 Existing Optical Caries Detection

In the first part of this chapter we discussed the desired improvements that newer technologies need in order to replace or to become a viable diagnostic supplement to existing conventional methods to detect early enamel lesions. Several different optical caries detection methods have become commercially available to address the desired improvements; notably, the Diagnodent™, Quantitative Light-induced Fluorescence (QLF) systems, and fiber optic transillumination systems (FOTI), such as the DiFoti™.

1.4.1 Fluorescence-based Caries Detection

The Diagnodent (Kavo, Germany) is a novel caries detection system, which was developed to detect occlusal lesions at an earlier stage than conventional methods. The Diagnodent uses a red diode (655-nm) illuminating laser and has a fiber optic bundle that can detect the fluorescence signal of carious tooth structure. Fluorescence occurs when the illuminating light at 655-nm is absorbed and re-emitted at a longer wavelength (>680 nm) with lower energy. A

fluorescent signal is not generated from sound dental tissue but from porphyrins, synthesized by bacteria, which populate a carious lesion (Koenig *et al.*, 1994). Thus, it is not a change in the tissue structure or depth that is directly related to the signal, rather the volume concentration of porphyrins. In addition, porphyrins are not significantly produced by cariogenic Mutans Strep. They are produced by other co-populating bacteria (Lennon *et al.*, 2006). These are the likely reasons that enamel lesions are detected by the Diagnodent with very low sensitivity (~ 0.4) (Shi *et al.*, 2000). Early white spot lesions with no bacteria involvement do not produce a fluorescence signal above the background sound enamel since these lesions lack porphyrin metabolites (Lussi *et al.*, 2001). However, the Diagnodent has been shown to have high sensitivity and specificity for dentinal lesions (Lussi *et al.*, 1999) and can detect occlusal dentinal lesions with greater success than conventional methods (Lussi *et al.*, 2001). The Diagnodent is a practical supplemental diagnostic technology to detect hidden occlusal dentinal lesions. Therefore, the Diagnodent, at best, aids with the practice of minimally invasive dentistry since decay can be detected at an earlier stage than conventional methods. Along with the additional complication of possible false positives from exogenous porphyrins (Lussi *et al.*, 2001; Shi *et al.*, 2000), the Diagnodent does not provide the needed diagnostic values for non-surgical therapy to treat or monitor the depth and severity of early enamel lesions.

Quantitative Light-induced Fluorescence (QLF) is a yellow/green fluorescence based caries detection system that is distinctly different from the

aforementioned red fluorescence based Diagnodent (Alfano and Yao, 1981; Bjelkhagen and Sundstrom, 1981; Spitzer and Bosch, 1976). Part of the mechanism of QLF involves the autofluorescence of sound teeth, which is likely caused by aromatic amino acids (Spitzer and ten Bosch, 1977) in dentin collagen after illumination of high intensity blue light. Enamel caries, particularly on smooth surfaces, can be identified from sound tissue because the demineralized lesion scatters, and attenuates, the returning fluorescence signal (Angmar-Mansson and ten Bosch, 1987). Whereas the Diagnodent measures an increase in fluorescence from bacteria porphyrins, QLF measures a decrease in the returning fluorescence signal: lesions appear dark from attenuation in the QLF images. There has been extensive research to show a positive empirical correlation between a loss of fluorescence and overall loss of mineral volume with shallow uniform lesions (de Josselin de Jong *et al.*, 1995; Hafstrom-Bjorkman *et al.*, 1992); and *in vivo* studies have shown to be able to longitudinally assess incipient enamel lesions on smooth surfaces (Al-Khateeb *et al.*, 1998; Tranaeus *et al.*, 2001). On smooth surfaces, an *in vitro* QLF study found the sensitivity and specificity to be 0.94 and 0.63 for enamel lesions <0.5 mm (Ando, 1999). When occlusal and smooth surfaces were grouped together in a recent *in vivo* validation study, the sensitivity and specificity were reduced to 0.64 and 0.55 (Ferreira-Zandona *et al.*, 2004), which may indicate the difficulty with QLF's assessment of occlusal lesions. There has been limited work on validating the diagnostic abilities on occlusal surfaces, and QLF has also not been successfully applied to imaging interproximal decay. QLF is unable to

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provide information on the structure and depth of the lesion. This lack of depth resolved information is a reason for skepticism over the effectiveness of QLF to measure remineralization and determine lesion activity (Stookey, 2005b). One reason that QLF is not easily applied to non-smooth surfaces is that the change in fluorescence is a measured relative ratio, comparing the loss of fluorescence of caries demineralization to a reference sound area on the tooth. This method implies that the path length of the returning fluorescence signal is comparable, which is impossible to ascertain in the highly convoluted occlusal region. Distinctly different fluorescence signals from comparable lesions can be produced from variations in the total enamel thickness (Ando *et al.*, 2003) and the imaging angle (Ando *et al.*, 2004). QLF's limitation in being sensitivity to irregular tooth topography and a lack of depth resolved information on the structure of the lesion make QLF unsuitable to assist dentists in the practice of both minimally invasive restorative and non-surgical dental treatment. QLF does not meaningfully improve upon the diagnostic abilities of conventional methods beyond smooth enamel surfaces.

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1.4.2 Optical Transillumination Caries Detection

Fiber optic transillumination (FOTI) and the digital counterpart, DiFoti system, are visible light based diagnostic systems designed to detect interproximal, smooth, and occlusal carious lesions (Schneiderman *et al.*, 1997). The biologically compatible visible light transillumination has been cited as a useful diagnostic supplement in caries clinical trials (Chesters *et al.*, 2002; Cumow *et al.*, 2002).

Early enamel demineralization from cariogenic bacteria leads to a direct increase in tissue scattering, with the scattering effects being more sensitive than the decrease in attenuation of x-rays measured in bite-wing radiographs. Thus, optical transillumination has the potential to detect decay at an earlier stage than x-rays. However, the principal limiting factor with FOTI and DiFoti is the use of visible light for transillumination. Visible light is highly attenuated by dental enamel, and therefore, light that exits the tooth has undergone multiple scattering events that inhibit the diagnostic ability of the technology. For example, bitewing radiographs effectiveness is related to the ability for a high number of ballistic photons to travel unimpeded through carious lesions and sound tissue. With a lower mineral volume and density, carious lesions attenuate the photons to a lesser degree, and there is a contrast between the attenuation differences between well established lesions and sound enamel in dental radiographs. Unfortunately, more subtle changes in the mineral volume of early caries do not significantly affect caries diagnostics in bitewing x-rays. For visible light transillumination, it is the lack of penetration depth through sound tissue that has the greatest effect on lowering the diagnostic ability. Research has shown that DiFoti is unable to accurately detect lesion depth of early enamel lesions, that have the potential to be treated with non-surgical therapy (Young and Featherstone, 2005), and the high scattering of visible light explains this observation. Therefore, visible light transillumination, such as FOTI and DiFOTI, is unsuited for minimally invasive and non-surgical dentistry due to the overwhelming effects of light scattering.

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2 SIGNIFICANCE

The existing commercially available optical technologies do not improve upon the diagnostic abilities of conventional methods. Although using these existing optical technologies with conventional methods increases the total information available, a clinician can be faced with a complicated scenario of conflicting signs for diagnosis. Newer technologies that give depth resolved information and images need to be developed and thoroughly tested to help detect early enamel caries, especially occlusal caries, and to prevent substantial loss of tooth structure. The first part of this chapter reviewed the key points to improve upon existing methods in order to facilitate the goals of minimally invasive dentistry. Newer technologies need to aid a clinician in decisions to either treat decay with minimally invasive restorative surgery or non-surgical therapy. Currently, the ideal practices of minimally invasive restorative dentistry and avoiding conventional restorative techniques are challenging with existing diagnostic methods. The diagnostic standards of new technologies will need high sensitivity, specificity, and predictive values for more subtle changes in enamel mineral volume and structure than currently available with existing technologies. Clinicians will require more subtle diagnostic information about the severity and status of a lesion to determine if minimally invasive restorative or non-surgical treatment is necessary. Two optical imaging systems, NIR transillumination and PS-OCT, will be studied in this work, to address the desired improvements in early enamel caries detection and lesion assessment.

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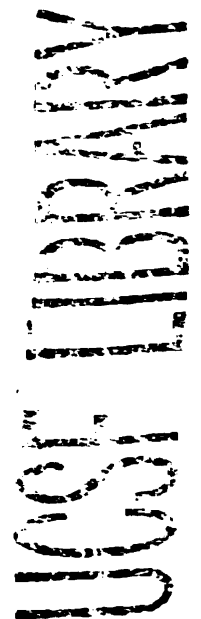
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acoustic echoes with different intensities values. The transducer both emits an acoustic pulse and records the series of echo pulses. Depending on the time delay between the pulses, the distance between the echoes can be estimated. The measured differences in intensity and distance between reflected structures generate information of the biological tissue. A small transducer can record the acoustic depth resolved echo signal in a narrow tissue dimension (A-scan), and an array of transducers can produce a two dimensional acoustic image within a larger area of tissue (B-scan).

An important principle in ultrasound is that acoustic sound waves travel in soft tissue at approximately 1540 meters per second. The echo time delays between different reflected tissues can be recorded by the piezoelectric transducers themselves. In contrast, light travels orders of magnitude faster than sound at $\sim 3 \times 10^8$ meters per second. The speed of light means that even a short pulse of light can not be directly measured in the same manner as an acoustic pulse. The solution to measuring depth resolved reflections of light is through a method called interferometry.

An OCT system is based on a low coherence interferometer, specifically a Michelson interferometer. A Michelson interferometer can measure a narrow tissue dimension in the same manner as a single transducer in ultrasound; OCT adds a scanning component that collects a series of depth resolved measurements to create a b-scan depth resolved optical image. A diagram of a Michelson interferometer is shown (Fig.1). The basic components are an emitting light source, a beam splitter, a sample arm, a reference arm with a



movable mirror, and a photodetector. In a brief description, the emitted light from the source is split equally by the beam splitter between the sample and reference arm. Light is then reflected from the sample and a mirror on the reference arm, and the two returning reflected light signals will recombine by the optics of the beam splitter and be measured by the photodetector. An interferometer will behave differently depending on the nature of the light source used. In OCT a low coherence light source is used and is very different than a laser because low coherence implies that there isn't a narrow set wavelength, like a laser, and the phase of the light goes through random shifts just like the 'white' light that our eyes can see. In the next section, the properties of an ideal source for OCT imaging will be discussed.

A low coherence interferometer relies on the interference of light between the reference and sample arm. The phase of the light from the emitting source is constantly changing after a small amount of time called the coherence time, T_c , and this time can be converted to a length by:

$$L_c = T_c \times V_g \quad (1)$$

Where L_c is the coherence length and V_g is the velocity of light in the tissue. V_g can be determined by:

$$V_g = \frac{c}{n} \quad (2)$$

where the speed of light, c , is divided by the index of refraction of the tissue, n .

For the reflected light from the sample and reference arm in the interferometer, the two beams will have a maximum interference when the total relative path lengths are perfectly matched. When both light beams travel the same distances, it means that their phases will always be similar and the two optical fields can be added together and interfere. For purposes of understanding, it is convenient to use an example where the sample arm has a single reflective signal and the reference arm has a movable mirror. This example shows the autocorrelation of the light (Fig. 2). If the reference arm mirror is moved to a much greater distance from the emitting source than the sample arm (illustrated in Fig. 1 by point 'a'), the reflected beam in the reference arm will travel a much greater distance than the beam in the sample arm. This means that when the two beams are recombined, the phase elements of the light will not be related and the statistical randomness of phases in the light will cancel out toward zero. There will essentially be a lack an interference effect (point 'a' in Fig. 2). If the mirror in the reference arm is moved closer, so that the relative path length is within the coherence length, the two beams will begin to interfere. The peak value of interference will be when the relative path lengths are equal (point 'b' in Fig. 1 & 2). And then if the reference mirror is moved closer so that the total distance is less than the sample arm distance, the intensity of the interference will decrease until the mirror is moved again to a distance beyond the coherence length (point 'c' in Fig. 1 & 2). During the time of moving the mirror, a fringe pattern will occur because of the constructive and destructive interference from peaks and troughs of the wavelengths of light. There will be an

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interference envelope signal, and this envelope is Gaussian in distribution when the source bandwidth is Gaussian. The example of the autocorrelation of a single reflective element with a movable mirror is very helpful when discussing the influences of the source bandwidth and resolution of the system and will be discussed later in the next section.

For biological tissue, the refractive index mismatches between different boundaries act as reflection and scattering points for the emitting beam. In ultrasound, reflections at tissue interfaces of the acoustic pulse produce an echo 'train' that can be recorded. But in interferometric detection a low coherence light source continuously illuminates the sample, and a 'superposition' of reflection signals make a return trip in the sample arm. The sample arm is composed of the 'superposition' or overlapping but independent reflection signals. Light traveling in the tissue will travel at a lower speed than light in air because of the difference in the index of refraction, n . The relative path lengths between the sample and reference arm need to account for this difference, where:

$$\text{Optical depth} = (\text{real depth}) \times n \quad (3)$$

If the reference mirror is moved so that it corresponds to a certain optical depth within the tissue in the sample arm, the relative path lengths will coincide and there will be interference between those two signals. The sample beam contains the 'superposition' of reflection signals from different depths, but only the reflection signal from the depth that travels back to the beam splitter and matches the relative path length from the reference signal will interfere. If the

mirror is moved, the interference effect will be the same as the single point reflection example, discussed previously, if the two path lengths are within the coherence length. But the movement of the reference mirror can capture other reflection signals throughout the depth of the tissue if the mirror scans farther throughout the depth of the tissue. The coherence length determines the axial resolution of measuring different reflection signals within the depth of the tissue.

The velocity of the path length shift, from moving the mirror, also causes a Doppler shift in the reference beam, and the recombined interference signal is heterodyned with a lower carrier frequency. Since the lower carrier frequency is known, the interference signal can be electronically demodulated after the power of the interference beam is recorded by the photodiode. The importance of the heterodyne technique is that it increases the sensitivity against background noise to detect small changes in the interference signal.

In summary for this section, a Michelson interferometer can detect the depth resolved reflected or backscattered signals at a specific position within a tissue. Optical Coherence Tomography adds a scanning method, such a lateral scanning device, to collect a series of depth resolved signals to produce a b-scan image.

3.3 Optical Coherence Tomography: Axial Resolution

Optical coherence tomography has the distinctive advantage of being a reflection based imaging modality that can resolve tissue structures on the order of microns. The lateral and axial resolutions are very important properties for

imaging structures within tissues, such as dental hard tissue. The lateral resolution of the system is based on the system optics, whereas, the axial resolution of the system is based on the bandwidth properties of the illuminating source.

OCT imaging can use a variety of illuminating sources; the type used throughout this work is a superluminescent diode (SLD). A SLD is very similar to a diode laser, except that the mirrors within the diode cavity are adjusted to prevent the output of coherent high energy light. A SLD instead emits low coherence light with a center wavelength (or frequency) and a distribution or range of wavelengths above and below the center wavelength.

The example of the interference signal from a single reflective point and a movable mirror within the interferometer is the autocorrelation function (Fig. 2). The autocorrelation function can be thought of the point spread function common in other medical imaging modalities and defines the resolution of the system. If the envelope or 'general distribution' of this function looks Gaussian (Fig. 2) it means that the bandwidth or distribution of frequencies of the source spectrum is Gaussian around the center wavelength. Before discussing this phenomenon, a basic principle must be reviewed. There is a general inverse relationship between time domain signals, i.e. a beam of light, and the frequency components within that signal, i.e. the spectrum of the light. We have discussed the coherence time, T_c , which is related to the frequency bandwidth, $\Delta\nu$, by:

$$T_c = \frac{1}{\pi \times \Delta\nu} \quad (4)$$

Converting the time domain signal into its frequency components is done through the use of a mathematical function called a Fourier transform. The Fourier transform of a Gaussian frequency spectrum yields a Gaussian time domain signal. Because of the inverse relationship between time and frequency, if the diode source bandwidth is a smooth *broad* Gaussian then the autocorrelation time domain function example is a smooth *narrow* Gaussian. Therefore, the axial resolution of the system can be determined by knowing the spectrum of the source. Resolution is usually defined by full width at half max (FWHM) of the Gaussian in the time domain because at the FWHM of two equal intensity reflections separated by the coherence length can be resolved visually. FWHM is a classical definition of resolution in medical imaging. The FWHM axial resolution, Δz , in an OCT a-scan is related to the FWHM of the bandwidth, $\Delta\lambda$, of a Gaussian source by:

$$\Delta z = \frac{2 \ln 2}{\pi} \left(\frac{\lambda^2}{\Delta\lambda} \right) \quad (5)$$

This equation signifies that if the center wavelength of the light, λ , is increased (such as using near infrared light opposed to visible light) the bandwidth of the source needs to increase in order to maintain the same resolution (Fujimoto, 2002). Also the equation shows that higher refractive index tissues, such as enamel ($n=1.63$) will have higher resolution than soft tissue ($n \sim 1.33$).

A broad Gaussian source bandwidth in a SLD can be difficult to achieve, especially as the power is increased. Diode source technology is advancing and becoming more streamlined for OCT applications, but it is important to select a diode with a smooth frequency spectrum. A noisy source with small fluctuations within the bandwidth (such as a small side lobe in the frequency distribution) can lead to large side lobes and ghosting of structure boundaries (the inverse relationship between frequency and time has a great impact in image resolution). Throughout the course of this thesis work, higher power diode sources continued to become available and the smooth spectrum of these diodes made the precise measurements of enamel and caries structures possible.

3.4 Optical Coherence Tomography: Lateral Resolution

For lateral resolution, the system optics can be focused with a plano-convex lens that has a certain focal point. The focal point, along with the spot size on the objective lens, determines the lateral resolution. But an important tradeoff to a smaller lateral resolution is a loss of depth of field (axial). Depth of field can be defined by the confocal parameter, b , and is related to lateral resolution, Δy , by:

$$b = \frac{\pi \times \Delta y^2}{2\lambda} \quad (6)$$

This relationship shows that a linear reduction in the lateral resolution causes an exponential decrease in the confocal parameter or depth of field. The beam will diverge rapidly from the focal point if high resolution is achieved by the lens.

One consequence is that if the beam is focused at the surface, the lateral resolution decrease as the illuminating beam penetrates deeper inside the tissue. In this work, the benefits of longer near infrared wavelengths over visible light will be discussed, but it is important to note that longer wavelengths, λ , have a greater beam divergence and a reduced focal parameter. The depth of field is very important in OCT imaging because it influences the axial imaging depth. The intensity of an illuminating beam is attenuated by scattering and absorption as it propagates in depth (axial). The attenuation is compounded if the beam diverges too rapidly and the beam intensity is dissipated over a greater lateral distance. The compromise in OCT, especially for medical or dental imaging, is to choose an acceptable lateral resolution so that the illuminating beam has the greatest depth of field for optimal imaging depth. Because the beam divergence changes the lateral resolution from the focal point, a conservative definition of lateral resolution was used in this work and measured by translating a knife edge across the beam waist (near focal point) and then calculating the $1/e^2$ diameter (Johnston and Sasnett, 2004).

3.5 Polarization Sensitive Optical Coherence Tomography: Introduction

Polarization-sensitive optical coherence tomography (PS-OCT) is a variant of conventional OCT, and the system uses polarized incident light and can record the polarization information from the backscattered signal in two separate channels (de Boer *et al.*, 1997; Hee *et al.*, 1992; Izatt *et al.*, 1993). PS-OCT has been found to be useful when imaging birefringent tissue, such as the collagen

fibers in the skin or muscle and also dental enamel (Baumgartner *et al.*, 2000; Colston *et al.*, 2002; de Boer *et al.*, 1997). Polarized light interacts uniquely with birefringent tissue because the index of refraction changes depending on the orientation of the tissue. Sound dental enamel is usually birefringent because the smaller enamel crystals are directionally organized along their c-axis into enamel rods. The c-axis has a small difference (~ 0.02) in the refractive index than both the a- & b-axes (Wang *et al.*, 1999). The organized collagen fibrils have similar effects in skin. What is significant with differences in refractive index is that it affects the speed of the light in the tissue (equation 2). For example, if illuminating polarized light (linear or circular) is orientated 45° to the c-axis, then half the electric field will interact with the c-axis and the other will interact with either the a-axis and/or b-axis. The two parts of the electric field will travel at different speeds and change the phase of the light (phase retardation), which in simple terms leads to a relative polarization rotation. As another example, if the polarized light is orientated 5° from normal to the c-axis, a majority of the electric field will interact with the a-axis and/or b-axis, and the relative polarization rotation (phase retardation) of the light will occur much slower than when the incidence was at 45° . When near-angle scattering and reflections occur within the tissue and return to be collected by OCT, the polarization information in two separate axes (parallel and perpendicular) can be recorded after the sample beam is recombined with a linearly (diagonal) polarized reference beam.

There are variety of PS-OCT methods to record and present polarization information. One method using circularly polarized light analyzes the degree of

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polarization, along with the conventional OCT intensity image. The degree of polarization at a specific depth, $D(z)$, is defined by:

$$D(z) = \frac{P_v(z) - P_h(z)}{P_v(z) + P_h(z)} \quad (7)$$

Where $P_v(z)$ is the intensity in the parallel axis and $P_h(z)$ is intensity in the perpendicular axis at a specific depth, z (Colston *et al.*, 2002). For sound enamel, the degree of polarization will vary from -1 to 1 as the light experiences phase retardation while propagating through the tissue. The degree of polarization PS-OCT image constructed will have a banded appearance in depth due to retardation caused from the birefringence.

Another PS-OCT method (Fried *et al.*, 2002) uses linearly polarized light and also records the polarization intensity in the P_v and P_h ; however, the degree of polarization information is not computed rather the 'raw' data of the intensities recorded in the separate parallel and perpendicular axes is presented in two separate images. This method, which will be discussed in greater detail in the next section, exploits two important properties of polarized light when imaging a high refraction index and birefringent tissue such as dental enamel. (1) The surface reflection (Fresnel reflection) at the air/enamel interface is much greater than the subsurface reflections and scattering. Linearly polarized light illuminating the enamel and aligned with the parallel axis will not depolarize (or rotate) into the perpendicular axis near normal incidence. The perpendicular axis will be less affected by a surface reflection that masks subsurface measurements. (2) As discussed previously, the linearly polarized light will

experience phase retardation as it propagates through enamel. The enamel rods are orthogonally aligned to the surface, and even considering a maximum incidence of 45° , the phase retardation occurs such that near the enamel surface the intensity is minimal in the perpendicular axis. In the next two sections and in Chapter III-VII, a novel method for quantifying early enamel caries is discussed and made possible because the background intensity of sound enamel in the perpendicular axis is close to zero. Both PS-OCT methods presented were utilized before this thesis work (Baumgartner *et al.*, 2000; Fried *et al.*, 2002) and will be discussed and compared in the next two sections.

3.6 Polarization Sensitive Optical Coherence Tomography System

The fundamentals of PS-OCT are detailed in numerous publications (de Boer, 2002; Schmitt, 1999), and an all fiber based PS-OCT system used throughout this work was first presented by Fried *et al.* (Fried *et al.*, 2002). Two-dimensional PS-OCT images are constructed by laterally scanning the system across a tooth and collecting a series of depth resolved backscattered signals. The all fiber based PS-OCT system integrated an Optical Coherence Domain Reflectometer (OCDR), which measured the backscattered signal defined as an *a*-scan, with a computer controlled high speed XY-scanning stage (Newport Corp., Irving, CA) to produce a two-dimensional optical tomographic image or *b*-scan. The PS-OCT system was completely controlled using Labview™ software (National Instruments, Austin, TX).

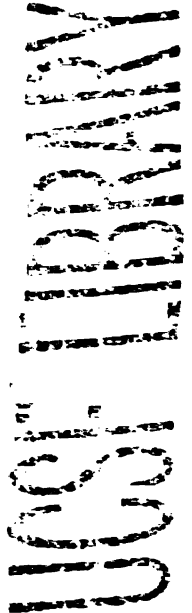
A schematic of the all fiber based OCDR system (Fig. 3, Optiphase Inc, Van Nuys, CA) and consisted of polarization maintaining (pm) optical fiber, high speed piezoelectric fiber stretchers and two balanced InGaAs photodetectors receivers. A broadband high power semiconductor optical amplifier (SOA), which is similar to a SLD, emitted linearly polarized light that was coupled into the slow or parallel-axis of a polarization maintaining (pm) -fiber and the focused beam illuminated the tooth sample. The reflected/back-scattered light from the sample was collected in two orthogonal polarization states of the pm-fiber: the slow or 'parallel' axis to the original incident plane and the fast or 'perpendicular' axis. An important difference from a traditional Michelson interferometer where the path length change is performed with a movable mirror within air is that the OCDR used two high speed piezoelectric fiber-stretchers, in a push-pull configuration, to alter the path length of the reference beam relative to the sample beam, and a 50/50 fiber coupler combined the two beams. A rapid change in the path length of the reference beam relative to the sample beam was needed to create a depth resolved interference signal. The velocity of the path length shift causes a Doppler shift in the reference beam, and the recombined interference signal is heterodyned with a lower carrier frequency. A polarized beam splitter (PBS) then split the two orthogonal components of the combined beam into the parallel and perpendicular axis signals. The two orthogonal signals were then measured by two balanced InGaAs receiver modules. The signals were then electronically demodulated and stored on a Labview™ controlled computer. In the next section, the benefits of the PS-OCT measuring

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the intensity of the interference signal in the two orthogonal axes will be discussed.

3.7 Optical Coherence Tomography: Dentistry

Conventional OCT and PS-OCT is capable of measuring backscattered light as a function of optical depth in up to 2-3 mm of dental enamel with ~ 10-30 μm resolution (Amaechi *et al.*, 2001; Colston *et al.*, 1998; Everett *et al.*, 1999). The early caries process usually involves the demineralization of the outer enamel layer, which is usually birefringent due to the rod-like organization of the hydroxyapatite crystals. Initial measurements of tooth enamel with PS-OCT emphasized characterization of the tissue birefringence (Baumgartner *et al.*, 1998; Wang *et al.*, 1999). PS-OCT images by Baumgartner et al (Baumgartner *et al.*, 2000) resolved enamel demineralization through an increase in backscattered intensity and changes in the enamel birefringence. The birefringence banding pattern is lost rather than subtly altered. Later studies have demonstrated that there is a 2-3 order of magnitude increase in the scattering coefficient of dental enamel upon demineralization (Huynh *et al.*, 2004). If the incident illuminating light is linearly polarized, the light scattered from the demineralized lesion area will rapidly depolarize (Everett *et al.*, 1999). The increase in backscattering and polarization changes caused by carious enamel demineralization has been thoroughly documented by many authors studying dental PS-OCT (Baumgartner *et al.*, 2000; Everett *et al.*, 1999; Fried *et al.*, 2002), although the theoretical principles behind the scattering and



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4 FIGURES AND FIGURE LEGENDS

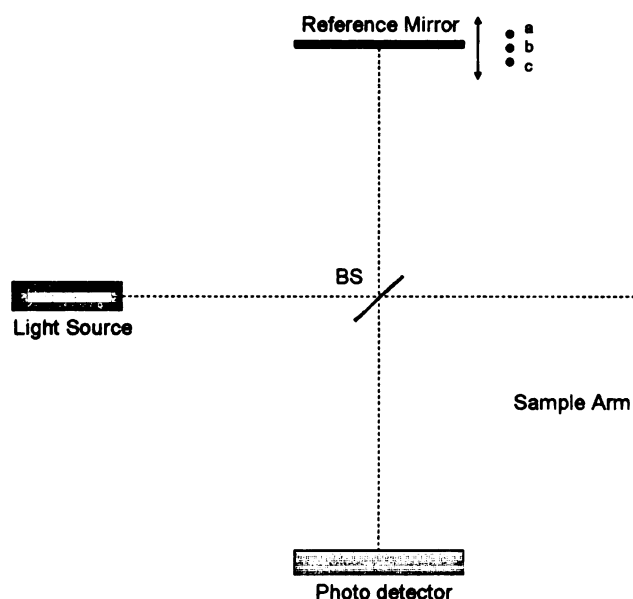


Fig. 1 (A) A low coherence Michelson interferometer. Emitted light is split equally by the beam splitter (BS) between the sample and reference arm. Light is then reflected from the sample and a mirror on the reference arm, and the two returning reflected light signals will recombine by the optics of the beam splitter and be measured by the photodetector. A Michelson interferometer can record depth changes in scattering from a tissue. If a single reflective point is in the sample arm, the recombine signal is the autocorrelation interferogram (shown in Fig. 2), which illustrates the axial resolution of the system.

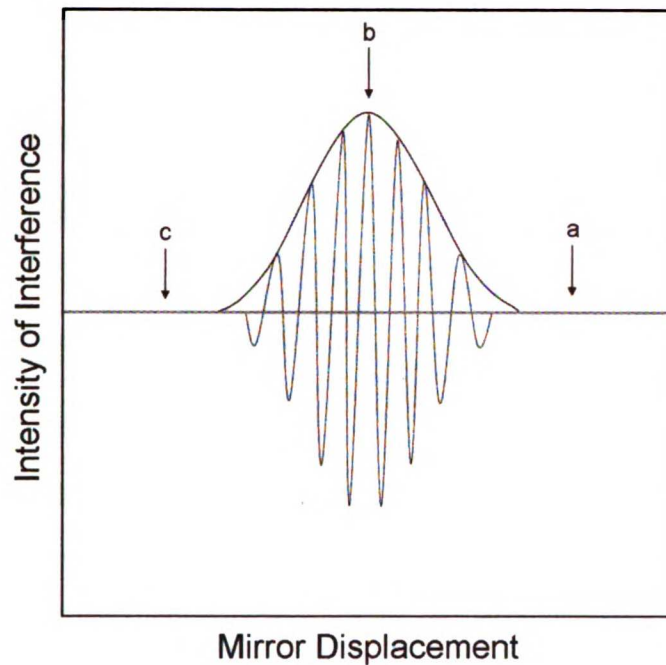


Fig. 2 The autocorrelation interferogram. If the reference arm mirror is moved to a much greater distance from the emitting source than the sample arm (Fig. 1 by point 'a'), the reflected beam in the reference arm will travel a much greater distance than the beam in the sample arm. This means when the two beams are recombined, the phase elements of the light will not be related and the statistical randomness of phases in the light will cancel out toward zero (point 'a'). If the mirror in the reference arm is moved closer, so that the relative path length is within the coherence length, the two beams will begin to interfere. The peak value will be when the relative path lengths are equal (point 'b'). If the reference mirror is moved closer so that the total distance is less than the sample arm distance, the intensity of the interference beam will decrease until the mirror is moved beyond the coherence length (point 'c'). Blue curve indicates the Gaussian profile of the interferogram.

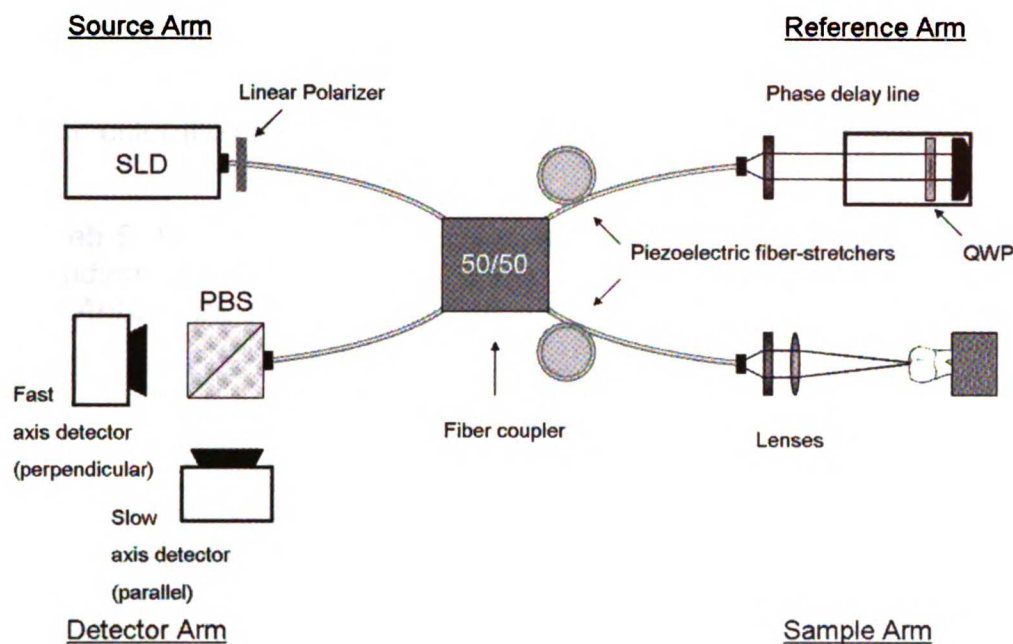


Fig. 3 PS-OCT system—light from the SLD is linearly polarized and coupled into the slow axis of a polarization maintaining fiber and equally split between the sample and reference arms of a polarization sensitive Optical Coherence Domain Reflectometer (OCDR). The path length difference between the sample and reference arms is varied using piezoelectric fiber stretchers. The quarter wave plate (QWP) in the reference arm splits the linearly polarized light equally between both polarization states. A polarizing beam splitter (PBS) in the detection arm splits the fast (perpendicular) and slow (parallel) axis components of the light onto the two detectors. The OCDR is integrated with a computer controlled scanning stage to produce tomographic PS-OCT images.

5 REFERENCES

Abell AK, Leinfelder KF, Turner DT (1983). Microscopic observations of the wear of a tooth restorative composite in vivo. *J Biomed Mater Res* 17(3):501-7.

Al-Khateeb S, Forsberg CM, de Josselin de Jong E, Angmar-Mansson B (1998). A longitudinal laser fluorescence study of white spot lesions in orthodontic patients. *Am J Orthod Dentofacial Orthop* 113(6):595-602.

Alfano RR, Yao SS (1981). Human teeth with and without dental caries studied by visible luminescent spectroscopy. *J Dent Res* 60(120-122).

Amaechi BT, Higham SM, Podoleanu AG, Rogers JA, Jackson DA (2001). Use of optical coherence tomography for assessment of dental caries: quantitative procedure. *J Oral Rehabil* 28(12):1092-3.

Ando M (1999). 4 Methods for Fissure Caries Detection Compared. Proceedings of the 4th Annual Indiana Conference, Early Detection of Dental Caries II, Indianapolis, IN.

Ando M, Schemehorn BR, Eckert GJ, Zero DT, Stookey GK (2003). Influence of enamel thickness on quantification of mineral loss in enamel using laser-induced fluorescence. *Caries Res* 37(1):24-8.

Ando M, Eckert G, Stookey GK, Zero D (2004). Effect of imaging geometry on evaluating natural white-spot lesions using quantitative light-induced fluorescence. *Caries Res* 38(1):39-44.

Angmar-Mansson B, ten Bosch JJ (1987). Optical methods for the detection and quantification of caries. *Adv. Dent. Res.* 1(1):14-20.

Baumgartner A, Hitzenberger CK, Dicht S, Sattmann H, Moritz A, Sperr W, Fercher AF (1998). Optical coherence tomography for dental structures. *Proc of SPIE, Lasers in Dentistry IV* 3248(1):130-136.

Baumgartner A, Dichtl S, Hitzemberger CK, Sattmann H, Robl B, Moritz A, Fercher AF, Sperr W (2000). Polarization-sensitive optical coherence tomography of dental structures. *Caries Res*. 34(1):59-69.

Bjelkhagen H, Sundstrom F (1981). A clinically applicable laser luminescence for the early detection of dental caries. *IEEE J Quant Electr* 17(266-268).

Bowen RL (1963). Properties of a silica-reinforced polymer for dental restorations. *J Am Dent Assoc* 66(57-64).

Bowen RL, Cobb EN, Rapson JE (1982). Adhesive bonding of various materials to hard tooth tissues: improvement in bond strength to dentin. *J Dent Res* 61(9):1070-6.

BRER (2005). Board on Radiation Effects Research, Health Risks from Exposure to Low Levels of Ionizing Radiation: BEIR VII Phase 2 Washington D.C.: The National Academies Press.

Buonocore MG (1955). A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *J Dent Res* 34(6):849-53.

Chesters RK, Pitts NB, Matuliene G, Kvedariene A, Huntington E, Bendinskaite R, Balciuniene I, Matheson JR, Nicholson JA, Gendvilyte A, Sabalaite R, Ramanauskiene J, Savage D, Mileriene J (2002). An abbreviated caries clinical trial design validated over 24 months. *J Dent Res* 81(9):637-40.

Colston BW, Sathyam US, DaSilva LB, Everett MJ, Stroeve P, Otis LL (1998). Dental OCT. *Optics Express* 3(6):230-238.

Colston BW, Everett M, Sathyam US, Da Silva Dominguez L (2002). Optical Coherence Tomography in Dentistry. In: Handbook of Optical Coherence Tomography. GJT Brett E. Bouma editor. New York: Marcel Dekker AG, pp. 591-612.

Curnow MM, Pine CM, Burnside G, Nicholson JA, Chesters RK, Huntington E (2002). A randomised controlled trial of the efficacy of supervised toothbrushing in high-caries-risk children. *Caries Res* 36(4):294-300.

de Boer J (2002). Polarization-Sensitive Optical Coherence Tomography: Introduction. In: Handbook of Optical Coherence Tomography. B Bouma and G Tearney editors. New York: Marcel Dekker AG, pp. 1-40.

de Boer JF, Milner TE, vanGemert MJC, Nelson JS (1997). Two-dimensional birefringence imaging in biological tissue by polarization-sensitive optical coherence tomography. *Optics Letters* 22(12):934-936.

de Josselin de Jong E, Sundstrom F, Westerling H, Tranaeus S, ten Bosch JJ, Angmar-Mansson B (1995). A new method for in vivo quantification of changes in initial enamel caries with laser fluorescence. *Caries Res* 29(1):2-7.

Dirks OB (1974). The benefits of water fluoridation. *Caries Res* 8(0):suppl:2-15.

Dodds MW (1996). Dental caries diagnosis -- toward the 21st century. *Nat Med* 2(3):283.

Ekstrand K, Qvist V, Thystrup A (1987). Light microscope study of the effect of probing in occlusal surfaces. *Caries Res* 21(4):368-74.

Ekstrand KR, Ricketts DN, Kidd EA (1997). Reproducibility and accuracy of three methods for assessment of demineralization depth of the occlusal surface: an in vitro examination. *Caries Res* 31(3):224-31.

Everett MJ, Colston BW, Sathyam US, Silva LBD, Fried D, Featherstone JDB (1999). Non-invasive diagnosis of early caries with polarization sensitive optical coherence tomography (PS-OCT). *Proc of SPIE, Lasers in Dentistry V* 3593(1):177-183.

Featherstone JD (2000). The science and practice of caries prevention. *J Am Dent Assoc* 131(7):887-99.

Fercher AF, Hitzengerger CK, Drexler W, Kamp G, Sattmann H (1993). In vivo optical coherence tomography. *Am J Ophthalmol* 116(1):113-4.

Ferreira-Zandona M, Ando M, Eggertsson H, Kelly S, Katz B, Eckert G, Ofner S, SA L, Lukantsova L, Wefel J, Stookey G (2004). Clinical Validation of Caries

Detection Methodologies: Preliminary Results. *J Dent Res* 83(Special Issue A):2812a.

Fried D, Xie J, Shafi S, Featherstone JDB, Breunig T, Lee CQ (2002). Early detection of dental caries and lesion progression with polarization sensitive optical coherence tomography. *J. Biomed. Optics* 7(4):618-627.

Fujimoto JG, Boppart SA, Tearney GJ, Bouma BE, Pitris C, Brezinski ME (1999). High resolution in vivo intra-arterial imaging with optical coherence tomography. *Heart* 82(2):128-33.

Fujimoto JG (2002). Optical Coherence Tomography: Introduction. In: Handbook of Optical Coherence Tomography. GJT Brett E. Bouma editor. New York: Marcel Dekker AG, pp. 1-40.

Gratt B (1994). Radiographic Interpretation of Pathology: Dental Caries. In: Oral Radiology: Principles and Interpretation P Goaz and S White editors. St. Louis: Mosby, pp. 312.

Hafstrom-Bjorkman U, Sundstrom F, de Josselin de Jong E, Oliveby A, Angmar-Mansson B (1992). Comparison of laser fluorescence and longitudinal microradiography for quantitative assessment of in vitro enamel caries. *Caries Res* 26(4):241-7.

Hee MR, Huang D, Swanson EA, Fujimoto JG (1992). Polarization-Sensitive Low-Coherence Reflectometer for Birefringence Characterization and Ranging. *Journal of the Optical Society of America B-Optical Physics* 9(6):903-908.

Hennon DK, Stookey GK, Muhler JC (1969). Prevalence and distribution of dental caries in preschool children. *J Am Dent Assoc* 79(6):1405-14.

Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA, Fujimoto JG (1991). Optical Coherence Tomography. *Science* 254(1178-1181).

Huynh G, Darling CL, D. Fried (2004). Changes in the optical properties of dental enamel at 1310-nm after demineralization. *Proc of SPIE, Lasers in Dentistry X* 5313(1):118-124.

ICDASII (2005). Rationale and Evidence for the International Caries Detection and Assessment System (ICDASII).

Izatt JA, Hee MR, Huang D, Fujimoto JG, Swanson EA, Lin CP, Schuman JS, Puliafito CA (1993). Optical Coherence Tomography for Medical Applications. In: Medical Optical Tomography: Functional Imaging and Monitoring. G Muller, B Chance, R Alfano, S Arridge, J Beuthan, E Gratton, M Kaschke, B Masters, S Svanberg and Pvd Zee editors. Bellingham, WA: SPIE, pp. 450-472.

Johnston TF, Sasnett MW (2004). Characterization of Laser Beams: The M² model. In: Handbook of Optical and Laser Scanning. GF Marshall editor. New York, Basel: Marcel Dekker, Inc, pp. 1-70.

Kidd EA, Beighton D (1996). Prediction of secondary caries around tooth-colored restorations: a clinical and microbiological study. *J Dent Res* 75(12):1942-6.

Kidd EAM, Ricketts DNJ, Pitts NB (1993). Occlusal caries diagnosis: a changing challenge for clinicians and epidemiologists. *J Dent Res* 72(3):323-331.

Koenig K, Schneckenburger H, Hemmer J, Tromberg BJ, Steiner RW, Rudolf W (1994). In-vivo fluorescence detection and imaging of porphyrin-producing bacteria in the human skin and in the oral cavity for diagnosis of acne vulgaris, caries, and squamous cell carcinoma. *Advances in Laser and Light Spectroscopy to Diagnose Cancer and Other Diseases*, San Jose: SPIE.

Leinfelder KF (1996). A conservative approach to placing posterior composite resin restorations. *J Am Dent Assoc* 127(6):743-8.

Lennon AM, Buchalla W, Brune L, Zimmermann O, Gross U, Attin T (2006). The ability of selected oral microorganisms to emit red fluorescence. *Caries Res* 40(1):2-5.

Lireka J (1987). The selection of patients for X-ray examinations: dental radiographic examinations. Rockville, Md: U.S. Food and Drug Administration, DHHS publication 88-8273.

Loesche WJ, Walenga A, Loos P (1973). Recovery of *Streptococcus mutans* and *Streptococcus sanguis* from a dental explorer after clinical examination of single human teeth. *Arch Oral Biol* 18(4):571-5.

Lussi A (1993). Comparison of different methods for the diagnosis of fissure caries without cavitation. *Caries Res* 27(5):409-16.

Lussi A, Imwinkelried S, Pitts N, Longbottom C, Reich E (1999). Performance and reproducibility of a laser fluorescence system for detection of occlusal caries in vitro. *Caries Res* 33(4):261-6.

Lussi A, Megert B, Longbottom C, Reich E, Francescut P (2001). Clinical performance of a laser fluorescence device for detection of occlusal caries lesions. *European Journal of Oral Sciences* 109(1):14-19.

McDonald SP, Sheiham A (1992). The distribution of caries on different tooth surfaces at varying levels of caries—a compilation of data from 18 previous studies. *Community Dent Health* 9(1):39-48.

Murdoch-Kinch CA, McLean ME (2003). Minimally invasive dentistry. *J Am Dent Assoc* 134(1):87-95.

NIH (2001). Diagnosis and Management of Dental Caries throughout Life, Report No.: 18: NIH Consensus Statement.

Nyvad B, Machiulskiene V, Baelum V (1999). Reliability of a new caries diagnostic system differentiating between active and inactive caries lesions. *Caries Res* 33(4):252-60.

Pitts NB (1997). Diagnostic tools and measurements—impact on appropriate care. *Community Dent Oral Epidemiol* 25(1):24-35.

Pitts NB, Stamm JW (2004). International Consensus Workshop on Caries Clinical Trials (ICW-CCT)—final consensus statements: agreeing where the evidence leads. *J Dent Res* 83 Spec No C(C125-8).

Schmitt JM (1999). Optical coherence tomography (OCT): a review. *IEEE Journal of Selected Topics in Quantum Electronics* 5(4):1205 - 1215.

Schneiderman A, Elbaum M, Schultz T, Keem S, Greenebaum M, Driller J (1997). Assessment of dental caries with Digital Imaging Fiber-Optic Transillumination (DIFOTI): in vitro study. *Caries Res*. 31(2):103-110.

Shi XQ, Welander U, Angmar-Mansson B (2000). Occlusal caries detection with Kavo DIAGNOdent and Radiography: An in vitro comparison. *Caries Res* 34(151-158).

Spitzer D, Bosch JJ (1976). The total luminescence of bovine and human dental enamel. *Calcif Tissue Res* 2):201-8.

Spitzer D, ten Bosch JJ (1977). Luminescence quantum yields of sound and carious dental enamel. *Calcif Tissue Res* 24(3):249-51.

Stewart RE, Hale KJ (2003). The paradigm shift in the etiology, prevention, and management of dental caries: its effect on the practice of clinical dentistry. *J Calif Dent Assoc* 31(3):247-51.

Stookey G (2005a). Should a dental explorer be used to probe suspected carious lesions? No—use of an explorer can lead to misdiagnosis and disrupt remineralization. *J Am Dent Assoc* 136(11):1527, 1529, 1531.

Stookey GK (2005b). Quantitative light fluorescence: a technology for early monitoring of the caries process. *Dent Clin North Am* 49(4):753-70, vi.

Tearney GJ, Brezinski ME, Bouma BE, Boppart SA, Pitris C, Southern JF, Fujimoto JG (1997). In Vivo endoscopic Optical Biopsy with Optical Coherence Tomography. *Science* 276(2037-2039).

ten Cate JM, van Amerongen JP (1996). Caries Diagnosis: Conventional Methods. Early Detection of dental caries, Indianapolis: Indiana University.

Thompson VP, Kaim JM (2005). Nonsurgical treatment of incipient and hidden caries. *Dent Clin North Am* 49(4):905-21, viii.

Tranaeus S, Al-Khateeb S, Bjorkman S, Twetman S, Angmar-Mansson B (2001). Application of quantitative light-induced fluorescence to monitor incipient lesions in caries-active children. A comparative study of remineralisation by fluoride varnish and professional cleaning. *Eur J Oral Sci* 109(2):71-5.

Tyas MJ, Anusavice KJ, Frencken JE, Mount GJ (2000). Minimal intervention dentistry—a review. FDI Commission Project 1-97. *Int Dent J* 50(1):1-12.

Wang XJ, Milner TE, de Boer JF, Zhang Y, Pashley DH, Nelson JS (1999). Characterization of dentin and enamel by use of optical coherence tomography. *Applied Optics* 38(10):2092-2096.

Wenzel A (2004). Bitewing and digital bitewing radiography for detection of caries lesions. *J Dent Res* 83 Spec No C(C72-5).

Young DA, Featherstone JD (2005). Digital imaging fiber-optic trans-illumination, F-speed radiographic film and depth of approximal lesions. *J Am Dent Assoc* 136(12):1682-7.

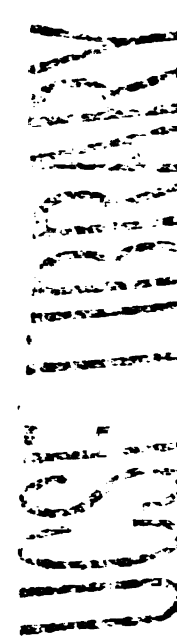
CHAPTER II

NEAR INFRARED TRANSILLUMINATION FOR IMAGING EARLY ENAMEL CARIES

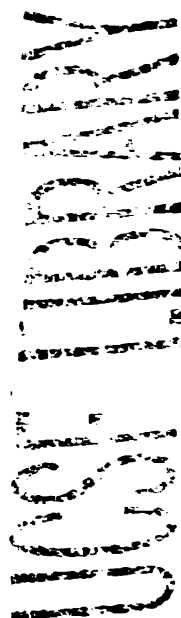
1 INTRODUCTION

Increased interest in early caries detection has followed the advancement of non-surgical methods for arresting or reversing early dental decay. The nature of dental caries has changed dramatically over the last 50 years with the addition of fluoride to the drinking water and the widespread use of fluoride dentifrices. Today, almost all new decay occurs in the occlusal pits and fissures of the posterior dentition and the interproximal contact sites between teeth. The treatment for early dental decay or caries is shifting away from aggressive cavity preparations that attempt to completely remove demineralized tooth structure toward minimally invasive restorative or non-surgical techniques. In non-surgical therapy, a clinician prescribes antibacterial rinses, fluoride treatments, and dietary changes in attempt to remineralize the decay before it becomes irreversible (Featherstone and Young, 1999). The success of this type of therapy is contingent on early caries detection and also requires imaging modalities that can safely and accurately monitor the success of such treatment.

Enamel caries, particularly in the early stages, are difficult to detect using the dentist's existing armamentarium of dental x-rays and the dental explorer. Both methods do not precisely measure the lesion depth of early dental decay, and due to ionizing radiation exposure are not indicated for regular monitoring. These constraints and limitations are the impetus for investigating optical imaging systems that could detect early dental decay, while providing the biologically compatible wavelengths that facilitate frequent screening.

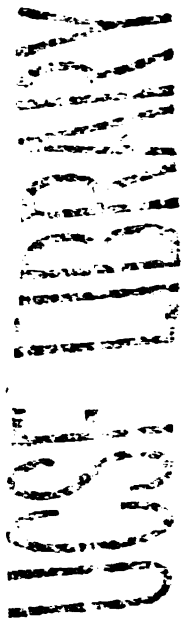


Before the advent of x-rays, dentists used light for the detection of caries lesions. In the past 30 years, the development of high intensity fiber-optic illumination sources has resurrected this method for caries detection (Barenie *et al.*, 1973; Holt and Azevedo, 1989; Mitropoulos, 1985; Peers *et al.*, 1993; Pine, 1996; Vaarkamp *et al.*, 2000). Fiber-optic transillumination (FOTI) has been shown to be promising for the detection of interproximal lesions. One digital-based system, DIFOTI™ (Digital Imaging Fiber-Optic Transillumination) from Electro-Optical Sciences, Inc., that utilizes visible light, has recently received FDA approval (Schneiderman *et al.*, 1997). Optical imaging of dental hard tissue at visible wavelengths, 400-700-nm is principally limited by light scattering. Light scattering has been shown to be high in the visible range (Angmar-Mansson and ten Bosch, 1987; Fried *et al.*, 1995; Spitzer and ten Bosch, 1975). Previous measurements of enamel optical properties extending to 1053-nm indicate that scattering in enamel is primarily determined by a combination of Mie and Rayleigh scattering (Fried *et al.*, 1995). According to theory, the magnitude of Rayleigh scattering is expected to decrease with increasing wavelength as λ^{-4} . The scattering coefficient decreases from 60 cm⁻¹ at 632-nm to 15 cm⁻¹ at 1053-nm (Fried *et al.*, 1995). Since attenuation or extinction is dominated by scattering, we postulated that attenuation of longer wavelengths in the near infrared (NIR) is far less than in the visible range. Based on low attenuation, the NIR may be an ideal spectral region to use in optical imaging. In the first section of this chapter, we measured the attenuation coefficient at 1310-nm and 1550-



nm to determine that 1310 nm near infrared light is the optimal imaging wavelength.

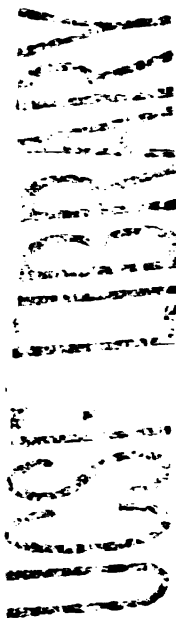
In the second section of this chapter, we demonstrate that NIR light at 1310-nm is well suited for the detection and imaging of interproximal caries lesions. Simulated lesions, which represent the increased scattering of dental caries, were placed in plano-parallel dental enamel sections of various thicknesses. These samples were then illuminated with a polarized broadband NIR light source, and images through each section were acquired using an InGaAs focal plane array (FPA). The objective of these measurements was to establish the effective NIR imaging depth through sound tooth structure and to determine the image contrast between simulated caries lesions with sound enamel. The 1310-nm transillumination using an InGaAs FPA, of the tooth sections, was compared to dental radiographs and transillumination in the visible range and NIR at 830-nm. Subsequent analysis of the acquired projection images, indicated high contrast between sound enamel and simulated lesions in the NIR, especially at 1310-nm. In addition, NIR 1310-nm images of extracted teeth containing natural caries lesions were collected to further demonstrate the clinical potential for imaging interproximal lesions, secondary decay around composite restorations, and cracks and defects in the tooth enamel.



2 MATERIALS AND METHODS

2.1 Attenuation Measurements at 1310-nm and 1550-nm

Plano-parallel sections approximately 100- μm to 2-mm were cut from extracted first premolars that were then highly polished to a 0.3- μm diamond grit finish. Enamel integrity was confirmed under microscopic inspection at up to 500x magnification, and the samples were stored in a 1% thymol solution to prevent decay. Samples were then placed inside a polystyrene cuvette (index of refraction, $n \sim 1.60$) containing an index matched viscous gel (enamel, $n = 1.63$) in order to remove the influence of surface scattering. Laser light ($\sim 2\text{-mW}$) from 1310-nm and 1550-nm single-mode pigtailed diode lasers with a LDC-2000 diode driver from Thorlabs (Newton, NJ) were used to illuminate specific areas of the sample with a spot size of approximately 100- μm (Fig. 1). By changing the optical fiber on a lens mount, the two wavelengths could be interchanged to illuminate identical areas of the sample (Fig. 2). Before illuminating the sample, the laser light was spatially filtered using a beam expander and an aperture to create a uniform Gaussian beam and achieve a small focal spot. The spot size was confirmed with knife edge profiling techniques. The amount of light transmitted through the illuminated area of enamel was measured using a InGaAs photodiode and an SRS-850 digital lock-in amplifier, Stanford Research Systems (Stanford, CA) for phase sensitive detection. Enamel measurements targeted cusp regions close to the halfway point between the surface and the DEJ to minimize scattering by either interface, possible surface demineralization, and protein structures like enamel tufts. Five measurements of collimated transmission were recorded at various sections within each sample (Fig.2). The collimated signal (I) at the detector was compared to the initial intensity of the



beam (I_0). Using this ratio and the thickness of the sample (T), the attenuation coefficient (μ_t) was calculated using Beer's law and a Beer-Lambert plot:

$$\mu_t = - \frac{\ln (I/I_0)}{T} \quad (1)$$

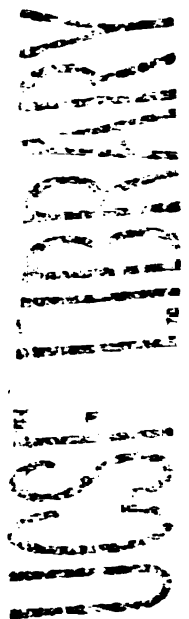
Beer's law states that the intensity of light passing through a sample decreases exponentially as thickness increases. This overall decrease is known as attenuation or extinction, which is caused by internal scattering and absorption.

2.2 Sample Preparation for NIR Imaging at 1310-nm

Thirty plano-parallel sections of enamel of various thickness, 2, 3, 4, 5, 6, 6.75-mm, were prepared from sound human teeth. These sections were stored in a moist environment to preserve tissue hydration with 0.1% thymol added to prevent bacterial growth. Uniform scattering phantoms simulating dental decay were produced midway through each section by drilling 1-mm diameter x 1.2-mm deep cavities in the proximal region of each sample and filling the cavities with hydroxyapatite paste. A thin layer of unfilled composite resin was applied to the outside of the filled cavity to seal the hydroxyapatite within the prepared tooth cavity.

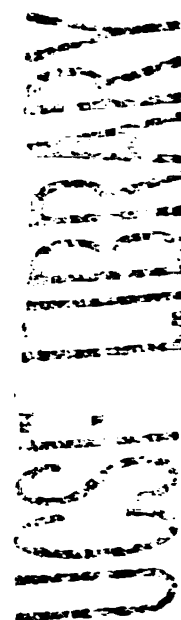
2.3 NIR Imaging at 1310 nm

The NIR imaging set-up is shown schematically in Fig. 3. Light from either a fiber-optic bundle coupled to a 150-watt halogen lamp, Visar™ (Den-Mat, Santa Maria, CA) or a 1310-nm super luminescent diode (SLD) with an output power of



3.5 mW and a bandwidth of 25-30 nm, Model QSDM-1300-5 (Qphotonics Inc., Chesapeake, VA) was used as the illumination source. We found that the speckle of narrower bandwidth Fabry-perot diode lasers such as a 50-mW 1310-nm source, Model QLD-1300-50 (Qphotonics Inc., Chesapeake, VA) interfered significantly with image resolution and was not useful for this application. Crossed near-IR polarizers, Model K46-252 (Edmund Scientific, Barrington, NJ), were used to remove light that directly illuminated the array without passing through the tooth. Dental enamel is birefringent, therefore, the polarization state of the light passing through the tooth is altered, thus reducing extinction by the orthogonal linear polarizer. An InGaAs focal plane array (318 x 252 pixels) the Alpha NIR™ (Indigo Systems, Goleta, Ca) with an Infinimite™ lens (Infinity, Boulder, Co) was used to acquire all the images. A plot of the camera responsivity is shown in Fig. 4. A 50-nm bandpass filter centered at 1310-nm, Model BP-1300-090B (Spectrogon US, Parsippany, NJ), was used to remove all light outside the spectral region of interest. The acquired 12-bit digital images were analyzed using IRVista™ software (Indigo Systems, Goleta, Ca). The illuminating light intensity, source to sample distance, and the aperture diameter were adjusted for each sample to obtain the maximum contrast between the lesion and the surrounding enamel without saturation of the InGaAs FPA around the lesion area.

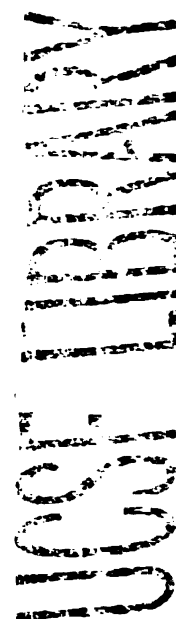
Although the 3.5-mW SLD source provided similar image quality to the halogen lamp source, all the images shown in this section were acquired using the fiber-optic illuminator. Due to the natural tooth contours, the sides near the



simulated lesions in the tooth sections were masked with putty to ensure that light traveled the full width of the sample. This masking is not applicable in a clinical situation and was not necessary to acquire images of whole teeth.

2.4 NIR Imaging at 830 nm

The NIR imaging set-up at 830-nm is shown schematically in Fig. 5, which is similar to the set-up used at 1310-nm. Light from a fiber-optic bundle coupled to a 150-watt halogen lamp, (E Licht Company, Denver, CO). Crossed near-IR polarizers, Model K46-252 (Edmund Scientific, Barrington, NJ), were used to remove light that directly illuminated the array without passing through the tooth. A 70-nm bandpass filter centered at 830-nm, Model P70- 850-F (Corion, Franklin, MA), was used to remove all light outside the spectral region of interest. A near-IR sensitive CCD camera (Model DMK-3002-IR, Imaging Source, Charlotte, NC) was used to acquire the images. A plot of the camera responsivity is shown in Fig. 6. A National Instruments (Austin, TX) IMAQ PCI-1409 frame-grabber card was used with 9-bit resolution. The illuminating light intensity, source to sample distance, and the aperture diameter were adjusted for each sample to obtain the maximum contrast between the lesion and the surrounding enamel without saturation of the CCD camera around the lesion area.



2.5 Visible and X-ray Imaging

The tooth sections imaged with the NIR transillumination systems were compared to conventional visible light transillumination FOTI and dental x-rays.

For visible light transillumination, the same fiber-optic illuminator was used to illuminate the sections and a color 1/3" CCD camera with a resolution of 450 lines, Model DFK 5002/N (Imaging Source, Charlotte, NC), equipped with the same Infinimite™ lens recorded the projection image. X-ray images were acquired by placing the section directly on Ultra-Speed™ D-speed film (Kodak, Rochester, NY) using 75 kVp, 15 mA, and 12 impulses.

2.6 Image Analysis

The coordinates of each simulated lesion were known prior to analyzing the contrast of each lesion. The mean pixel intensity of the lesion and the enamel above and below the lesion was measured using the IRvista™ and Matlab™ software. Lesion contrast was calculated for each sample as follows:

$$\text{Lesion contrast}(C) = (I_E - I_L) / I_E \quad (2)$$

where I_E is the mean intensity of the enamel bordering the lesion and I_L is the mean intensity of the lesion. Lesion contrast is defined as a ratio that will vary from 0 to 1. For each of the six sample thicknesses measured, the mean lesion contrast was calculated and plotted versus sample thickness.

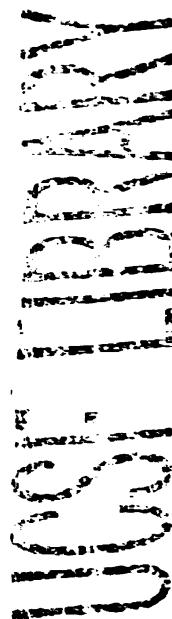
Although contrast is important, the boundary or edge between the lesion and the sound tooth structure is central to detection of the lesion. Therefore, the spatial intensity profile of a lesion with its surrounding enamel was analyzed. An

intensity profile was mapped from a distinct line in six sample images representing each thickness.

3 RESULTS

3.1 Attenuation Coefficients at 1310 nm and 1550 nm

Using a Beer-Lambert plot with linear regression gives mean attenuation values of $3.1 \pm 0.17 \text{ cm}^{-1}$ and $3.8 \pm 0.17 \text{ cm}^{-1}$ for 1310-nm and 1550-nm (Fig. 7 & 8). The Pearson product moment correlation coefficient (P_r) calculated from the sample data shows a very high (>0.90) and positive correlation. As with our previous measurements (Fried *et al.*, 1993), we found index matching to be paramount to acquiring accurate values of the bulk attenuation coefficients. Since the bulk attenuation in the enamel is an order of magnitude less than attenuation from surface scattering, index matching was significantly more difficult than our previous measurements in the visible range. To illustrate this problem, there was no difference in the measured attenuation for a sample thickness of 100- μm versus 2-mm if index matching was not employed. A Beer-Lambert plot of our samples without index matching produces roughly a horizontal line indicating that the attenuation was dominated by surface scattering, since the surface area does not increase with increasing sample thickness. Even highly polished samples still possess surface roughness that completely masks the true bulk attenuation properties. In order to resolve this problem, samples had to be placed in cuvettes filled with a high-viscosity index-matched fluid (Fig. 9). The cuvettes were polycarbonate with a refractive index of 1.6; therefore, the only Fresnel reflection

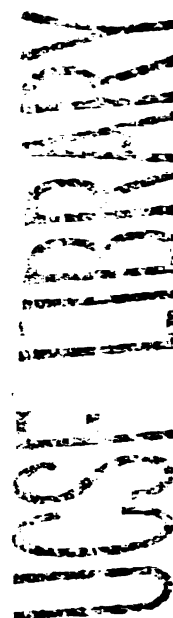


losses are at the cuvette/air boundaries and do not influence the Beer-Lambert plot, i.e., since each sample is presumably equally affected by surface reflection, the plotted data should produce a consistent slope, or mean attenuation value. This holds true as long as the surface scattering is significantly less than the true bulk attenuation.

3.2 Near-IR Transillumination

Visible light, 1310-nm NIR and x-ray images of a simulated lesion placed in one of the 3-mm thick tooth sections are shown in Fig. 10. The lesion cannot be seen using visible light transillumination; however the lesion is clearly visible with high contrast using 1310-nm NIR light transillumination. A radiographic image of the tooth section using D-speed film shows poor contrast between the lesion and the surrounding enamel. For the 1310-nm transillumination, representative spatial intensity profiles from six of the samples of each thickness and the corresponding images are shown in Fig. 11. From these profiles, the edge or boundary between the sound enamel and the lesion is clearly demarcated in all six of the sections. A steep intensity gradient is visible between the surrounding enamel and the lesion. This gradient is less pronounced for sections greater than 4-mm thick, especially on the lower border of the lesion.

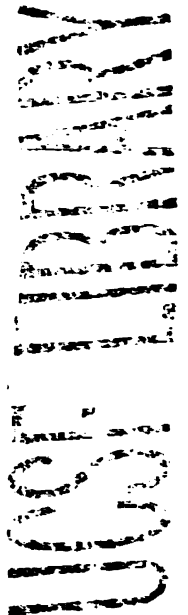
The 830-nm transillumination, using the optimized DMK-3002-IR CCD array, is compared to the 1310-nm InGaAs transillumination, visible light, and x-rays, in Fig. 12. The simulated lesion in the left side of the incisor is best seen in the 1310-nm image; however, it is clearly resolved at 830-nm. The contrast of the lesion with the surrounding enamel is much lower in the D-speed x-ray image,



and the lesion is irresolvable in the visible light image. In the visible light image, there is a stain and possibly slight surface demineralization on the facial surface of the tooth (right-side) that is indistinguishable from the deep lesion on the left-side. The 1310-nm and 830-nm images indicate that the right side stain does not penetrate in depth, as does the x-ray image.

The lesion contrast was calculated for all thirty of the enamel sections under both NIR wavelengths and visible illumination. The image contrast plotted vs. section thickness is shown in Fig. 13. The 1310-nm transillumination offered the greatest lesion contrast, and a lesion contrast of greater than 0.30 was seen in all the sections with the exception of the 6-mm samples. A 0.30 lesion contrast is equivalent to a lesion intensity that is 70% of the surrounding enamel. The 830-nm transillumination did not provide as high of a contrast as 1310-nm and could not resolve lesions when the enamel was greater than 5-mm. 830-nm with the optimized CCD array did produce superior contrast to that of the visible transillumination, where the visible light and standard CCD could not resolve lesions greater than 2-3 mm.

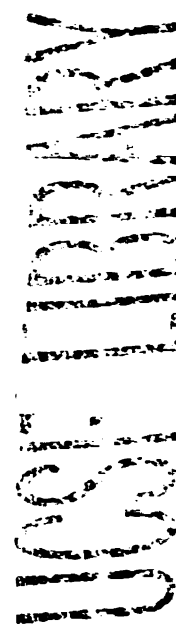
A 1310-nm NIR image of a whole tooth sample with a natural lesion, depicted in Fig. 14, illustrates that a natural lesion can be resolved with the same success as the simulated lesions placed in plano-parallel sections. A composite filling is also visible on the opposite side of the tooth in Fig. 14, indicating that there is also high contrast between composite filling materials and sound tooth structure. In a supplemental multimedia file that was included in the original publication (Jones *et al.*, 2003), tooth1, a metal rod was inserted between the



illuminating source and the tooth and rotated approximately 45° to demonstrate that the tooth enamel is transparent. The rod is clearly resolved through the enamel, but the base of the rod that extends beneath the dentin cannot be resolved because of the high opacity of dentin, even in the NIR.

4 DISCUSSION

The fundamental optical property measurements and transillumination of dental enamel at wavelengths in the NIR, especially near 1310-nm, clearly suggest that the NIR is ideal for the optical imaging of early enamel caries. The attenuation coefficients measured at 1310-nm and 1550-nm are 1-2 orders of magnitude less than those in the visible range: 105cm^{-1} at 543-nm and 60cm^{-1} at 632-nm (see Fig. 15). The inverse of the attenuation coefficient is the mean free path. The mean free path is the distance an average photon travels before it is scattered or absorbed. The mean free path is ~3mm for 1310-nm and 1550-nm compared to less than a tenth of a millimeter for wavelengths in the visible range. As stated earlier, the magnitude of Rayleigh scattering decreases markedly with increasing wavelength. Based on scattering alone, attenuation should be lower at 1550-nm than at 1310-nm; however, absorption by water in enamel (8-12% by volume) contributes to attenuation at longer wavelengths. The absorption coefficient of water is low at 1310-nm (1.0cm^{-1}). Absorption continues to increase with wavelength, one order of magnitude at 1550-nm (10cm^{-1}), reaching a peak at $2.92\mu\text{m}$ ($1.3 \times 10^4\text{cm}^{-1}$) (Hale and Querry, 1973). This not only accurately explains why the attenuation is higher at 1550-nm than 1310-nm, but suggests that longer



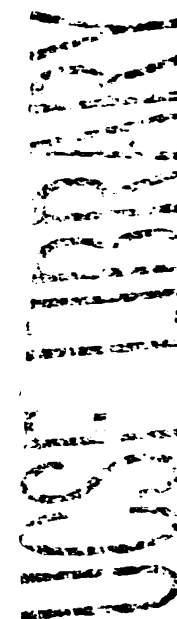
wavelengths toward the mid-IR will have higher attenuation coefficients because of water absorption.

The clinical potential of NIR transillumination for imaging dental caries is illustrated by the high contrast and intensity profiles of the simulated caries lesions with the surrounding enamel. Since one of main clinical uses of transillumination is to detect interproximal lesions, it is important to note that forty of the sixty interproximal surfaces in the mouth would require imaging through less than 5-mm of enamel. This study suggests that resolving caries lesions through 5-mm of enamel is clinically feasible. This is further demonstrated by the NIR imaging of whole teeth with natural decay.

During the transillumination of whole tooth samples, polarization gating with crossed polarizers was critical for preventing the illuminating light from saturating the InGaAs array near the area of the lesion 'shadow'. This technique will also be important in a clinical setting where adjacent tooth surfaces will reflect, but not depolarize the light, and could interfere with the accuracy of the projection image.

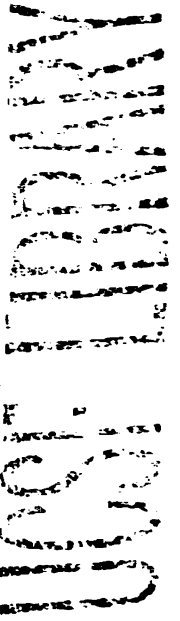
Previous groups pursuing visible light transillumination, have used or proposed more advanced imaging techniques like temporal or coherence gating and sophisticated image processing algorithms to enhance the imaging and detection of dental decay (Keem and Elbaum, 1997; Schneiderman *et al.*, 1997). With NIR transillumination, it is likely that these techniques may not be necessary.

During demineralization of enamel in the caries process, preferential dissolution of the mineral phase creates pores that highly scatter light. The



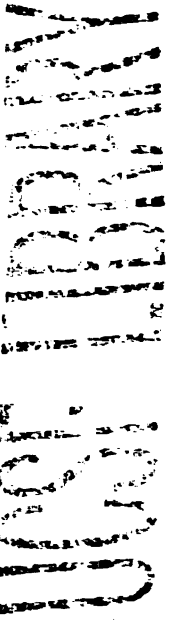
simulated lesions in our study are primarily made up of isotropic scatterers, with scattering occurring at the grain boundaries in the hydroxyapatite powder (Fried *et al.*, 1995). Therefore, such simulated lesions may possibly overestimate the magnitude of scattering in natural caries lesions; however, creating more accurate optical simulated lesions requires an intimate understanding of the fundamental optical properties of carious tissue that has yet to be determined. This study shows that 1310-nm is optimal for both high transmission through sound dental enamel and for achieving high contrast between caries lesions and sound enamel. Simulated lesions composed of an unorganized paste of hydroxyapatite, strongly scatter the 1310-nm light, which provides high contrast with the transparent sound enamel.

Optical transillumination is similar to other projection imaging modalities like conventional x-rays, however; the image contrast arises from changes in tissue scattering as opposed to variations in tissue density. Therefore, NIR transillumination can be more sensitive than x-rays for resolving early caries lesions. Clinicians are trained to diagnose at the low lesion contrast depicted in the radiograph of Fig. 3, but the high contrast in the NIR image suggests that the simulated lesions are more sensitive to optical detection. This is due to the fact that the simulated lesions have only slightly lower density than the sound enamel but strongly scatter NIR light. The contrast and intensity profile data signifies the advantage of 1310-nm versus 830-nm. A caries lesions can be detected with greater contrast and through thicker samples at 1310-nm. Another important benefit of 1310 nm images is that the lesion boundary or “edge” of the lesion is



sharper than at 830 nm as illustrated in Fig. 12. This sharpness, which is the result of the low scattering at 1310-nm, may allow the dentist to more accurately discern the dimensions of the lesion. In dental radiology, image sharpness is one reason why many dentists still prefer to use lower speed films, such as D-speed film, over higher speed films despite the increase in radiation exposure to patients. There is no increased health risk with using non-ionizing wavelength of 1310 nm over 830 nm or visible light with the increased benefits. Presently, there is a substantial difference in cost between the InGaAs detector technology in 1310-nm transillumination with that of the inexpensive NIR sensitive CCD cameras used with 830-nm. Since NIR transillumination is a considerably improvement to the present visible light based transillumination systems, both 830 nm and 1310 nm based systems may be useful to the dental profession in the future.

This study focused primarily on caries detection with NIR transillumination, and more in depth analysis will be done to investigate the relationship between density loss in caries lesions and their optical properties. A detailed understanding may aid in discriminating dental decay at different stages of the caries and remineralization process so that a dentist can also assess the severity of a caries lesion.



5. FIGURES AND FIGURE LEGEND

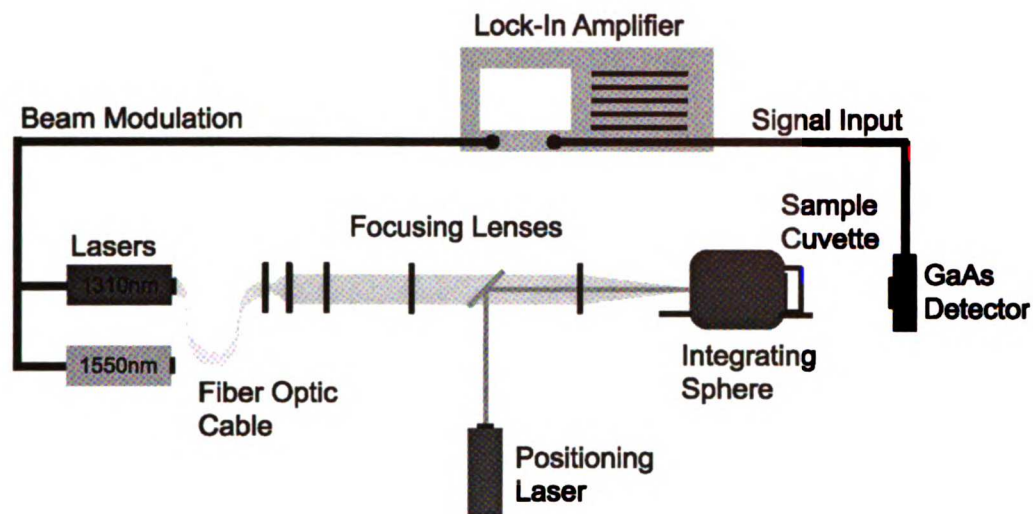


Fig. 1 The experimental setup, which measures the optical attention of a section of enamel placed in the sample cuvette. Two diode lasers of 1310-nm and 1550-nm were interchanged in the setup with the use of single-mode optical fiber.

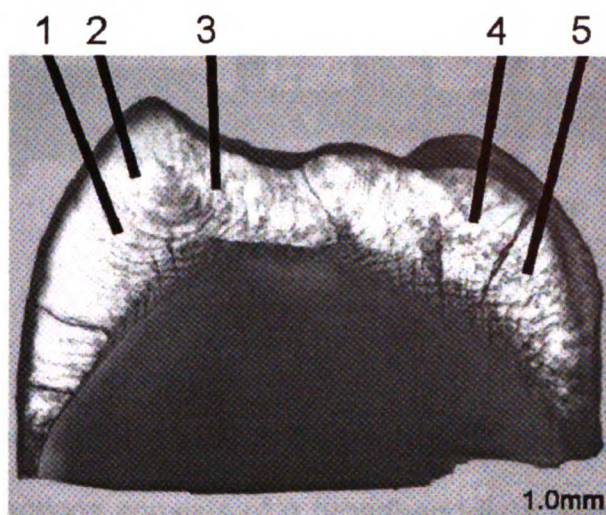


Fig. 2 A diamond grit finished plano-parallel tooth section of 1.0mm thickness is shown. Five measurements of the collimated signal through specific enamel points were recorded at 1310-nm and 1550-nm using the set-up in Fig. 1.

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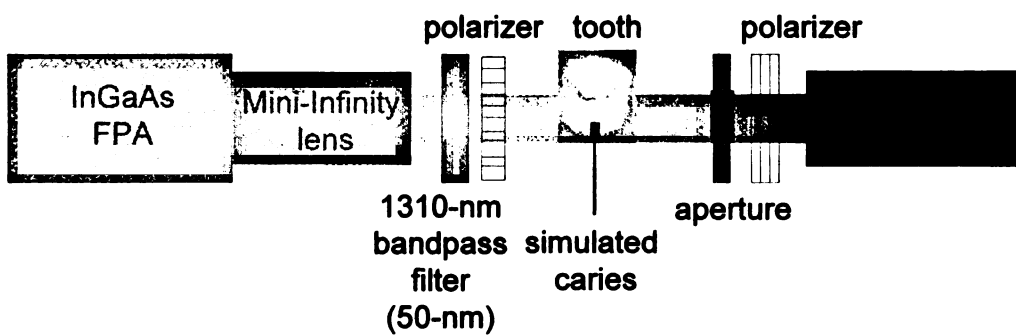


Fig. 3 Setup used for the 1310-nm Near-Infrared Transillumination of whole teeth and tooth sections consists of a broadband light source, crossed linear polarizers, a bandpass filter and a NIR InGaAs focal plane array (FPA).

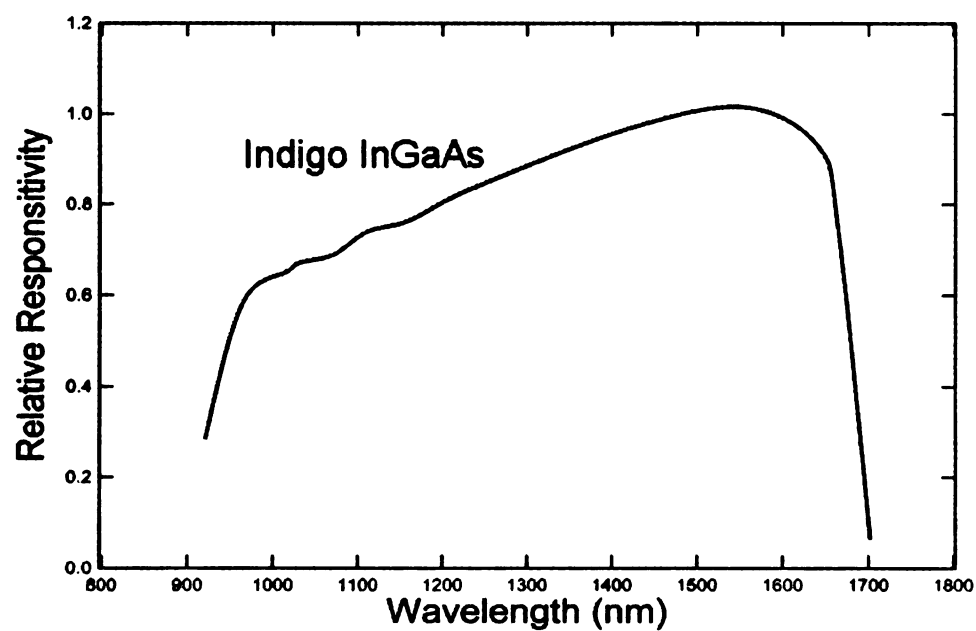


Fig. 4 A plot of the sensitivity versus wavelength of the Alpha NIR™ (Indigo Systems, Goleta, Ca) camera demonstrates that the InGaAs FPA array is well-suited for 1310-nm transillumination.

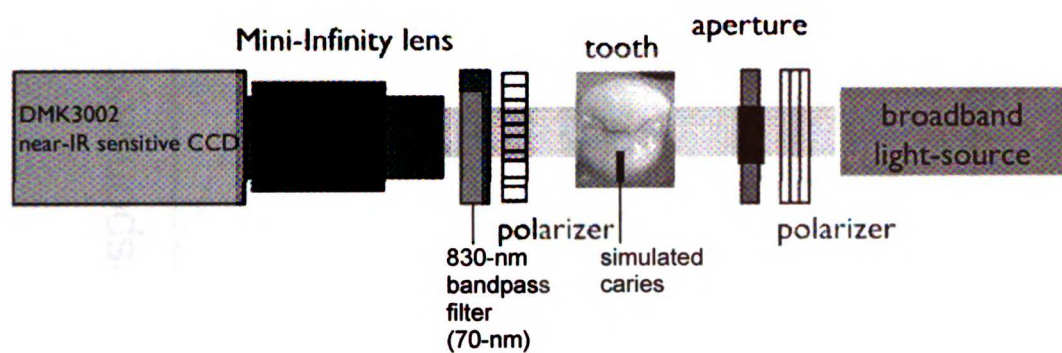


Fig. 5 Setup used for the 830-nm Near-Infrared Transillumination of whole teeth and tooth sections consists of a broadband light source, crossed linear polarizers, a bandpass filter and a NIR sensitive CCD array.

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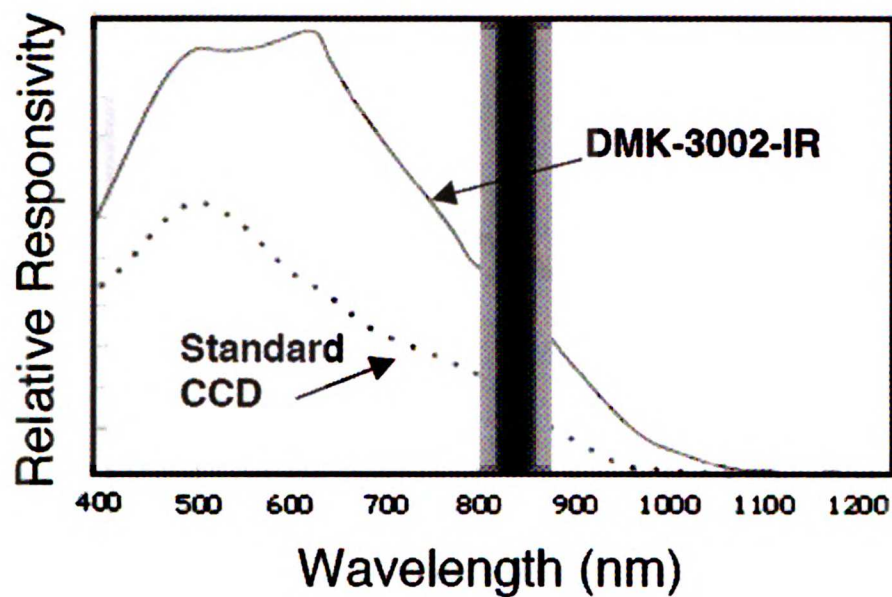


Fig. 6 A plot of the relative sensitivity versus wavelength of the DMK-3002-IR (Imaging Source, Charlotte, NC) camera shows the enhanced response in the near-IR wavelength. The center column denotes the region where the 830-nm bandpass filter is used to collect the transillumination projection image.

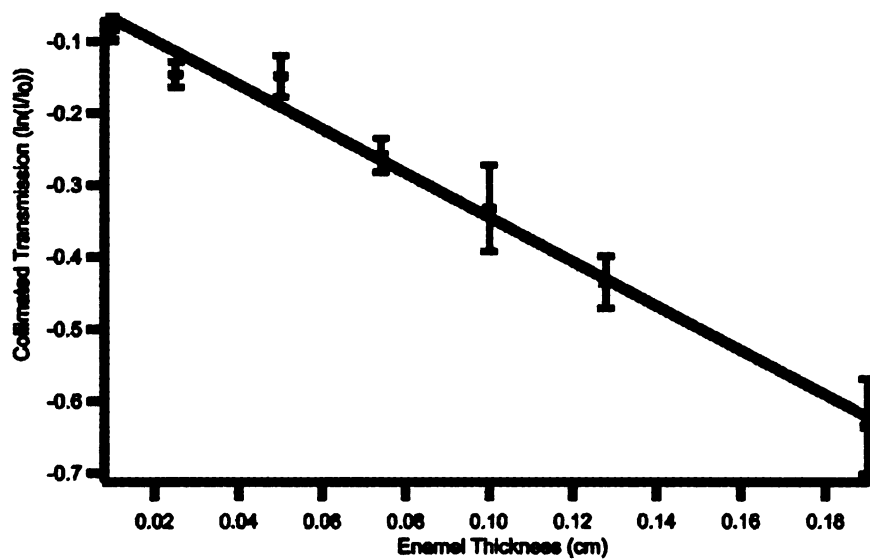


Fig. 7 A Beer-Lambert plot with linear regression gives mean attenuation values of $3.1 \pm 0.17 \text{ cm}^{-1}$ for 1310-nm.

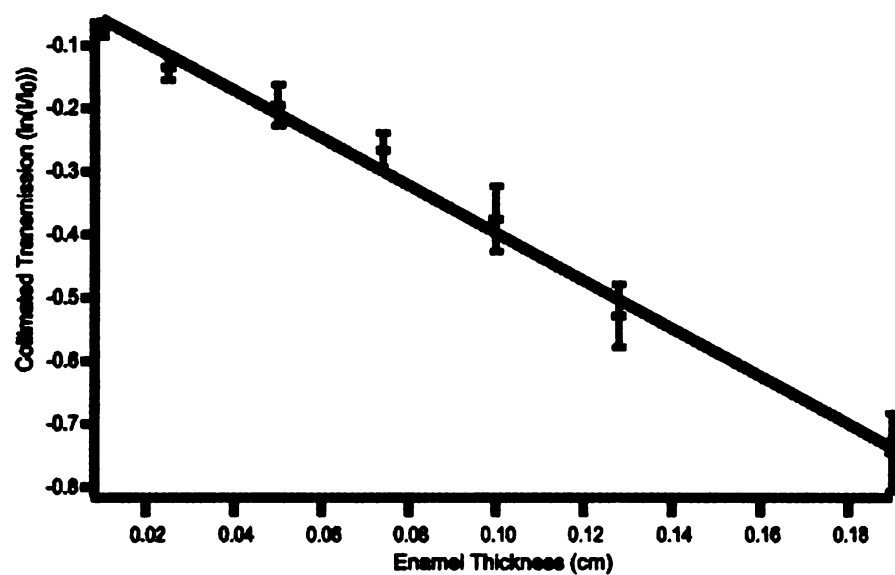
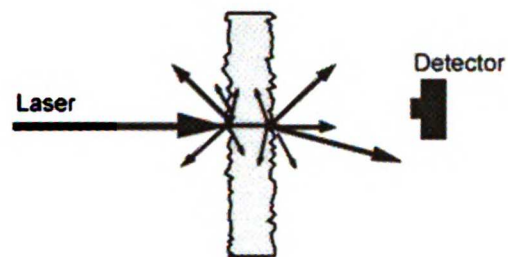
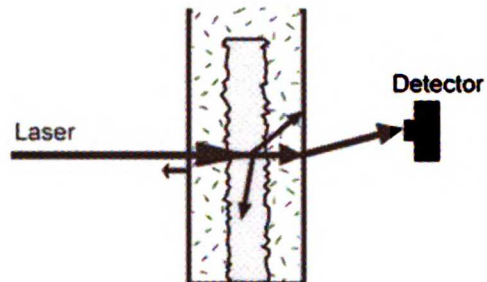


Fig. 8 A Beer-Lambert plot with linear regression gives mean attenuation values of $3.8 \pm 0.17 \text{ cm}^{-1}$ for 1550-nm.



0.3 μ m polished enamel sample still possesses surface roughness that completely masks the bulk behavior.



0.3 μ m polished sample in an index matched solution and cuvette eliminates surface scattering. Light is attenuated (scattered or absorbed) by internal structures.

Fig. 9 The true bulk attenuation properties are revealed in an index matched gel and cuvette.

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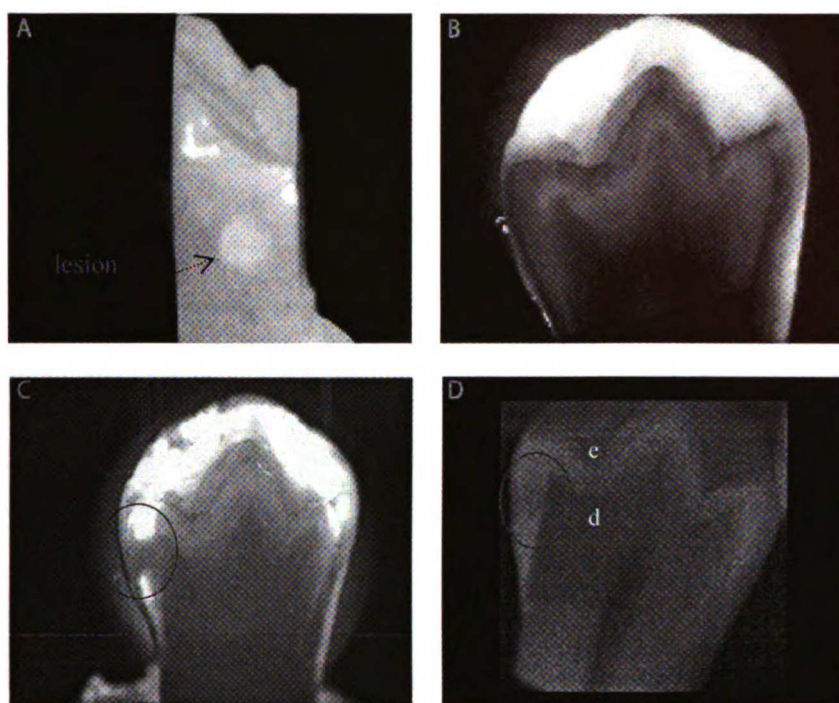
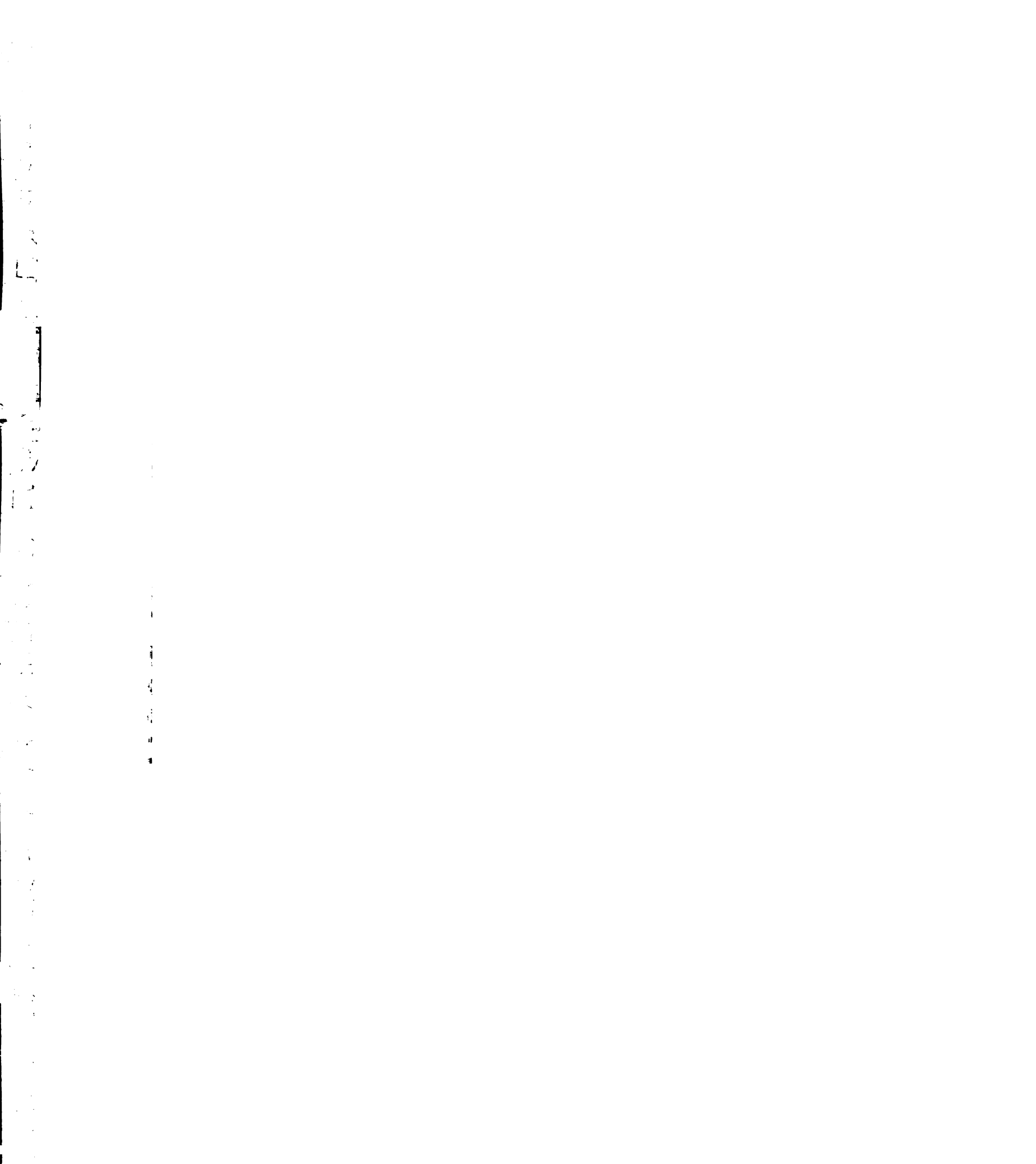


Fig. 10 A) Side-view of a 3-mm thick tooth section with a simulated lesion. B) The lesion cannot be seen using transillumination with visible light and a CCD camera. C) The lesion is clearly visible under NIR transillumination D) An x-ray of the section using D-speed film indicates the small contrast difference between the simulated lesion and sound enamel. The sound enamel [e] and dentin [d] layers are distinguishable in all the projection images.



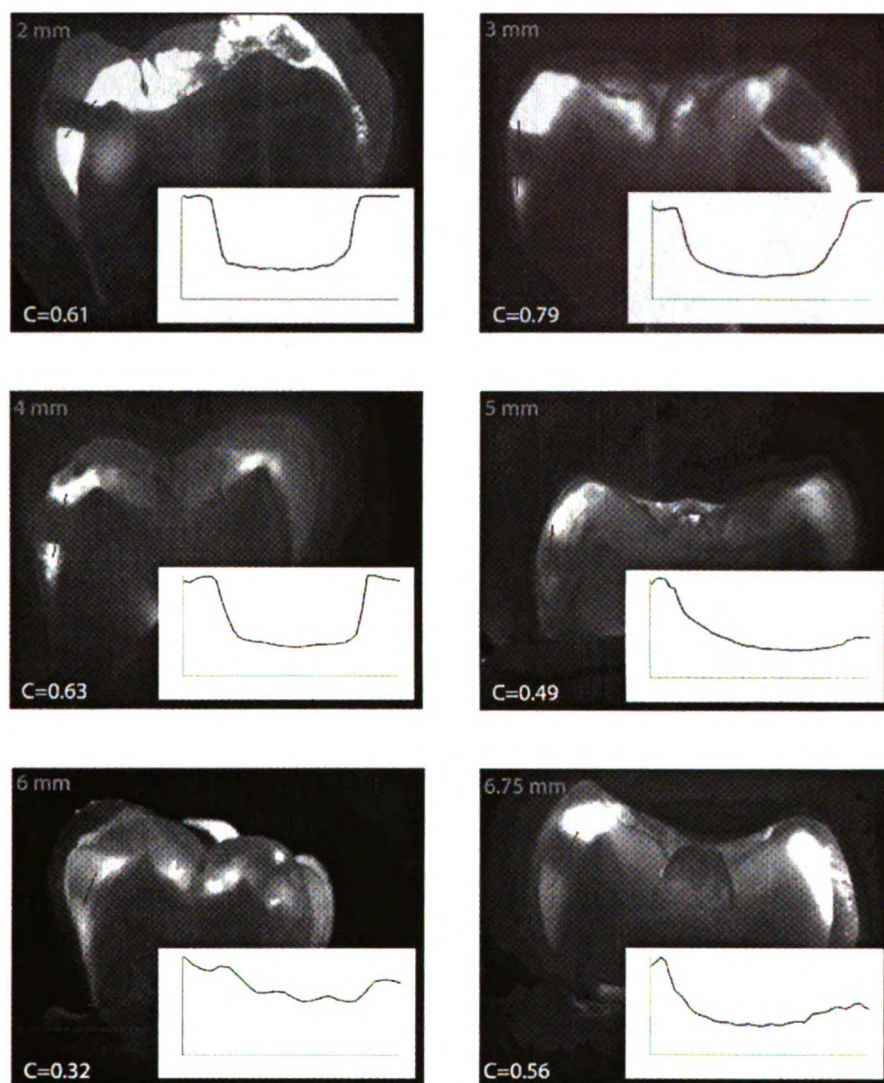
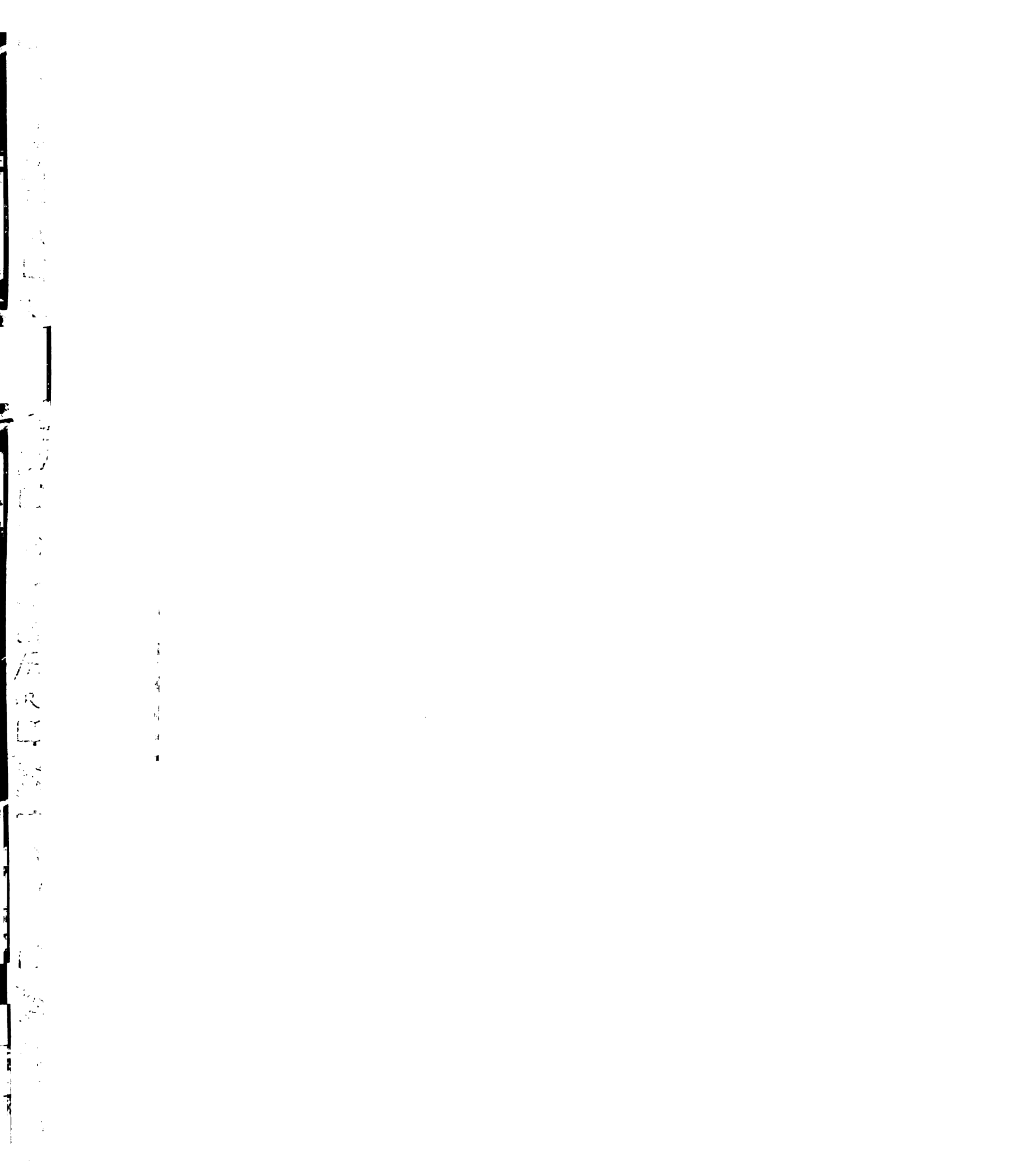


Fig. 11 NIR transillumination images of tooth sections with simulated lesions are shown for each representative sample thickness. The corresponding spatial line profiles demarcated in red are shown on the inset in the lower right of each image, and the measured lesion contrast is shown in the lower left. The left axis represents the pixel intensity ranging from 0 – 4096, and the bottom axis the pixel position through the lesion.



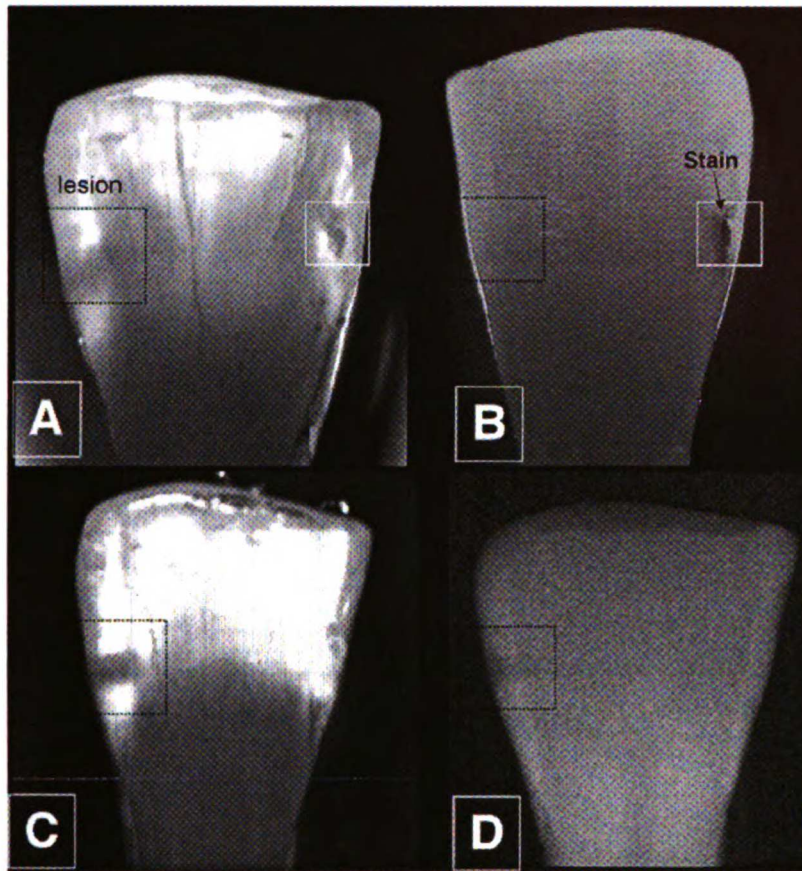


Fig. 12 Images of a whole human incisor (A) 830-nm image, (B) visible light image, (C) 1310-nm image and (D) D-speed dental x-ray. The dotted line box on the left marks the region where the lesion is located. Visible light transillumination does not reveal the lesion, which is evident in the 830-nm image (A) and with even greater contrast in the 1310-nm (C). A stain on the right-side of the tooth appears to be a lesion in the visible light image (B).



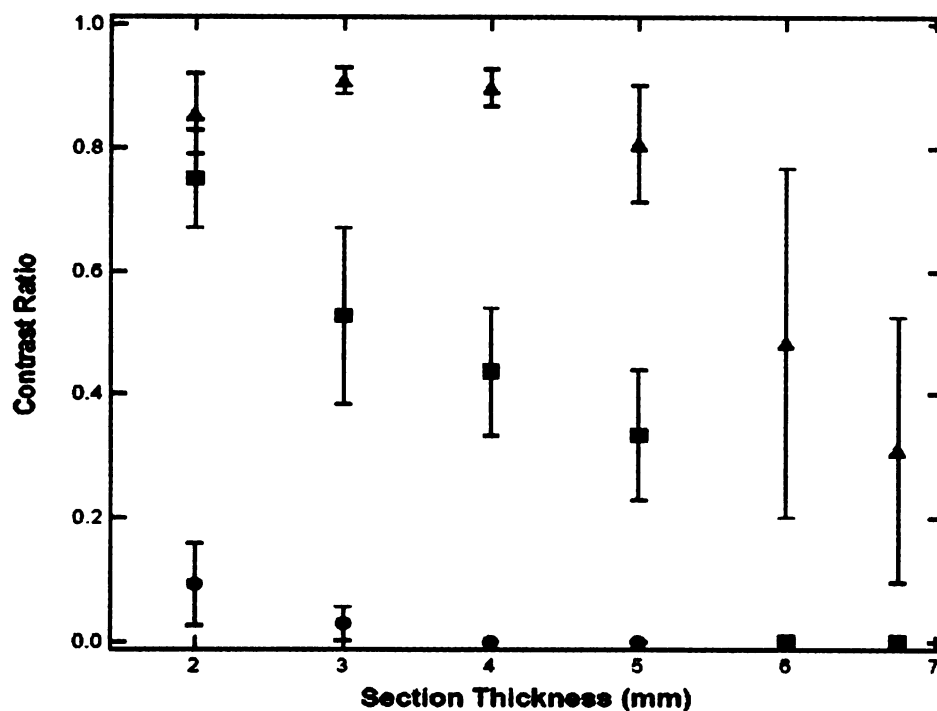


Fig. 13 Contrast ratio between sound enamel and the simulated lesion for the three imaging systems for varying section thickness, (triangles) – 1310-nm, (squares) – 830-nm, and (circles) – visible standard CCD no filters. The lesion contrast was significantly greater in the NIR than the visible range with 1310-nm producing the highest lesion contrast. 1310-nm transillumination was also able to fully resolve lesions in the thickest 6.75-mm samples, whereas 830-nm was unable to resolve the lesions in thicknesses greater than 5-mm.

[illegible]

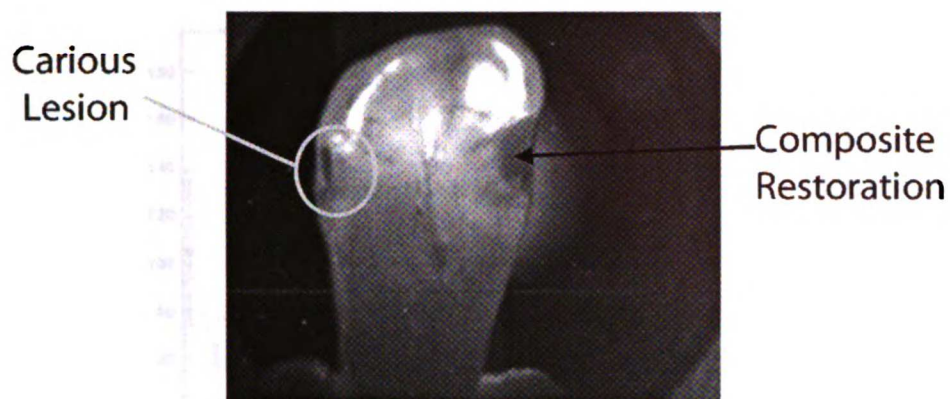


Fig. 14 1310-nm NIR transillumination image of a whole tooth sample. A natural carious lesion and a composite restoration are seen on the left and right, respectively. The tooth is slightly rotated to present different viewing angles. A crack is also visible in the center of the tooth.

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[illegible]

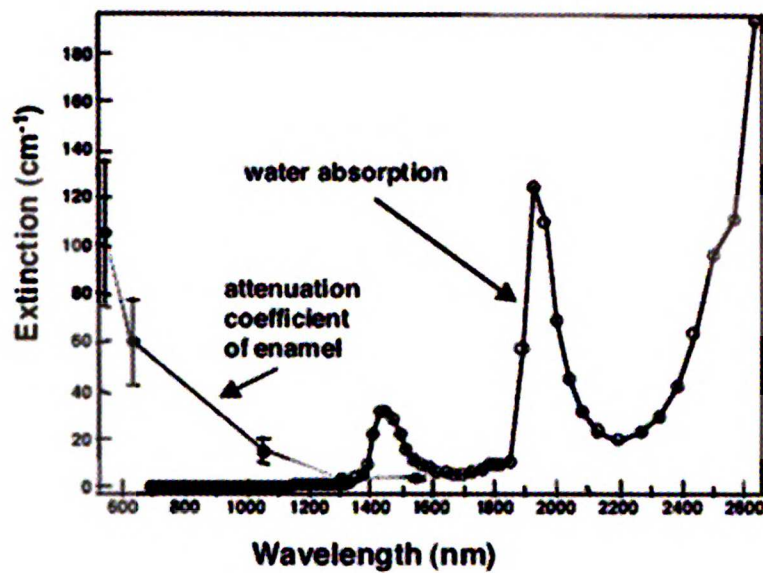


Fig. 15 Plot of the attenuation coefficient of dental enamel measured using this data and data taken from reference (Fried *et al.*, 1995) (filled-circles) and the absorption coefficient of water (Hale and Querry, 1973) (open-circles) vs. wavelength.

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6 REFERENCES

Angmar-Mansson B, ten Bosch JJ (1987). Optical methods for the detection and quantification of caries. *Adv. Dent. Res.* 1(1):14-20.

Barenie J, Leske G, Ripa LW (1973). The use of fiber optics transillumination for the detection of proximal caries. *Oral Surg Oral Med Oral Pathol* 36(6):891-7.

Featherstone JDB, Young D (1999). The need for new caries detection methods. *Lasers in Dentistry V*, San Jose, CA: SPIE.

Fried D, Featherstone JDB, Glana RE, Bordyn B, Seka W (1993). The light scattering properties of dentin and enamel at 543, 632, and 1053 nm. *Lasers in orthopedic, dental, and veterinary medicine II*, Los Angeles: SPIE.

Fried D, Featherstone JDB, Glana RE, Seka W (1995). The nature of light scattering in dental enamel and dentin at visible and near-IR wavelengths. *Appl. Optics* 34(7):1278-1285.

Hale GM, Querry MR (1973). Optical constants of water in the 200-nm to 200- μ m wavelength region. *Appl. Optics* 12(3):555-562.

Holt RD, Azevedo MR (1989). Fibre optic transillumination and radiographs in diagnosis of approximal caries in primary teeth. *Community Dent Health* 6(3):239-47.

Jones RS, Huynh GD, Jones GC, Fried D (2003). Near-infrared transillumination at 1310-nm for the imaging of early dental decay. *Optics Express* 11(18):2259-2265.

Keem S, Elbaum M (1997). Wavelet representations for monitoring changes in teeth imaged with digital imaging fiber-optic transillumination. *IEEE Trans Med Imaging* 16(5):653-63.

Mitropoulos CM (1985). The use of fibre-optic transillumination in the diagnosis of posterior approximal caries in clinical trials. *Caries Res* 19(4):379-84.

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Peers A, Hill FJ, Mitropoulos CM, Holloway PJ (1993). Validity and reproducibility of clinical examination, fibre-optic transillumination, and bite-wing radiology for the diagnosis of small approximal carious lesions: an in vitro study. *Caries Res* 27(4):307-11.

Pine CM (1996). Fiber-Optic Transillumination (FOTI) in Caries Diagnosis. Early Detection of dental caries, Indianapolis: Indiana University.

Schneiderman A, Elbaum M, Schultz T, Keem S, Greenebaum M, Driller J (1997). Assessment of dental caries with Digital Imaging Fiber-Optic Transillumination (DIFOTI): in vitro study. *Caries Res*. 31(2):103-110.

Spitzer D, ten Bosch JJ (1975). The absorption and scattering of light in bovine and human dental enamel. *Calcif. Tiss. Res*. 17(2):129-37.

Vaarkamp J, Bosch JJt, Verdonschot EH, Bronkhorst EM (2000). The real performance of bitewing radiography and fiber-optic transillumination for approximal caries diagnosis. *J Dent Res* 79(10):1747-1751.

CHAPTER III

IMAGING ARTIFICIAL CRIES UNDER COMPOSITE SEALANTS AND RESTORATIONS

1 INTRODUCTION

The occlusal pits and fissures of posterior teeth continue to be the principal area where dental decay (caries) develops. Occlusal caries are difficult to detect and monitor, particularly in the early stages, using the conventional armamentarium of the dental explorer and bitewing x-rays. Since pit and fissures are highly susceptible to caries, clinicians often place preventive resin sealants on the occlusal surfaces of posterior teeth on patients with deep fissures or a history of caries. Although resin sealants prevent decay by acting as a physical barrier blocking out acid producing bacteria, the adhesion of the sealant to the tooth enamel can eventually fail and dental caries can develop underneath the sealant restorations (Kidd and Beighton, 1996). No current diagnostic method is capable of evaluating the structural integrity of resin sealants and detecting caries under these materials.

Polarization Sensitive Optical Coherence Tomography (PS-OCT) has been used to image the artificial caries progression on smooth enamel surfaces by measuring the high backscattered reflectivity and depolarization of carious lesions (Fried *et al.*, 2002). In chapter III, optical property measurements established that dental enamel is nearly transparent in the near-IR, notably 1310 nm (Fried *et al.*, 1995; Jones and Fried, 2002; Jones *et al.*, 2003), and OCT systems can operate with 1310-nm to improve the axial imaging penetration depth over wavelengths in the visible range. In this study, a PS-OCT system operating at 1310-nm utilized linearly polarized incident light and recorded images in axes parallel or perpendicular to the incident light. Since most new

caries lesions are not found on smooth surfaces but rather involve diagnostically challenging regions like the occlusal surfaces, this chapter will investigate the ability of PS-OCT to image demineralization on occlusal surfaces. The hypothesis of this work is that PS-OCT will be able to evaluate lesion severity of artificial occlusal caries and be insensitive to the surface topography of the tooth. In addition, recent investigations of dental composite materials, Otis et al, have used OCT to discriminate composite sealants from sound enamel tissue (Otis *et al.*, 2003). Feldchtein et al observed that PS-OCT can not only identify composites, but image dentinal caries beneath these materials (Feldchtein *et al.*, 1998). However, a method to quantify the severity of decay in occlusal surfaces and beneath composite sealants and restorations has not been demonstrated. Another objective of this study was to determine if a PS-OCT system operating at 1310-nm can image and quantify artificial caries progression on the occlusal surfaces of posterior teeth and artificial occlusal caries underneath resin sealants.

2 MATERIALS AND METHODS

2.1 Artificial Occlusal Caries

Forty-five sound human posterior teeth were mounted on 12 x 12-mm acrylic blocks after root resection. Teeth were sterilized using gamma irradiation and stored in a moist environment to preserve tissue hydration with 0.1% thymol added to prevent bacterial growth. Teeth were not chemically or mechanically

pretreated before the study. An acid resistant varnish was applied on the teeth on all areas outside the 4 x 3-mm pit and fissure region, which was scanned using the PS-OCT system. Artificial occlusal lesions on twenty teeth were formed using a well-characterized pH cycling model replicating the cycle of demineralization and remineralization that takes place naturally in the oral environment (Featherstone *et al.*, 1986; White and Featherstone, 1987). Teeth were exposed for 14 days to a daily regimen of 6 hrs demineralization and 17 hrs remineralization (Featherstone *et al.*, 1990). Teeth were exposed 6 hours each day to a demineralizing solution of 40 mL aliquots of a buffer solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate and 0.075 mol/L acetate maintained at pH 4.3 and a temperature of 37°C. After demineralization, teeth were immersed for 17 hrs in a 20 mL remineralization solution of 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L KCl, 20 mmol/L cacodylate buffer maintained at pH 7.0 and 37°C. Five teeth were imaged periodically at day 0,1,3,6,9,14 during the pH cycling regimen. Twenty teeth were used as non-treated controls.

2.2 Sealant Restorations

After artificial caries were created on the occlusal surfaces, approximately 1 mm thick layer of resin sealant was placed over the pits and fissures of the twenty teeth. Two sealant materials, Aeliteflo™ (Bisco) (n=10) and Concise™ (3M ESPE) (n=10), were used in this study. Both sealants are primarily composed of a matrix resin methacrylate monomer. Concise™ is a visibly opaque sealant and has 5-10 % by weight of silica filler (3M, 2003), which provides strength and

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resistance to long term wear. A tooth colored dental shade (A3) was selected for the Aeliteflo™ sealant (40-70 % filler (Bisco, 2003). For the twenty control teeth, teeth were etched with 35 % phosphoric acid for 15 seconds, rinsed with water, and dried, before sealant application. This acid etching is required for adequate bonding of the sealant material to the dental enamel. In addition to sealants, composite materials used in restorative cavity preparations were also studied by fabricating 1.25 mm thick blocks of Z-100™ (A3, 3M ESPE) and Herculite™ (A2, Kerr) composites. The composite materials, along with blocks of Aeliteflo™ and Concise™, were placed over an intense scattering surface of barium sulfate.

2.3 Polarization Sensitive Optical Coherence Tomographic Imaging

The PS-OCT system used in this study is presented in Chapter I. The PS-OCT system used one of two diode illuminating sources. The PS-OCT system was coupled with a 3.5-mW power SLD source, centered at 1310-nm, possessed a spectral bandwidth (FWHM) of 25-nm. This diode source was used for the samples which were monitored throughout the pH cycling regimen. The theoretical free space resolution with this 25-nm bandwidth source was ~ 34 μm . For samples in the sealant experiment, the PS-OCT system was coupled with a super luminescent diode (SLD), SLED-1310, from COVEGA (Jessup, MD), with an output power of 20 mW, a center wavelength of 1310 nm, and a spectral bandwidth (FWHM) of 50 nm. The theoretical free space resolution with this 50-nm bandwidth source was ~17 μm . The free space axial resolution for this source was experimentally measured to be 20 μm when a single reflective peak



in the time domain was measured at the -3 dB level or the full-width at half max (FWHM). The incident beam was focused onto the sample surface using a 20 mm focal length AR coated plano-convex lens. This optical configuration produced a 30 μm lateral resolution, which is independent of the source bandwidth and axial resolution, that was measured by translating a knife edge across the beam waist and then calculating the $1/e^2$ diameter (Johnston and Sasnett, 2004). The system was found to have a signal to noise ratio (SNR) of 40 dB, which was calculated using a single reflective signal from a mirror that was just below that saturation intensity of the InGaAs detectors.

Two-dimensional OCT intensity plots were obtained by collecting a series of depth resolved signals by laterally scanning the beam across the tooth. The acrylic blocks attached to each tooth fit into a custom metal jig that allowed orientation and coordinates to be precisely replicated, within 50 μm , for every scan, see Chapter V Fig. 1 (Jones *et al.*, 2004).

Transverse b-scan images from both polarization states were acquired at day 0,1,3,6,9,14 during the 14 day pH cycling regimen for five teeth. In the perpendicular-axis images, the mean reflectivity of the enamel was determined by integrating the enamel 200 μm deep and dividing by the total pixels integrated. After the 14th day PS-OCT scans, teeth were sectioned and examined under a polarized light microscope (100x) to compare the artificial lesion depth with the OCT perpendicular-axis images. For the other forty sample teeth, images were acquired at day 0, and for the treatment samples, also after 14 days of pH cycling. Following the PS-OCT scans of the artificial lesions (n=20) and control

1. *Introduction*
 2. *Background*
 3. *Methodology*
 4. *Results*
 5. *Discussion*
 6. *Conclusion*
 7. *References*
 8. *Appendix*
 9. *Index*
 10. *Table of Contents*
 11. *Abstract*
 12. *Keywords*
 13. *Summary*
 14. *Notes*
 15. *References*
 16. *Appendix*
 17. *Index*
 18. *Table of Contents*
 19. *Abstract*
 20. *Keywords*
 21. *Summary*
 22. *Notes*
 23. *References*
 24. *Appendix*
 25. *Index*
 26. *Table of Contents*
 27. *Abstract*
 28. *Keywords*
 29. *Summary*
 30. *Notes*
 31. *References*
 32. *Appendix*
 33. *Index*
 34. *Table of Contents*
 35. *Abstract*
 36. *Keywords*
 37. *Summary*
 38. *Notes*
 39. *References*
 40. *Appendix*
 41. *Index*
 42. *Table of Contents*
 43. *Abstract*
 44. *Keywords*
 45. *Summary*
 46. *Notes*
 47. *References*
 48. *Appendix*
 49. *Index*
 50. *Table of Contents*
 51. *Abstract*
 52. *Keywords*
 53. *Summary*
 54. *Notes*
 55. *References*
 56. *Appendix*
 57. *Index*
 58. *Table of Contents*
 59. *Abstract*
 60. *Keywords*
 61. *Summary*
 62. *Notes*
 63. *References*
 64. *Appendix*
 65. *Index*
 66. *Table of Contents*
 67. *Abstract*
 68. *Keywords*
 69. *Summary*
 70. *Notes*
 71. *References*
 72. *Appendix*
 73. *Index*
 74. *Table of Contents*
 75. *Abstract*
 76. *Keywords*
 77. *Summary*
 78. *Notes*
 79. *References*
 80. *Appendix*
 81. *Index*
 82. *Table of Contents*
 83. *Abstract*
 84. *Keywords*
 85. *Summary*
 86. *Notes*
 87. *References*
 88. *Appendix*
 89. *Index*
 90. *Table of Contents*
 91. *Abstract*
 92. *Keywords*
 93. *Summary*
 94. *Notes*
 95. *References*
 96. *Appendix*
 97. *Index*
 98. *Table of Contents*
 99. *Abstract*
 100. *Keywords*
 101. *Summary*
 102. *Notes*
 103. *References*
 104. *Appendix*
 105. *Index*
 106. *Table of Contents*
 107. *Abstract*
 108. *Keywords*
 109. *Summary*
 110. *Notes*
 111. *References*
 112. *Appendix*
 113. *Index*
 114. *Table of Contents*
 115. *Abstract*
 116. *Keywords*
 117. *Summary*
 118. *Notes*
 119. *References*
 120. *Appendix*
 121. *Index*
 122. *Table of Contents*
 123. *Abstract*
 124. *Keywords*
 125. *Summary*
 126. *Notes*
 127. *References*
 128. *Appendix*
 129. *Index*
 130. *Table of Contents*
 131. *Abstract*
 132. *Keywords*
 133. *Summary*
 134. *Notes*
 135. *References*
 136. *Appendix*
 137. *Index*
 138. *Table of Contents*
 139. *Abstract*
 140. *Keywords*
 141. *Summary*
 142. *Notes*
 143. *References*
 144. *Appendix*
 145. *Index*
 146. *Table of Contents*
 147. *Abstract*
 148. *Keywords*
 149. *Summary*
 150. *Notes*
 151. *References*
 152. *Appendix*
 153. *Index*
 154. *Table of Contents*
 155. *Abstract*
 156. *Keywords*
 157. *Summary*
 158. *Notes*
 159. *References*
 160. *Appendix*
 161. *Index*
 162. *Table of Contents*
 163. *Abstract*
 164. *Keywords*
 165. *Summary*
 166. *Notes*
 167. *References*
 168. *Appendix*
 169. *Index*
 170. *Table of Contents*
 171. *Abstract*
 172. *Keywords*
 173. *Summary*
 174. *Notes*
 175. *References*
 176. *Appendix*
 177. *Index*
 178. *Table of Contents*
 179. *Abstract*
 180. *Keywords*
 181. *Summary*
 182. *Notes*
 183. *References*
 184. *Appendix*
 185. *Index*
 186. *Table of Contents*
 187. *Abstract*
 188. *Keywords*
 189. *Summary*
 190. *Notes*
 191. *References*
 192. *Appendix*
 193. *Index*
 194. *Table of Contents*
 195. *Abstract*
 196. *Keywords*
 197. *Summary*
 198. *Notes*
 199. *References*
 200. *Appendix*
 201. *Index*
 202. *Table of Contents*
 203. *Abstract*
 204. *Keywords*
 205. *Summary*
 206. *Notes*
 207. *References*
 208. *Appendix*
 209. *Index*
 210. *Table of Contents*
 211. *Abstract*
 212. *Keywords*
 213. *Summary*
 214. *Notes*
 215. *References*
 216. *Appendix*
 217. *Index*
 218. *Table of Contents*
 219. *Abstract*
 220. *Keywords*
 221. *Summary*
 222. *Notes*
 223. *References*
 224. *Appendix*
 225. *Index*
 226. *Table of Contents*
 227. *Abstract*
 228. *Keywords*
 229. *Summary*
 230. *Notes*
 231. *References*
 232. *Appendix*
 233. *Index*
 234. *Table of Contents*
 235. *Abstract*
 236. *Keywords*
 237. *Summary*
 238. *Notes*
 239. *References*
 240. *Appendix*
 241. *Index*
 242. *Table of Contents*
 243. *Abstract*
 244. *Keywords*
 245. *Summary*
 246. *Notes*
 247. *References*
 248. *Appendix*
 249. *Index*
 250. *Table of Contents*
 251. *Abstract*
 252. *Keywords*
 253. *Summary*
 254. *Notes*
 255. *References*
 256. *Appendix*
 257. *Index*

samples (n=20), the Aeliteflo™ and Concise™ sealants were applied to the occlusal surfaces of the posterior teeth. To determine how deep the lesions could be detected under the sealants, the mean reflectivity of the demineralized samples and the controls was calculated under different optical depths of sealant: 250, 500, 750 and 1000 µm. PS-OCT was also used to image an intense scattering surface of barium sulfate through 1.25 mm thick blocks of Concise™, Aeliteflo™, Herculite™, and Z-100™. Image analysis was performed using Matlab™ (Mathworks, Natick, MA) and Igor Pro™ (Wavemetrics Inc, Lake Oswego, OR).

3 RESULTS

3.1 Imaging Artificial Caries Progression on Occlusal Surfaces

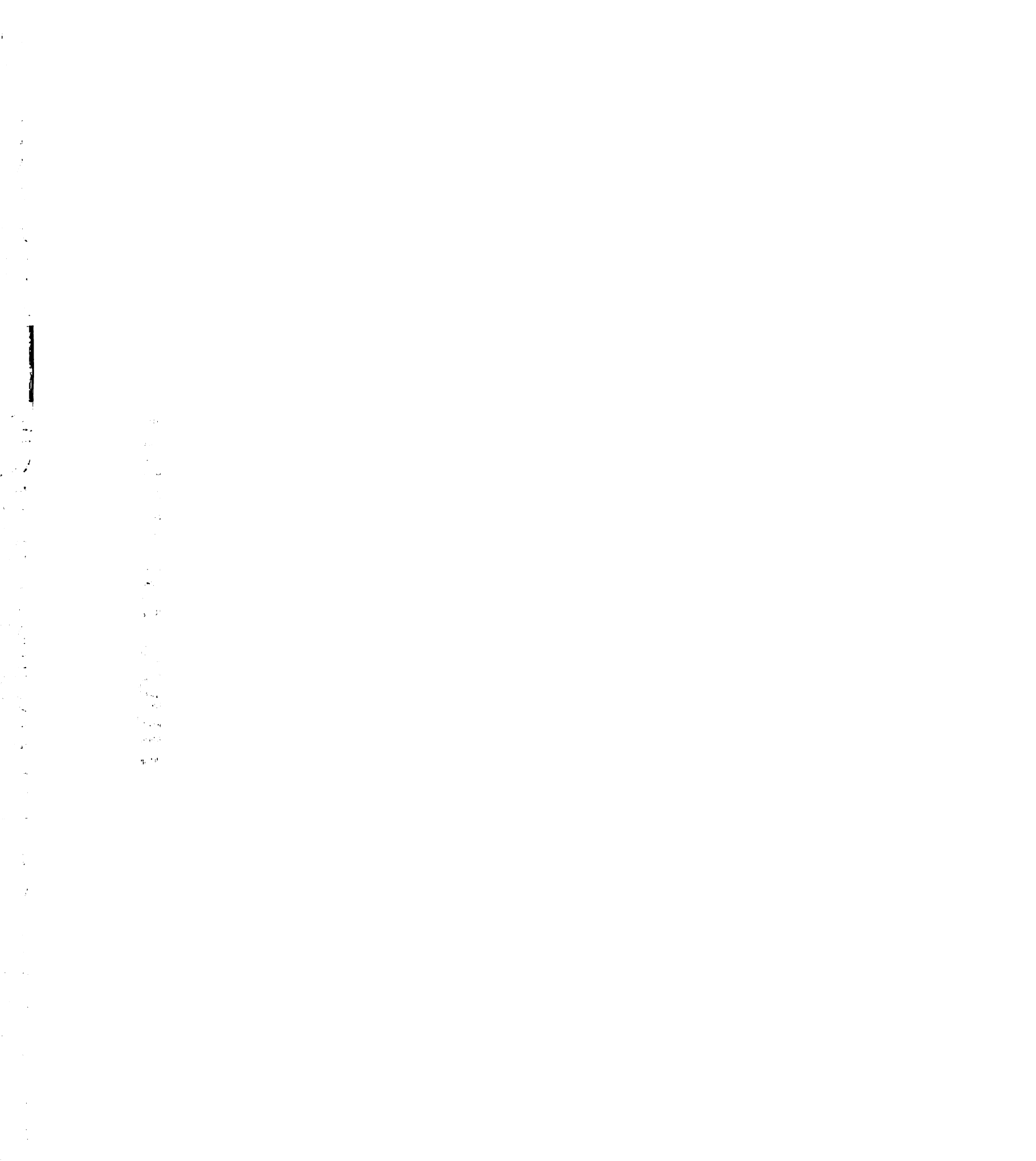
Initial, day 0, images of an occlusal surface illuminated with the 3.5 mW source are shown in Fig. 1. Incident light on a sample is linearly polarized, and the backscattered light was recorded in both the parallel and perpendicular axes to the original incidence. The parallel and perpendicular axes images are presented in a logarithmic grayscale, recorded in decibels (dB), where white represents high backscattering or reflection. Images of the dental enamel at day 0 constructed from the parallel-axis show an intense surface reflection at the enamel-air interface and 1-1.5 mm of depth penetration. Figure 2 (bottom-rows) shows the progression of the lesion severity in depth and reflectivity in the perpendicular-axis images. The parallel-axis image at day 14 (Fig. 1, top-row) reveals the higher reflectivity or scattering from the subsurface lesion but

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compared with perpendicular-axis images does not provide as high of contrast with day 0. The mean reflectivity was calculated for each time point in the perpendicular-axis images for all five samples illuminated with the 3.5 mW source. In the pH cycling model, the depth and severity of the lesion increases as the square root of the time;(Featherstone *et al.*, 1983; Fried *et al.*, 2002) therefore, the mean reflectivity $\pm$ s.d. was plotted versus square root of pH cycling time (Fig. 2). There was a linear increase in the mean reflectivity with the square root of time, $r=0.94$. This strong correlation indicates that the perpendicular-axis images represent the progressing severity of the artificial lesions. The mean reflectivity of the lesion became significant compared with day 0 after day 9 ($p<0.05$, Repeated-Measures ANOVA, Tukey-Kramer). The mean reflectivity increased from day 0 to day 14 was 5.1 dB ($p<0.01$), which is equivalent to a 3 times larger reflectivity intensity since decibels (dB) are log scale units of optical power. After sectioning the teeth, the samples under polarized light revealed that the real depth of the pH cycled artificial lesions were $\sim 100\ \mu\text{m}$, which validated that the $200\ \mu\text{m}$ optical depth integrations transverse the entire artificial lesions ($n=1.60$).

3.2 Imaging Artificial Caries Underneath Dental Sealants

PS-OCT b-scan images of the control samples with no occlusal decay are shown (Fig. 3). Concise™ sealant is placed over the occlusal surface, and from the parallel-axis image it is evident that the Concise™ sealant strongly backscatters the incident light. The backscattered light is also depolarized which is illustrated



by the high reflectivity of the sealant in the perpendicular-axis image. For the pH cycled samples, images of the artificial lesions on the occlusal surfaces were acquired (Fig. 5). After Concise™ sealant was placed over the occlusal surfaces, the PS-OCT b-scans were able to image the artificial lesions beneath the margin of the sealant but at a limited depth. Analyzing the perpendicular-axis images, the mean reflectivity of the enamel underneath 250, 500, 750 and 1000 µm of Concise™ sealant was calculated for the treatment and control samples (Fig. 7-top). The artificial lesions could be detected under 750 µm of the Concise™ sealant, with a 5.0 dB difference from the control samples (t-test, $p < 0.001$).

When examining the Aeliteflo™ samples, the PS-OCT b-scans of the control samples show an imaging penetration depth that was markedly greater than the Concise™ sealant (Fig. 4). This can be attributed to the weaker backscattering seen in the parallel-axis image. As indicated in the perpendicular-axis image, the Aeliteflo™ sealant does not significantly depolarize the incident light. For the pH cycled and control samples, the mean reflectivity of the enamel in the perpendicular-axis images was calculated underneath 250, 500, 750 and 1000 µm of Aeliteflo™ sealant (Fig. 7-bottom). The artificial lesions could be detected under the entire 1 mm of the Aeliteflo™ sealant (Fig. 6), with a 6.6 dB difference from the control samples (t-test, $p < 0.001$).

To examine the potential of imaging through composite restorative materials, PS-OCT b-scans were acquired through 1.25 mm thick sections of Concise™, Aeliteflo™ (A2, Bisco), Herculite™ (A2, Kerr), and Z-100™ (A3,3M)

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with an intense scattering surface underneath the materials. The scattering surface was resolved in both the parallel and perpendicular-axes images through all of the materials except the Concise™ sealant (Fig. 8).

4 DISCUSSION

This study demonstrates that PS-OCT can effectively monitor and quantify the artificial lesion progression on occlusal surfaces and image demineralized enamel under resin sealants. Initial studies have primarily studied the ability of OCT to image demineralization on bovine (Amaechi *et al.*, 2003) or human smooth surface enamel samples (Fried *et al.*, 2002). Although an important step in OCT development in dentistry, diagnosing smooth surface decay does not pose the same clinical challenges as detecting occlusal surface caries. Caries on the occlusal surfaces are also more prevalent in children and adults than on smooth surfaces. In addition, caries under dental sealants are a serious and widespread problem in dentistry, and this study demonstrates for the first time that decay under sealants can be quantified with OCT. This study further indicates the clinical utility of OCT, especially a polarization sensitive system.

By using linearly polarized incident light, PS-OCT can detect subsurface lesions by an increase in both backscattering and depolarization. Without a polarization sensitive system, the strong surface reflection, which also causes secondary coherence peaks, can both obscure and resemble the signal of subsurface demineralization of the lesion as illustrated in the parallel-axis images (Fig. 5-box). The signal in the perpendicular-axis is not sensitive to this surface

reflection because incident light is not depolarized upon surface reflection at normal incidence. In sound enamel, the images constructed from the perpendicular-axis are expected to be less intense than the parallel-axis, since the perpendicular-axis signal intensity arises from the phase retardation of the incident light by the enamel birefringence. As a caries lesion develops, the subsurface demineralized areas strongly scatter the incident light, contributing to a higher reflectivity signal in both axes. The strong scattering also causes rapid depolarization, which increases the signal in the perpendicular-axis to a greater degree than that caused by the sound enamel birefringence. Birefringence is particularly problematic in conventional OCT systems because it produces artifacts, such as banding, that confounds early caries diagnosis (Colston *et al.*, 1998).

This work demonstrates that occlusal lesion severity can be quantified by measuring the increased signal intensity of light depolarized into the perpendicular-axis. The enamel signal in the perpendicular-axis images can be directly integrated to determine the overall lesion severity. Other studies have reported that demineralization can be detected in a conventional OCT system by measuring the attenuation of the signal or reflectivity loss compared with the surrounding healthy tissue (Amaechi *et al.*, 2003). This approach assumes that imaging depths are similar throughout a b-scan. Imaging penetration and attenuation of a signal can be influenced by the varying angle of incidence seen when scanning occlusal surfaces. Polarization-sensitive OCT b-scans which are presented in total intensity and degree of polarization images have shown



improved contrast in detecting caries lesions than using only reflectivity (Baumgartner *et al.*, 1998). In healthy enamel, the degree of polarization, if measured as a difference between the two orthogonal axes divided by the total intensity, will alternate between -1 and 1 as the polarized light travels through the tissue. The birefringence of dental enamel is lost as it undergoes demineralization, and therefore, the degree of depolarization will be close to zero after it passes through the demineralization. Measuring the degree of depolarization gives better contrast between healthy and carious enamel than total intensity, but this method is also sensitive to the orientation of the incident light to the optical axis of the tissue. The PS-OCT system presented in this paper is affected by enamel birefringence that causes incident light to rotate into the perpendicular-axis. However, the contribution from the strong scattering of enamel demineralization in the perpendicular-axis signal is orders of magnitude greater than that caused by polarization rotation.

For the sealant samples, PS-OCT imaged the artificial caries in both polarization axes. This demonstrates that conventional OCT could detect the lesions by an increase in reflectivity, but it is important to note that the image would be sensitive to the marginal adaptation of the sealant to the underlying enamel. Defects in the contact of the sealant to the enamel would produce surface/air interfaces that could highly reflect the incident light. These reflections could only be discriminated with demineralization in a polarization sensitive system. PS-OCT can quantify the severity of a carious lesion under sealant restorations by directly integrating the backscattered perpendicular-axis signal in the enamel.

Sealant and composite materials that do not depolarize the incident light increase the diagnostic ability of PS-OCT to quantify the lesion severity. For the control samples, the surface acid etching needed for bonding did not produce noticeable backscattering signals, which indicates that the artificial lesions were not imaged only by surface scattering events. Intimate adaptation of the sealant to the etched enamel controls may have reduced the surface scattering and depolarization.

The lower imaging depth through Concise™ compared to Aeliteflo™ suggests that the visible light opacifier, titanium dioxide, which is found in Concise™, strongly attenuates even near-IR light (3M, 2003). Concise™ is produced in an opaque white shade because the conventional method for detecting caries underneath sealants is visible and tactile inspection. The opaque sealant is preferred by many clinicians since the margin can be easily differentiated from the enamel. The proprietary oxides that alters the Aeliteflo™ hue does not adversely affect imaging depth, nor does the increase in filler content of Herculite™ and Z-100™. Undoubtedly a future shift in diagnostic techniques will be accompanied with a change in material composition. Since Aeliteflo™, Herculite™, and Z-100™ are all used for larger cavity restorations in dentistry, this study illustrates the potential of PS-OCT to image and quantify enamel demineralization under sealant and composite restorations.

5 FIGURES AND FIGURE LEGEND

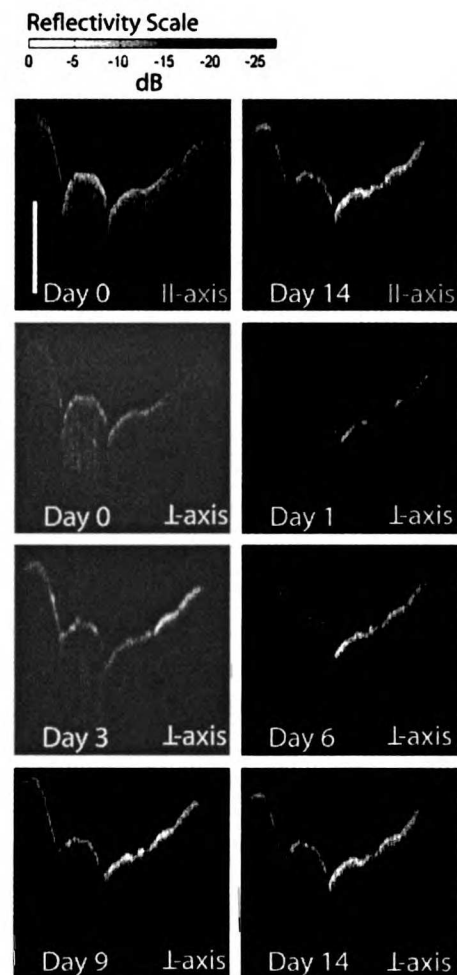


Fig. 1 PS-OCT images, illuminated with the 3.5 mW SLD, are viewed using a logarithmic grayscale measured in decibels. (Top-row) The reflectivity increase from day 0 to day 14 is difficult to quantify using the parallel ($\parallel$)-axis images due to the high surface reflection. (Bottom-rows) Perpendicular ($\perp$)-axis images at day 0,1,3,6,9,14 recorded the growing severity of the artificial lesions in both depth and reflectivity. The secondary coherence artifact or “ghost image” above the occlusal surface is caused by internal reflections within the optical fiber of the sample probe. The white bar is 1 mm of optical depth.



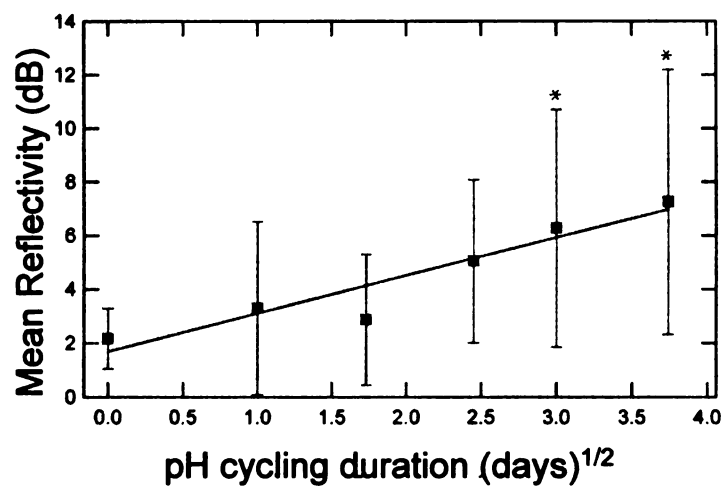


Fig. 2 The mean reflectivity (error bars = standard deviation) was plotted versus the square root of time for each of the illuminating sources. With the 3.5 mW 25 nm bandwidth SLD source, the mean reflectivity was plotted versus the square root of time and showed a linear relationship, $r=0.94$ ($n=5$).



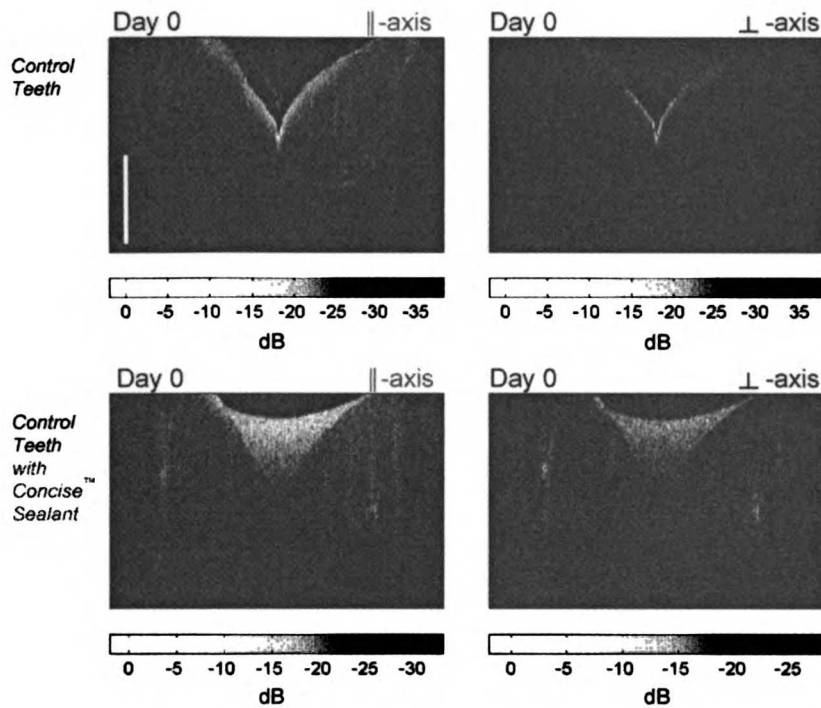
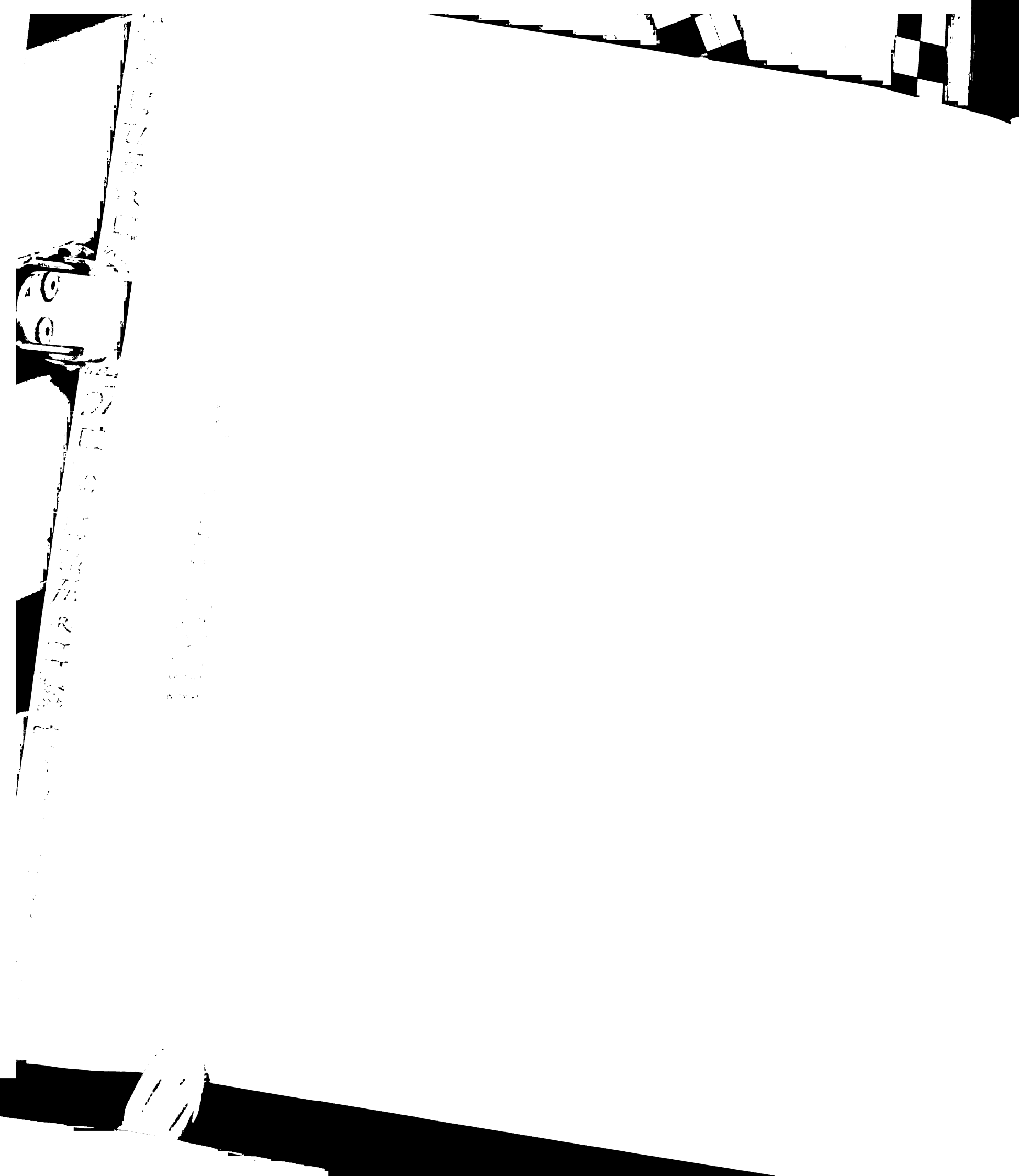


Fig. 3 (top) PS-OCT images of the control teeth, at day 0, are recorded in the parallel ($\parallel$) and perpendicular ($\perp$)-axes and were illuminated with the 20 mW SLD. (bottom) The images through Concise™ sealant show that the material highly reflects and depolarizes the incident light. Imaging penetration depth is limited. The white bar is 1-mm of optical depth.



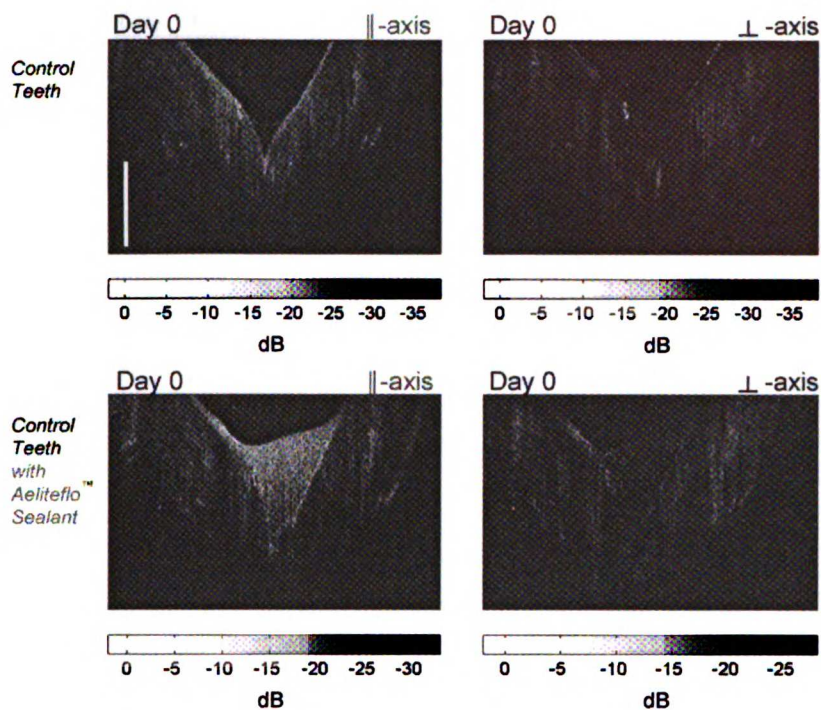


Fig. 4 (top) PS-OCT images of control teeth, at day 0, parallel (||) and perpendicular (⊥)-axes. (bottom) Images of the control teeth through Aeliteflo™ sealant show markedly higher imaging penetration depth and less reflectivity and depolarization than the Concise™ sealant in Fig. 4. The white bar is 1mm of optical depth.



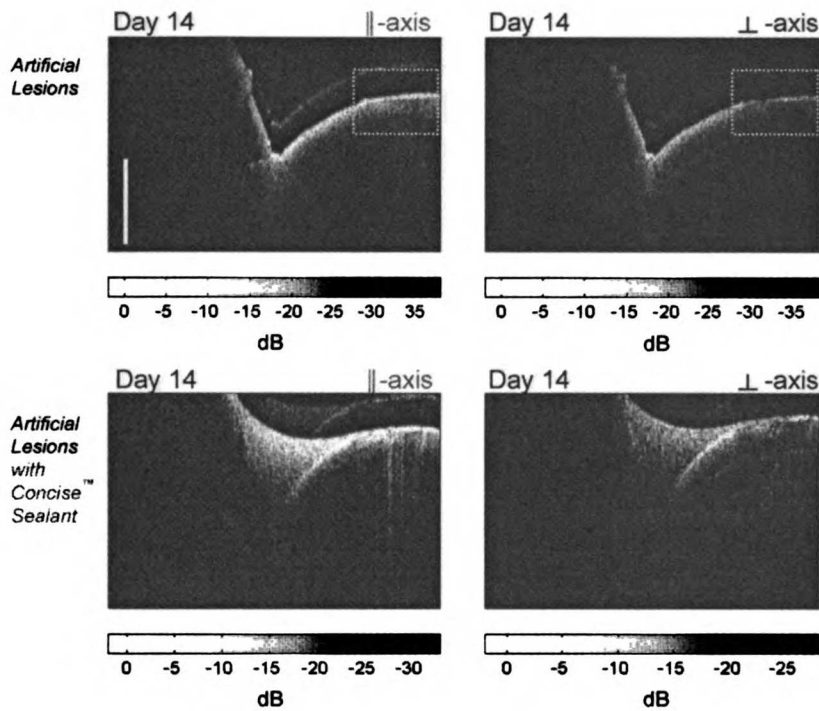
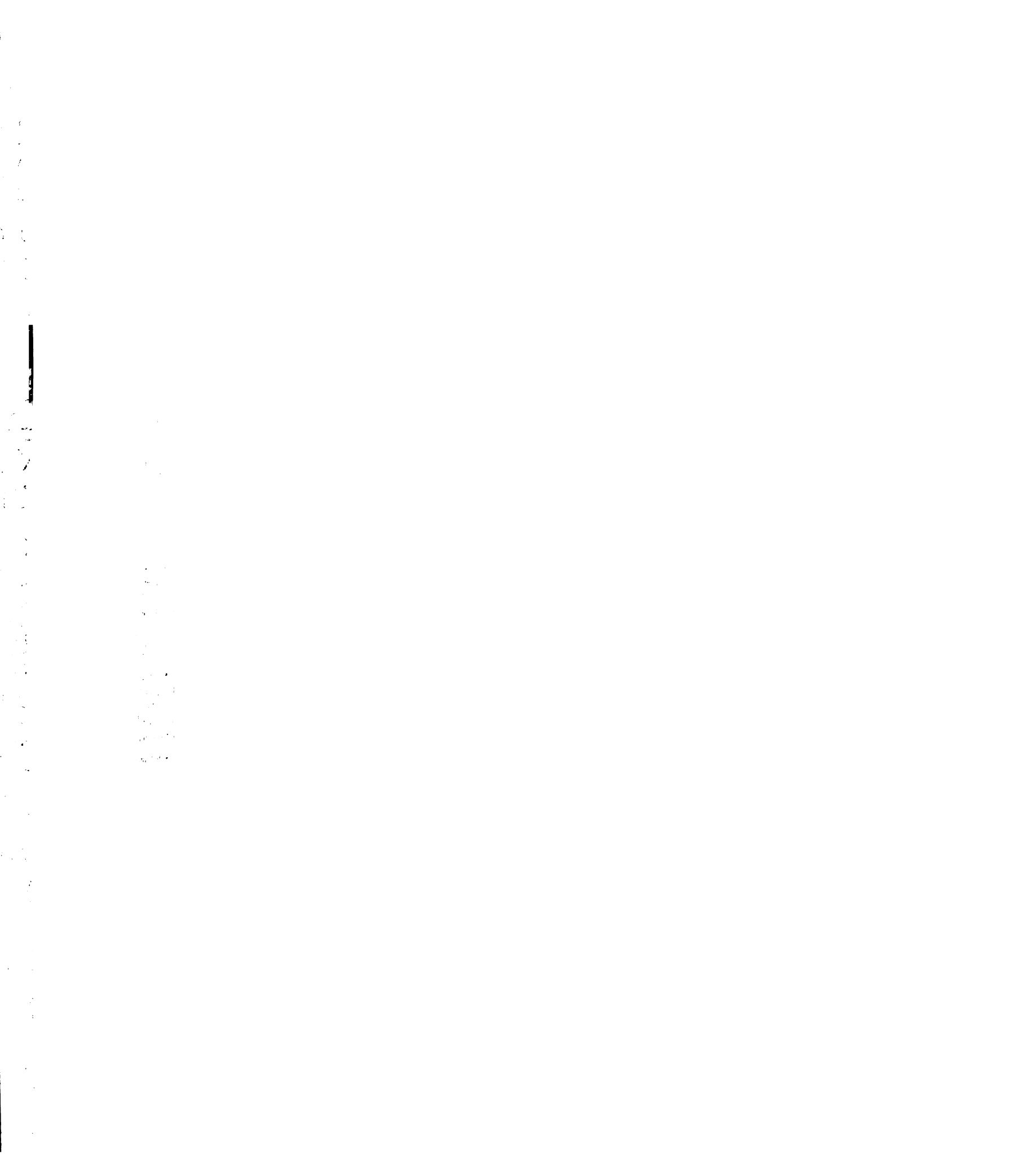


Fig. 5 (top) PS-OCT images of teeth that have artificial occlusal lesions from 14 day pH cycling. In the parallel ($\parallel$) axis, surface reflections (box) can mask the subsurface demineralization. The perpendicular ($\perp$)-axis records depolarization and the acid exposed lesion area is easily differentiated from the occlusal surface that was protected from demineralization. (bottom) PS-OCT is able to detect the artificial lesion beneath the margin of the Concise[™] sealant but at a limited depth.



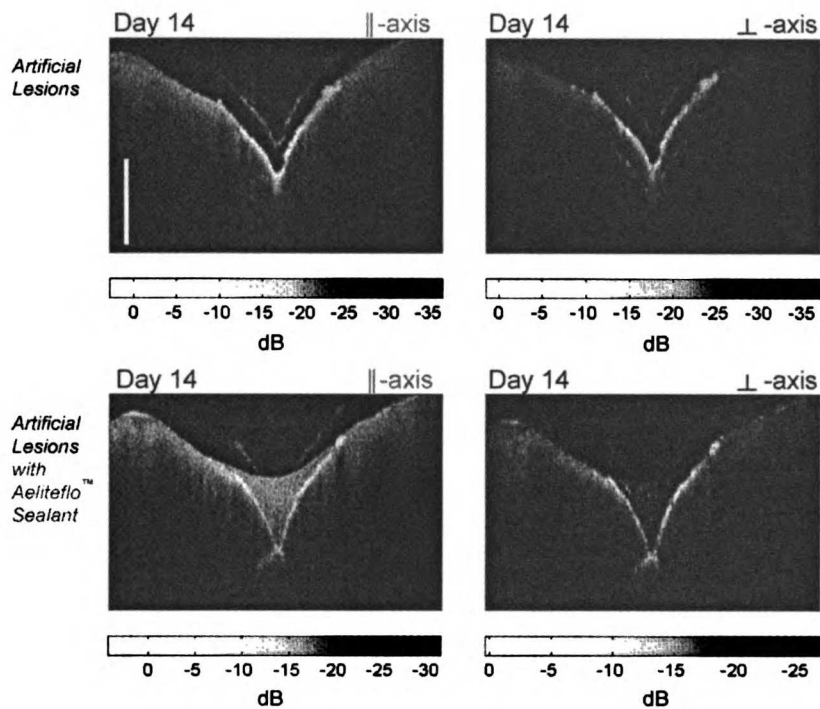


Fig. 6 (top) PS-OCT images of teeth that have artificial occlusal lesions from 14 day pH cycling. (bottom) The artificial lesions can be imaged through the full depth of the Aeliteflo™ sealant samples. Because of the limited reflectivity and minor depolarization of the Aeliteflo™ sealant, the measured lesion intensity is strong in both the parallel (||) and perpendicular (⊥)-axes.



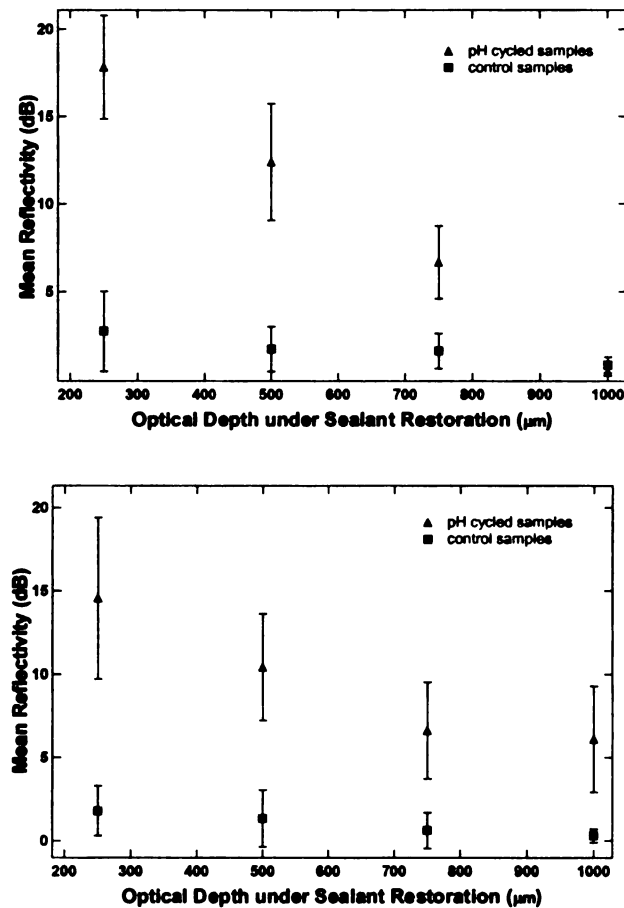


Fig. 7 The mean reflectivity of the artificial lesions and the controls under different optical depths of the sealants were calculated using the $\perp$ -axis image. In ($\perp$)-axis, the total reflectivity was integrated ~ 100 μm deep in the enamel and divided by the total pixels integrated. This mean reflectivity was calculated under different depths of the sealant. (top) For the Concise™ samples, the lesions could be imaged up to 750 μm deep with 5.0 dB difference from the control samples (t-test, $p < 0.001$). (bottom) For the Aeliteflo™ samples the artificial lesions could be detected under the full 1 mm depth of the sealant we placed, with a 6.6 dB difference from the control samples (t-test, $p < 0.001$).



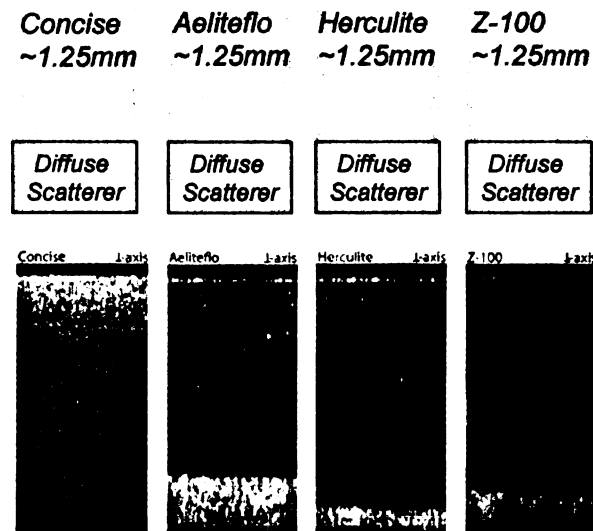
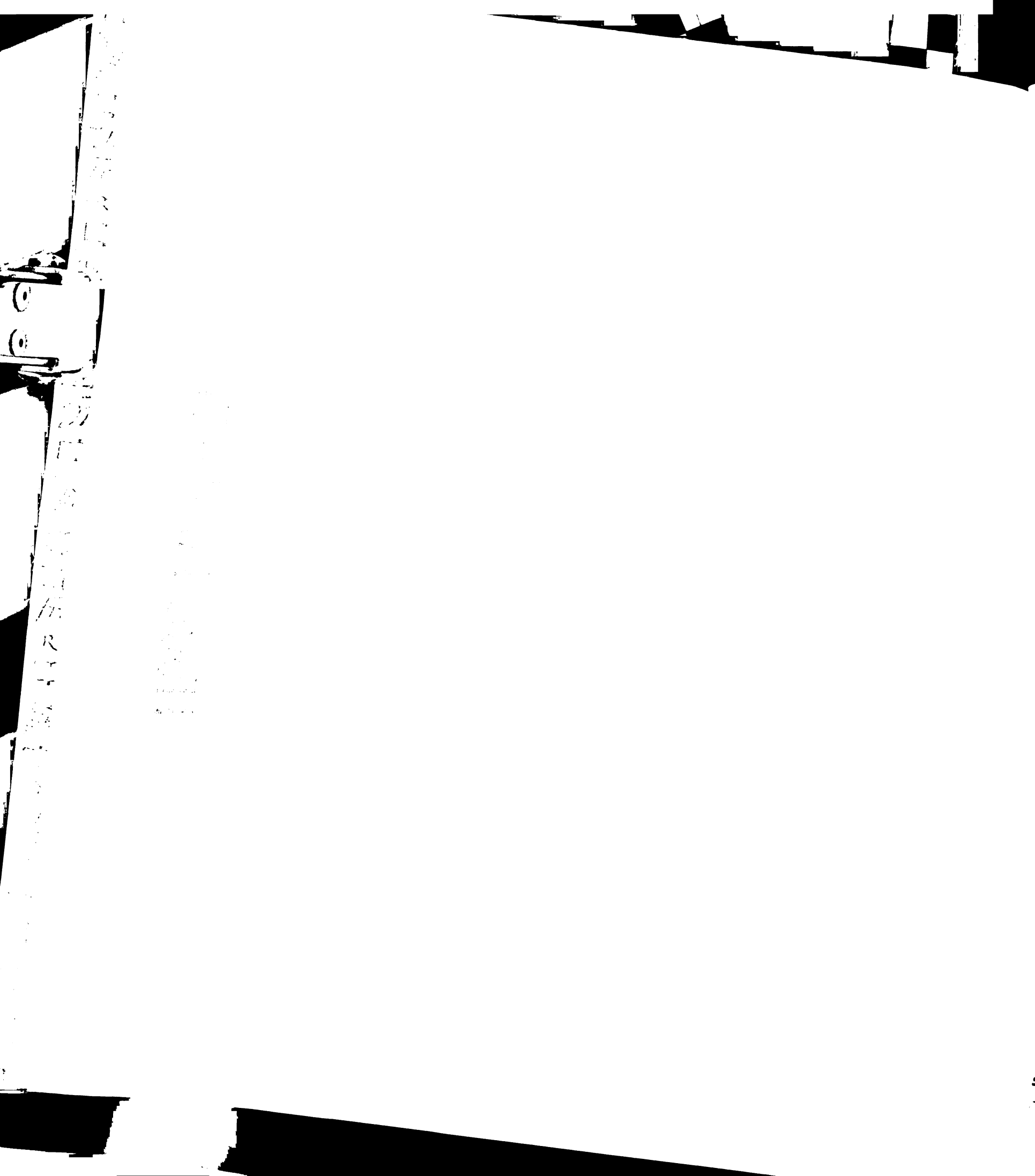


Fig. 8 (top) Approximately 1.25mm thick blocks of Concise™, Aeliteflo™, Herculite™, and Z-100™ were fabricated and an intense diffuse scattering surface of barium sulfate was placed underneath the composites. (bottom) PS-OCT images through each of the materials are shown in the perpendicular ($\perp$)-axis. The barium sulfate is revealed in all of the materials except the Concise™ sealant material.



6 REFERENCES

3M (2003). 3M MATERIAL SAFETY DATA SHEET 3M(TM) ESPE(TM) CONCISE(TM) WHITE SEALANT. St. Paul, MN: 3M.

Amaechi BT, Podoleanu A, Higham SM, Jackson DA (2003). Correlation of quantitative light-induced fluorescence and optical coherence tomography applied for detection and quantification of early dental caries. *Journal of Biomedical Optics* 8(4):642-647.

Baumgartner A, Hitzemberger CK, Dicht S, Sattmann H, Moritz A, Sperr W, Fercher AF (1998). Optical coherence tomography for dental structures. *Proc of SPIE, Lasers in Dentistry IV* 3248(1):130-136.

Bisco (2003). Aeliteflo/Aeliteflo LV Material Safety Data Sheet. Schaumburg, IL: Bisco, Inc.

Colston BW, Sathyam US, DaSilva LB, Everett MJ, Stroeve P (1998). Dental OCT. *Optics Express* 3(3):230-238.

de Boer J (2002). Polarization-Sensitive Optical Coherence Tomography: Introduction. In: Handbook of Optical Coherence Tomography. B Bouma and G Tearney editors. New York: Marcel Dekker AG, pp. 1-40.

Featherstone JDB, ten Cate JM, Shariati M, Arends J (1983). Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. *Caries Res.* 17(5):385-391.

Featherstone JDB, O'Reilly MM, Shariati M, Brugler S (1986). Enhancement of remineralization in *vitro* and in *vivo*. In: Factors Relating to Demineralization and Remineralization of the Teeth. SA Leach editor. Oxford, U.K: IRL Press.

Featherstone JDB, Glena R, Shariati M, Shields CP (1990). Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. *J Dent Res* 69(Spec No):620-625.

Feldchtein FI, Gelikonov GV, Gelikonov VM, Iksanov RR, Kuranov RV, Sergeev AM, Gladkova ND, Ourutina MN, Warren JA, Reitze DH (1998). In vivo OCT imaging of hard and soft tissue of the oral cavity. *Optics Express* 3(3):239-251.

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Fried D, Featherstone JDB, Glena RE, Seka W (1995). The nature of light scattering in dental enamel and dentin at visible and near-IR wavelengths. *Appl. Optics* 34(7):1278-1285.

Fried D, Xie J, Shafi S, Featherstone JDB, Breunig T, Lee CQ (2002). Early detection of dental caries and lesion progression with polarization sensitive optical coherence tomography. *J. Biomed. Optics* 7(4):618-627.

Harris N, Garcia-Godoy F (1999). Primary Preventive Dentistry. 5th ed. Stamford: Appleton & Lange.

Johnston TF, Sasnett MW (2004). Characterization of Laser Beams: The M^2 model. In: Handbook of Optical and Laser Scanning. GF Marshall editor. New York, Basel: Marcel Dekker, Inc, pp. 1-70.

Jones R, Fried D (2002). Attenuation of 1310 and 1550-nm Laser Light through Dental Enamel. *Proc of SPIE, Lasers in Dentistry VIII* 4610(1):187-190.

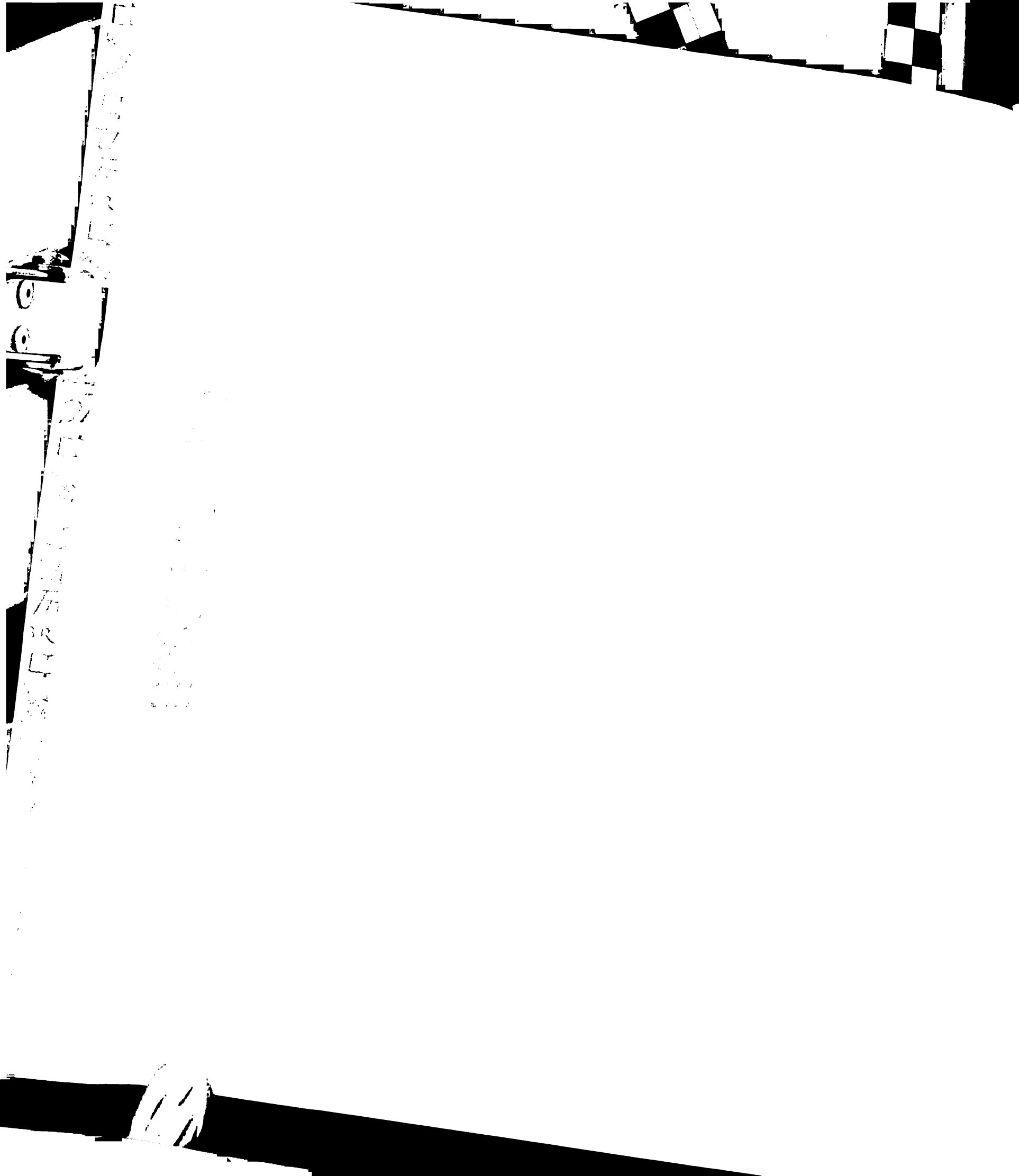
Jones RS, Huynh GD, Jones GC, Fried D (2003). Near-infrared transillumination at 1310-nm for the imaging of early dental decay. *Optics Express* 11(18):2259-2265.

Jones RS, Staninec M, Fried D (2004). Imaging secondary caries under composite sealants and restorations. *Proc of SPIE, Lasers in Dentistry X* 5313(1):7-16.

Kidd EA, Beighton D (1996). Prediction of secondary caries around tooth-colored restorations: a clinical and microbiological study. *J Dent Res* 75(12):1942-6.

Otis LL, al-Sadhan RI, Meiers J, Redford-Badwal D (2003). Identification of occlusal sealants using optical coherence tomography. *J Clin Dent* 14(1):7-10.

Schmitt JM (1999). Optical coherence tomography (OCT): a review. *IEEE Journal of Selected Topics in Quantum Electronics* 5(4):1205 - 1215.



White DJ, Featherstone JDB (1987). A longitudinal microhardness analysis of fluoride dentifrice effects on lesion progression *in vitro*. *Caries Res.* 21(6):502-512.



CHAPTER IV

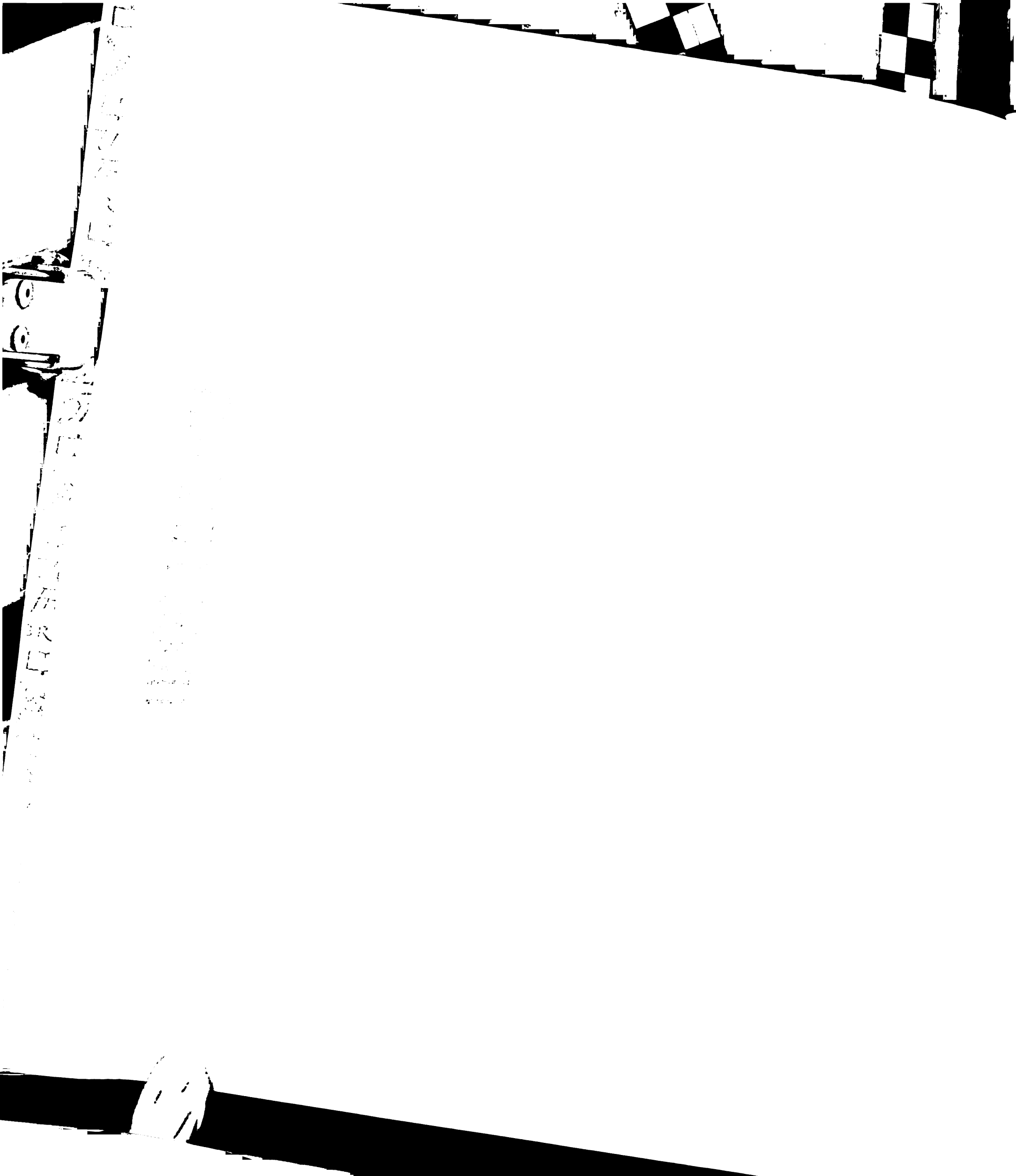
IMAGING ARTIFICIAL CARIES ON THE OCCLUSAL SURFACES WITH POLARIZATION SENSITIVE OPTICAL COHERENCE TOMOGRAPHY



1 INTRODUCTION

Conventional diagnostic tools for dental caries are not capable of detecting the early stages of decay, at which point, aggressive surgical treatment can be avoided and the early lesion can be arrested or reversed by fluoride therapy, anti-bacterial regimens, dietary changes, or by low intensity laser irradiation (Featherstone, 1999; Harris and Garcia-Godoy, 1999). The effectiveness of this non-surgical therapy is contingent on detecting decay in the outer enamel and requires imaging modalities that can safely and accurately monitor the success of such treatment. In order to monitor early dental decay and establish the type and degree of therapeutic intervention, the severity of a caries lesion needs to be determined.

Caries diagnosis and management requires more information than simply the detection of carious demineralization, which has been successfully demonstrated with intensity and degree of polarization images common in PS-OCT applications (Baumgartner *et al.*, 2000). A dentist needs to determine lesion severity in order to make the appropriate treatment decisions. In Chapter III, it was demonstrated that PS-OCT can image and quantify artificial caries progression on the occlusal surfaces of posterior teeth using a low power narrow bandwidth optical source with PS-OCT (Jones *et al.*, 2004a). An objective of this study is to use a higher power wider bandwidth source, which is expected to increase imaging depth and resolution, to track artificial caries on the occlusal surfaces. This will test the ability of the improved PS-OCT system to monitor the severity of the subsurface lesions, despite a possible increase in surface reflection and detector saturation.



Another objective of this work is to investigate the relationship between the magnitude of backscattered light and depolarization recorded by PS-OCT with changes in the volume % mineral of enamel upon demineralization. This is important for understanding the severity of caries lesions using PS-OCT imaging. In this study, an all fiber-based PS-OCT system operating at 1310 nm was used to image artificial lesions on the occlusal surfaces and compared to the quantitative mineral content profile and relative mineral loss, ΔZ (%vol x μm), obtained from high resolution Digital Microradiography (DM).

2 MATERIALS AND METHODS

2.1 Artificial Occlusal Caries

15 sound human posterior teeth were mounted on 12 mm³ acrylic blocks after root resection. Teeth were sterilized using gamma irradiation and stored in a moist environment to preserve tissue hydration with 0.1% thymol added to prevent bacterial growth. Teeth were not chemically or mechanically pretreated before the study. An acid resistant varnish was applied on the teeth on all areas outside the 3x2 mm pit and fissure region, which was scanned using the PS-OCT system. Artificial occlusal lesions on the teeth were formed using a well-characterized pH cycling model replicating the cycle of demineralization and remineralization that takes place naturally in the oral environment (Featherstone *et al.*, 1986; White and Featherstone, 1987). Teeth were exposed for 14 days to a daily regimen of 6 hrs demineralization and 17 hrs remineralization

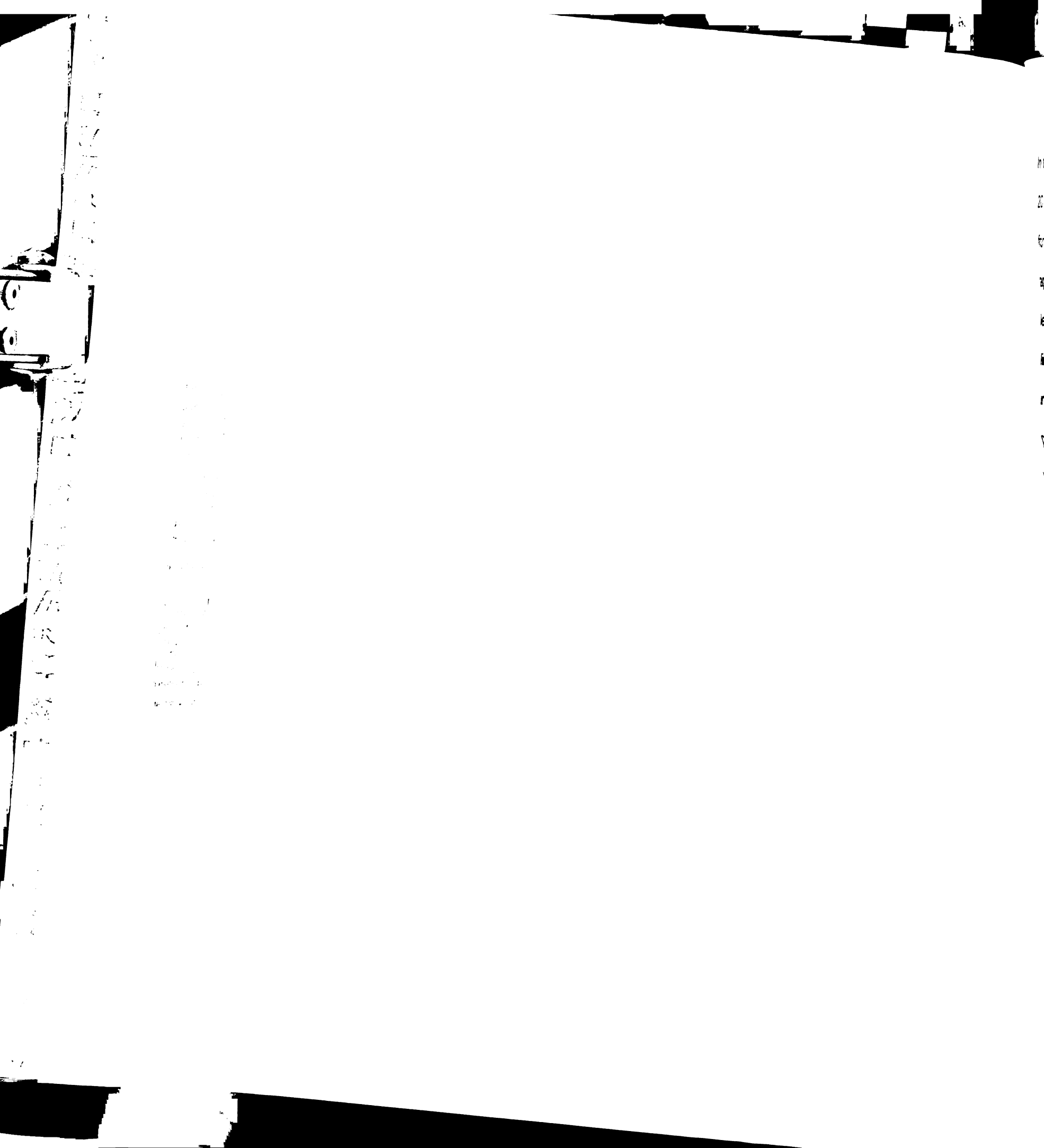


(Featherstone *et al.*, 1990). During demineralization, each tooth was exposed for 6 hours a day to a 40 mL aliquot buffer solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate and 0.075 mol/L acetate maintained at pH 4.3 and a temperature of 37°C. After demineralization, each tooth was immersed for 17 hrs in a 20 mL remineralization solution of 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L KCl, 20 mmol/L cacodylate buffer maintained at pH 7.0 and 37°C.

2.2 Polarization Sensitive Optical Coherence Tomographic Imaging

Two-dimensional OCT *b*-scans were obtained by collecting a series of depth resolved signals by laterally scanning (buccal-lingual) the beam across the moist tooth. The details of the PS-OCT system are presented in Chapter I. It is important to point out that samples were not pre-polished before the artificial lesions were created and an irregular pattern of lesions were created (Fig. 1). This made the acrylic blocks attached to each tooth were that allowed orientation and coordinates to be precisely replicated extremely necessary in order to take a specific topographic slice (Chapter V, Fig.1).

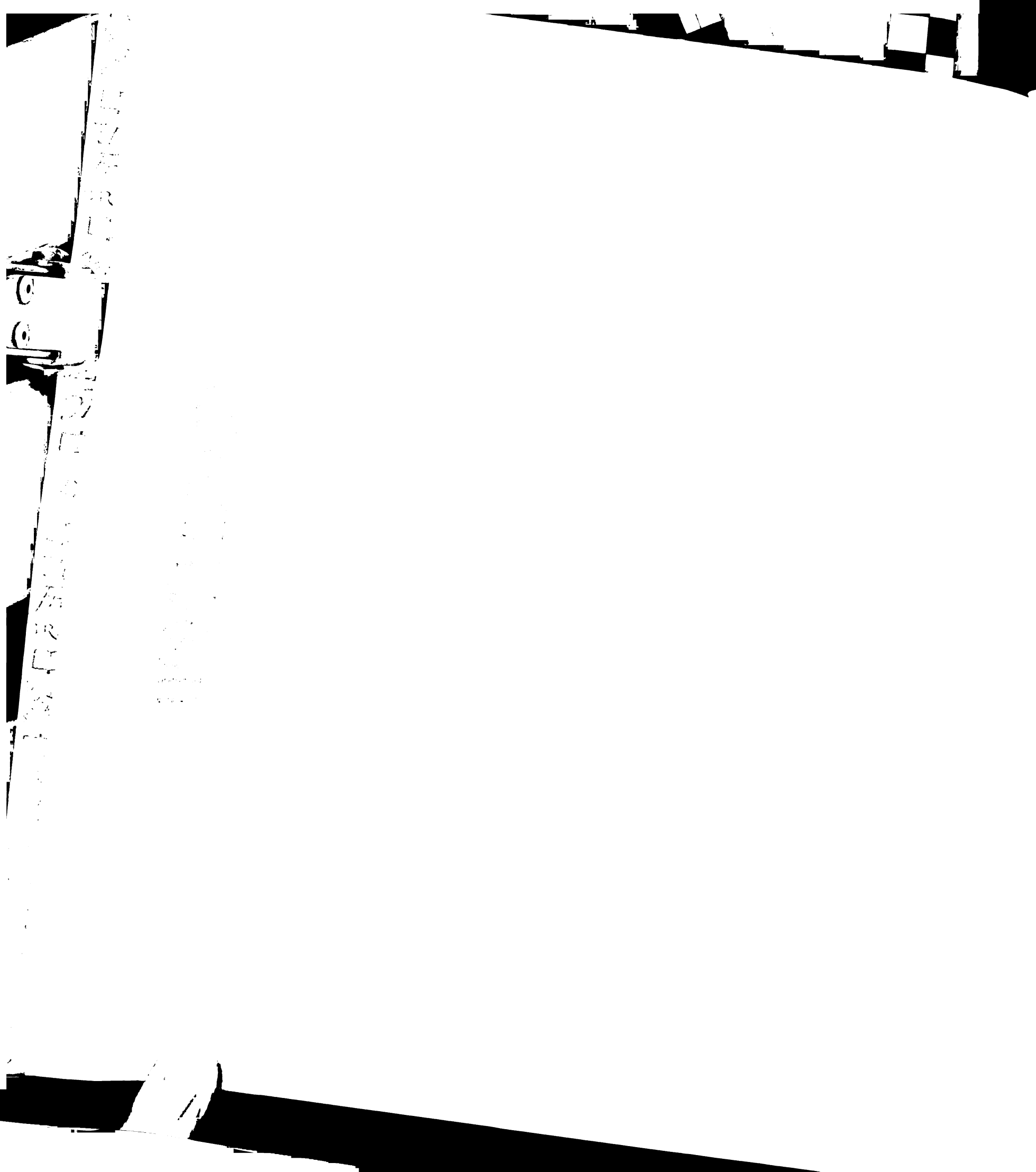
The PS-OCT *b*-scan images, using the 26 mW SOA diode source, were acquired at day 0,1,3,6,9,14 during the 14-day pH cycling regimen for 5 teeth. Using the perpendicular-axis images, the mean reflectivity of the enamel was determined by integrating the enamel 200-μm deep and dividing by the total pixels integrated. This was done for all the *a*-scan line profiles of the artificial caries, where an algorithm computed the mean reflectivity of the entire lesion.



In the other independent group, 10 teeth were scanned with PS-OCT, using the 20 mW SLD, at day 0 and 14. A total of 15 serial *b*-scan images were acquired for each tooth at 100 μ m intervals (distal-mesial), which encompassed approximately a 1.5 x 12-mm area of the occlusal surface (Fig. 1). The overall lesion severity of each sample at day 14 was quantified by simply integrating a line profile over the depth of each lesion from the perpendicular-axis image. This integration of the line profile, a summation of the logarithmic reflectivity (dB), is presented in arbitrary units (a.u.). Using the line profile of the lesion, the real lesion depth was determined by dividing the measured optical depth by the index of refraction of the enamel ($n = 1.6$). This refractive index correction is performed because the entire axial depth of each image is recorded in optical depth, which is a function of the real physical depth multiplied by the index of refraction of the tissue.

2.3 Histological Evaluation (Polarized Light Microscopy and Digital Microradiography)

After the PS-OCT scans of the 5 teeth monitored through the pH cycling regimen were completed, transverse thin sections were cut to approximately 100 μ m thickness using a hard tissue microtome. The tooth sections were examined under a polarized light microscope ($\times 100$, $NA = 0.25$) interfaced to a high resolution digital camera with Image Pro Plus image analysis software (Media Cybernetics, Silver Spring, MD) to compare the artificial caries depth with the PS-OCT perpendicular axis images.



For the other 10 samples, 200 μm thick transverse sections were cut using the microtome and a digital micrometer. Thicker samples were used for this group because a high success rate of sectioning was needed. No further polishing and finishing was performed to avoid changing the lesion mineral volume. Polarized light microscopy could not be used to properly assess lesion depth with the thicker samples but was useful ($\times 25$, $\text{NA} = 0.08$) for matching a specific section to one of the collected PS-OCT *b*-scan images.

Digital Microradiography (DM) was used to measure mineral loss or gain in the artificial caries lesions from each of the 10 transverse tooth sections. High-resolution microradiographs were taken using $\text{Cu K}\alpha$ radiation from a Philips 3100 X-ray generator. A Photonics Science FDI X-ray digital imager (Microphotonics, Allentown, PA) consisting of a 1392×1040 pixel interline CCD directly bonded to a coherent micro fiber-optic coupler that transfers the light from an optimized gadolinium oxysulphide scintillator to the CCD sensor was used to acquire the microradiographic images. Images were acquired in real-time at a frame rate of 10 fps. The image size was 2.99×2.24 mm with a pixel resolution of $2.15 \mu\text{m}$. The imaging system consisted of a high-speed motion control system with Newport UTM150 and 850G stages and an ESP300 controller coupled to a video microscopy and laser targeting system for precise positioning of the tooth sections in the field of view of the imaging system. The volume percent (%) mineral content of each section was determined by comparison with a calibration curve of x-ray intensity vs. sample thickness created using sound enamel sections of 86.3 ± 1.9 % volume percent mineral

varying from 50-300 μm in thickness. The calibration curve was validated via comparison with cross-sectional microhardness measurements. The volume % mineral determined using microradiography for section thickness ranging from 50-300- μm highly correlated with the volume percent mineral determined using microhardness ($R^2=0.99$).

From the DM technique, we obtained the quantitative mineral loss profiles (Angmar *et al.*, 1963). The relative mineral loss ($\text{vol}\% \times \mu\text{m}$), ΔZ , was calculated as the difference between the sound and carious profiles on the same sample. This method was valuable in correcting the edge effect seen in microradiography of thicker sections. Lesion depth was measured from 20% mineral volume at the enamel surface to approximately the 95% level of the sound enamel (Issa *et al.*, 2003). The mineral loss profiles were sampled from the selected region of the PS-OCT lineouts.

3 RESULTS

3.1 Artificial Occlusal Caries Progression

The mean reflectivity $\pm$ s.d. of the 5 samples was plotted versus the pH cycling time. From the PS-OCT perpendicular-axis images, the mean reflectivity increase from day 0 to day 14 was calculated to be 7.3 dB ($p < 0.001$, Repeated-Measures ANOVA, Tukey-Kramer) or 5 times larger, since dB is a logarithmic unit of optical power. The measured reflectivity of day 0 and day 1 may indicate that there was a lag period in the lesion progression. A visual observation of the

data plot signifies that the increase from day 1 to day 3 appears different than day 3 to day 14. Notably, the mean reflectivity of the lesion became significant compared with day 0 after day 3 ($p < 0.05$). Day 3 was also statistically different from day 14 ($p < 0.05$). Under polarized light microscopy, all five samples possessed substantial areas of simulated caries at least 100- μm deep.

3.2 Assessing Lesion Severity and Depth

PS-OCT *b*-scan images of the occlusal surface at day 0 and day 14 are shown in Fig. 3. The parallel-axis image at day 0 (Fig. 3a) shows the moderate intensity of backscattering that normally occurs in sound enamel as the linearly polarized light penetrates the tissue from 1 to 1.5 mm. The penetration depth varies with the topography of the tooth. Other parallel axis images (not shown) exhibited intense surface reflections. In the parallel-axis image of the occlusal region exposed to 14 days of pH cycling (Fig. 3b), the caries lesion can be differentiated from the adjacent protected sound enamel by the higher reflectivity. When examining the perpendicular-axis day 0 image (Fig. 3d), the sound enamel does not depolarize the incident linearly polarized light; however, there is a small reflectivity signal due to the native birefringence. This reflectivity is negligible when compared to the rapid depolarization caused by the artificial caries (Fig. 3e). The signal from the caries in the perpendicular-axis increases markedly from day 0 to day 14. The signal increase and the lesion depth profile is illustrated from lineouts extracted from the PS-OCT images (Fig. 3f). The lesion profiles from the parallel-axis are also displayed (Fig. 3c), illustrating that the

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image contrast between the sound (day 0) and the carious enamel (day 14) is less profound than in the perpendicular axis. Since sound tissue reflectivity in the perpendicular axis is minor, quantifying the lesion severity in the perpendicular axis is done by simply integrating the reflectivity of the day 14 lesion. This straightforward technique differs significantly to a subtraction based technique needed for the parallel-axis image where prior knowledge of the day 0 sound tissue reflectivity is needed.

Using line profiles from the perpendicular axis PS-OCT images of the 10 posterior teeth, the integrated reflectivity, quantifying lesion severity, and lesion depth of the artificial lesions were calculated, with a mean value of 450 a.u. $\pm$ 110 and 150 μm $\pm$ 30.

The high resolution digital micrographs obtained after tooth sectioning matched the PS-OCT scanned region, with polarized light aiding in matching DM sections with the PS-images (Fig. 4). The quantitative mineral content profiles were acquired (Fig. 5), and the relative mineral loss, ΔZ , was calculated for each sample. The mean ΔZ value was 4109 vol% $\times$ μm $\pm$ 2089, signifying the large variability in occlusal lesion severity. The mean lesion depth was 143 μm $\pm$ 27. The integrated reflectivity of the perpendicular-axis PS-OCT image, correlated to the ΔZ values ($r = 0.755$) of the artificial carious lesions (Fig. 6). There was also a strong correlation ($r = 0.811$) between the lesion depth calculated from both imaging modalities (Fig. 7).

4 DISCUSSION

1. The first part of the document is a list of names and addresses of the members of the committee who have been appointed to investigate the matter.

The results of this study suggest a number of advantages to employing PS-OCT for measuring demineralization over conventional OCT. Light scattering in the lesion area increases markedly with demineralization producing an increase in backscattering and depolarization of the scattered incident light, giving rise to the increase in signal in the perpendicular-axis of the PS-OCT system. This rise in signal in the perpendicular-axis enables straightforward integration of reflectivity in the perpendicular-axis image to easily quantify the severity of the occlusal lesion. The lesion severity can also be determined with a conventional OCT system by measuring the loss of penetration depth caused by increased attenuation due to demineralization (Amaechi *et al.*, 2003). However, this technique is limited to smooth surfaces with uniform penetration depths and it is difficult to implement in the occlusal surfaces due to varying optical penetration and surface reflectivity. Images of sound occlusal surfaces do not show uniform penetration, as represented in the parallel-axis images (Fig. 3a) because of the varying occlusal topography. The parallel-axis images are similar to images acquired with a conventional OCT system without polarization sensitivity since the reflectivity in that axis dominates the signal. One can also assess the lesion severity using only the parallel-axis image through image subtraction if the sound day 0 signal is known. Although the sound enamel signal at a specific point can be established on an extracted tooth, such an approach is impractical in most clinical situations. By integrating the perpendicular-axis PS-OCT image, we present a straightforward method for effectively quantifying caries lesions that is insensitive to the surface topography of the tooth.

PS-OCT is also insensitive to the confounding effects of the intense surface reflection, i.e., the Fresnel reflection at the surface of the tooth. Without a polarization sensitive system, the strong surface reflection can obscure the signal of subsurface carious demineralization (Fried *et al.*, 2002). The signal in the perpendicular-axis is not sensitive to this surface reflection because incident light is not depolarized upon surface reflection at normal incidence. In sound enamel, the images constructed from the perpendicular-axis are generally less intense than the parallel-axis, since the perpendicular-axis signal intensity arises from the phase retardation by the native enamel birefringence.

It is also important to note that the higher power optical sources used in this study did not present any confounding issues that interfered with the quality of the images, such as detector saturation or polarization cross-talk from strong surface reflection. Therefore, as higher power and wider bandwidth optical diode sources become available in the future, we expect better performance of PS-OCT, translating to deeper imaging depths of sound and carious enamel and increased image resolution of the system.

The longitudinal progression of the artificial lesions produced using the artificial caries model did not manifest the exact time dependence relationship found in other studies where, for instance, lesion depth was linearly dependent on the square-root (Featherstone and Rodgers, 1981; Poole *et al.*, 1981) or cube-root of time (Arends *et al.*, 1983; Christoffersen and Arends, 1982). The large deviation of the first time point may be attributed to a possible lag period in the measured reflectivity from day 0 to day 1. This lag period may be described

as the period of initial attack of the surface enamel before subsurface dissolution (Featherstone, 1984; Nieuw Amerongen *et al.*, 1987). These observations underscore the need to confirm the lesion severity measured by PS-OCT with the gold standard of mineral volume loss measured by microradiography.

It is encouraging that the correlation values for the lesion severity and depth were relatively high, even in light of the difficulty of precisely matching the PS-OCT and DM images. Artificial caries on the occlusal surfaces are typically highly varied in depth and severity, and the slice thickness of the PS-OCT image is 30 μm compared to the 200 μm section thickness in DM. This suggests that micron differences in section matching are a likely source of error in the correlation. The results of this study suggest that the severity of artificial lesions on the occlusal surfaces can be assessed with PS-OCT by either integrating the lesion profile or measuring the depth of the decay. We are careful to point out that the strong correlation between lesion severity and depth is only intrinsic to the particular artificial caries model employed in this study (Arends *et al.*, 1997). Optical attenuation measurements in our laboratory show that the magnitude of the scattering coefficient at 1310-nm increases by 2-3 orders of magnitude after demineralization providing high contrast (Darling and Fried, 2005; Huynh *et al.*, 2004); however, the degree of depolarization with scattering has yet to be established along with the precise relationship with volume % mineral loss. Therefore, the linear correlation observed for the model employed in this study may not apply to other artificial caries models or natural occlusal caries, since the optical properties may differ between the lesion types. The results of this study

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Figure 1

suggest that the approach taken utilizing PS-OCT for measuring lesion severity is applicable to shallow natural occlusal lesions; however, more work is needed to evaluate the diagnostic ability of PS-OCT to quantitatively assess the lesion severity and depth of deeper natural caries lesions within the narrow pit and fissures. The ability of PS-OCT to monitor occlusal enamel caries while delivering non-surgical therapy may also enable the *in vivo* testing of the effectiveness of different anti-caries agents without the necessity of waiting 2-3 years for cavitation to occur.

5 FIGURES AND FIGURE LEGEND

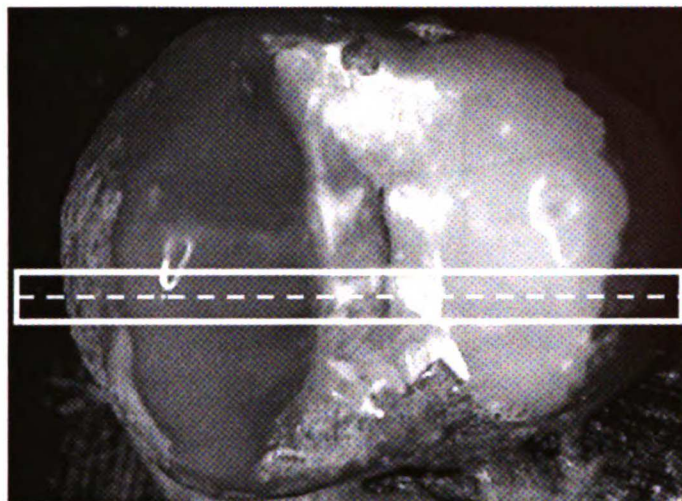


Fig. 1 Artificial caries were created on a select area, $\sim 3 \times 2 \text{ mm}^2$, of the occlusal surfaces. Sound enamel was protected by a nail polish varnish (left) that is removed (right) before PS-OCT imaging. Because the teeth were not pre-polished or treated, an irregular pattern of caries was observed (with minor leakage under the varnish near the marginal ridges). PS-OCT scans were performed within a uniform area of demineralization that also included the bordering sound enamel, which encompassed approximately a $1.5 \times 12\text{-mm}$ area (box). A total of 15 serial *b*-scan images were acquired for each tooth at $100 \text{ }\mu\text{m}$ intervals (distal-mesial). The path of a single *b*-scan is depicted (dashed line). Teeth are mounted on acrylic blocks that allowed reproducible imaging.

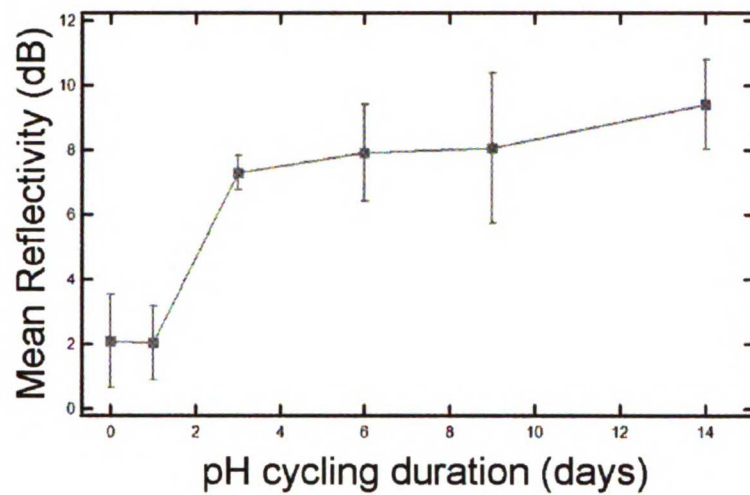


Fig. 2 The mean perpendicular-axis reflectivity was plotted versus pH cycling time for the artificial lesion progression on the occlusal surfaces. Error bars = standard deviation. The reflectivity was significantly different ($p < 0.05$) after 3 days, and then 14 days, of pH cycling.

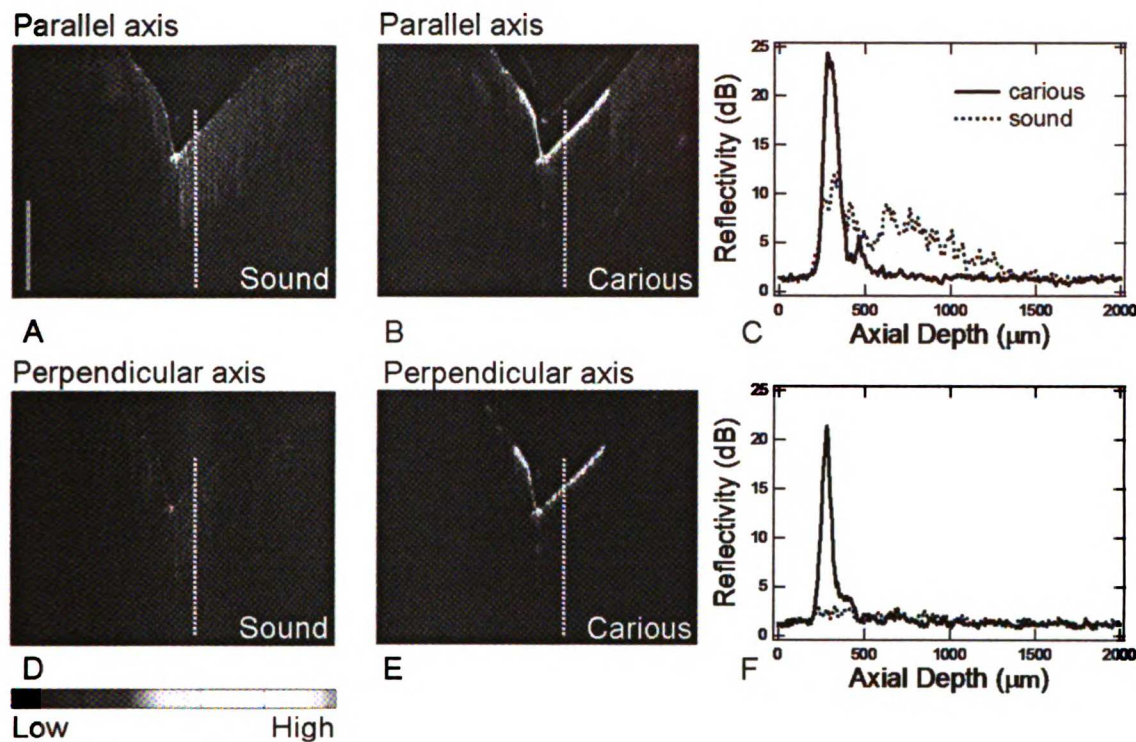


Fig. 3 PS-OCT *b*-scan images of a posterior tooth displayed in a false-color scale (bottom-left). This logarithmic scale was useful for discriminating areas of varying degrees of reflectivity. Parallel-axis images of the (A) sound (day 0) and (B) the 14 day pH cycled (cariou) tooth show an increase in backscattering of the area exposed to the acid attack. Perpendicular-axis images of the (D) sound and (E) cariou tooth show both the increase of backscattering and depolarization of the caries lesion. Depth profiles from the images of the sound and cariou tooth in both the (C) parallel and (F) perpendicular axes were extracted from the selected lineouts (white lines). The perpendicular-axis shows greater lesion contrast than the parallel-axis, and the lineout of the cariou region from (F) can be integrated to determine lesion severity. The blue scale bar in (A) is 1 mm of depth.

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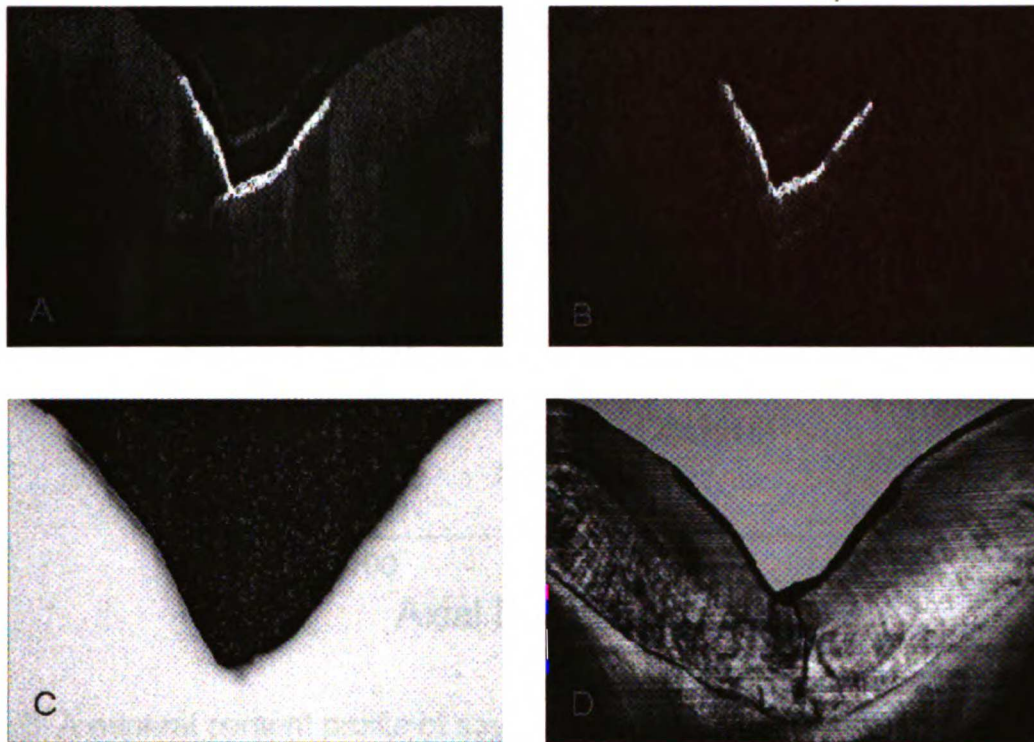


Fig. 4 PS-OCT images of the artificial occlusal caries in the two orthogonal polarization axes: (A) parallel and (B) perpendicular. Lesions are clearly represented in both axes and correspond to the transverse images of (C) Digital Microradiography (DM) and (D) polarized light (x25). Both PS-OCT and DM show the varying degree of severity of the artificial caries lesion, while polarized light only identifies the demineralized area.

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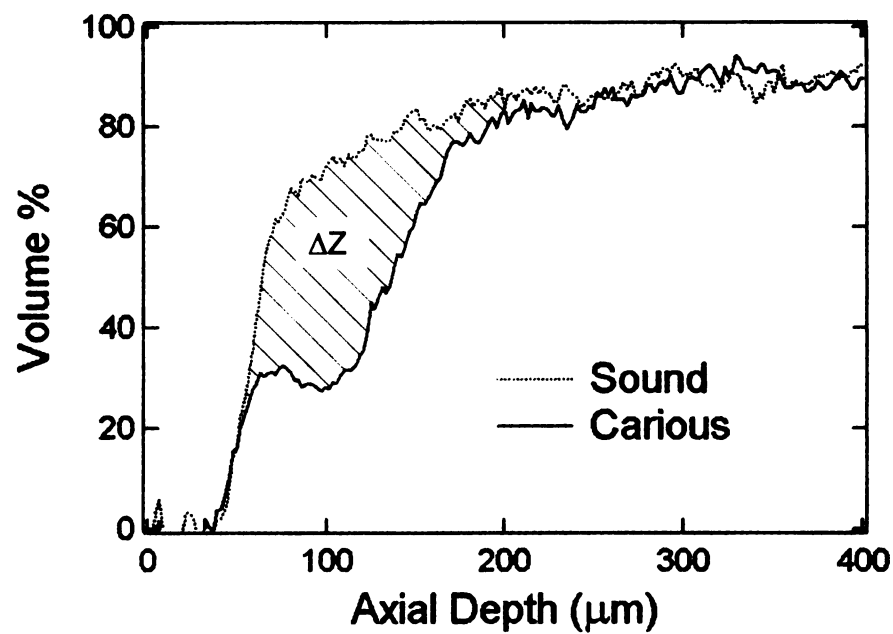


Fig. 5 A mineral content profile of sound and artificial occlusal carious enamel determined with digital microradiography. ΔZ was calculated as the difference between the sound and carious profiles on the same sample.

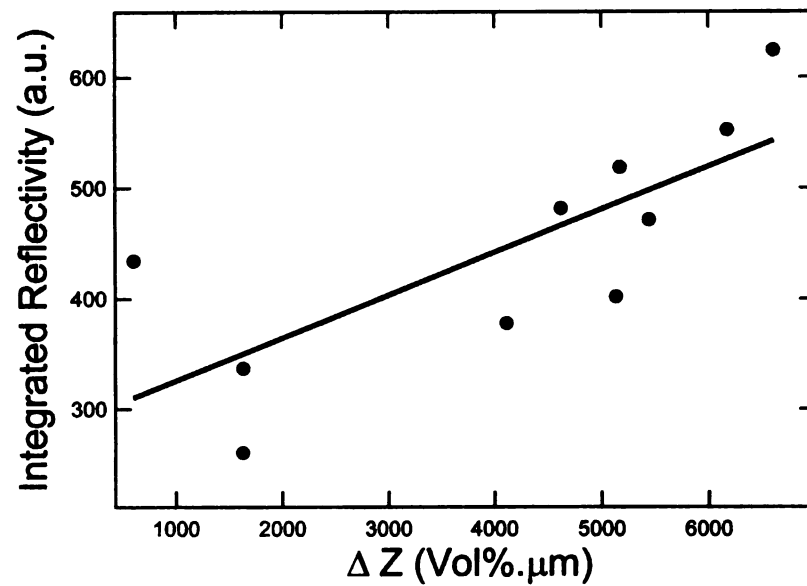


Fig. 6 Lesion Severity- The integrated reflectivity of the perpendicular axis PS-OCT image correlated to the ΔZ values ($r = 0.755$) of the artificial carious lesions.

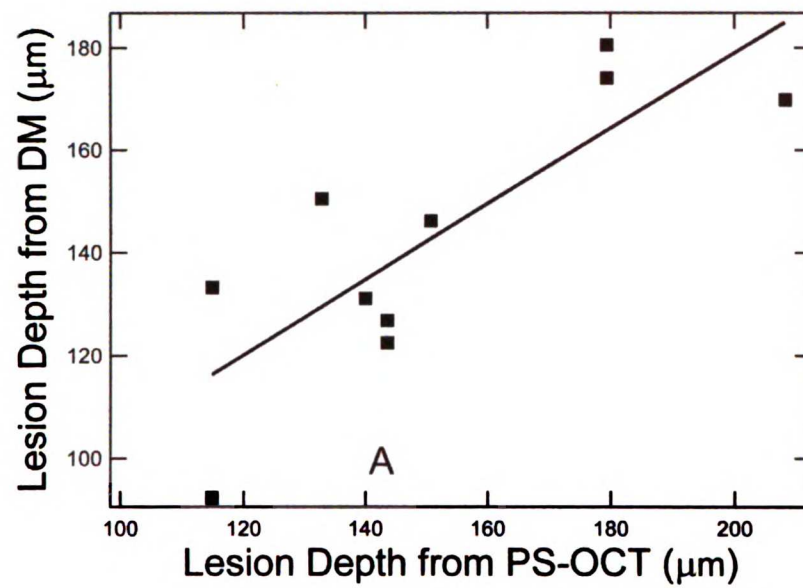


Fig. 7 Lesion Depth- There was a strong correlation ($r = 0.811$) between the lesion depth calculated from both digital microradiography (DM) and PS-OCT.

1. *Phragmites australis* (Cav.) Trin. ex Steud.

6 REFERENCES

- Amaechi BT, Higham SM, Podoleanu AG, Rogers JA, Jackson DA (2001). Use of optical coherence tomography for assessment of dental caries: quantitative procedure. *J Oral Rehabil* 28(12):1092-3.
- Amaechi BT, Podoleanu A, Higham SM, Jackson DA (2003). Correlation of quantitative light-induced fluorescence and optical coherence tomography applied for detection and quantification of early dental caries. *Journal of Biomedical Optics* 8(4):642-647.
- Angmar B, Carlstrom D, Glas JE (1963). Studies on the ultrastructure of dental enamel. IV. The mineralization of normal human enamel. *J Ultrastruct Res* 8(12-23).
- Arends J, Christoffersen J, Christoffersen MR, Schuthof J (1983). Influence of fluoride concentration on the progress of demineralization in bovine enamel at pH 4.5. *Caries Res* 17(5):455-7.
- Arends J, Ruben JL, Inaba D (1997). Major topics in quantitative microradiography of enamel and dentin: R parameter, mineral distribution visualization, and hyper-remineralization. *Adv Dent Res* 11(4):403-14.
- Baumgartner A, Hitzenberger CK, Dicht S, Sattmann H, Moritz A, Sperr W, Fercher AF (1998). Optical coherence tomography for dental structures. *Proc of SPIE, Lasers in Dentistry IV* 3248(1):130-136.
- Baumgartner A, Dichtl S, Hitzenberger CK, Sattmann H, Robl B, Moritz A, Fercher AF, Sperr W (2000). Polarization-sensitive optical coherence tomography of dental structures. *Caries Res*. 34(1):59-69.
- Christoffersen J, Arends J (1982). Progress of artificial carious lesions in enamel. *Caries Res* 16(6):433-9.
- Colston BW, Sathyam US, DaSilva LB, Everett MJ, Stroeve P, Otis LL (1998). Dental OCT. *Optics Express* 3(6):230-238.
- Darling CL, Fried D (2005). Optical properties of natural caries lesions in dental enamel at 1310-nm. *Proc. of SPIE, Lasers in Dentistry XI* 5687(1):102-110.

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The *Agrobacterium* strains were grown in YEA medium for 24 h at 28°C. The cell concentration of the strains was adjusted to 10⁸ cells/ml. The cell suspension was mixed with the plant tissue and the transformation efficiency was determined. The results were expressed as the mean ± SD of three independent experiments. The asterisks indicate significant differences between the strains at the same concentration of the cell suspension.

de Boer J (2002). Polarization-Sensitive Optical Coherence Tomography: Introduction. In: Handbook of Optical Coherence Tomography. B Bouma and G Tearney editors. New York: Marcel Dekker AG, pp. 1-40.

de Boer JF, Milner TE, vanGemert MJC, Nelson JS (1997). Two-dimensional birefringence imaging in biological tissue by polarization-sensitive optical coherence tomography. *Optics Letters* 22(12):934-936.

Everett MJ, Colston BW, Sathyam US, Silva LBD, Fried D, Featherstone JDB (1999). Non-invasive diagnosis of early caries with polarization sensitive optical coherence tomography (PS-OCT). *Proc of SPIE, Lasers in Dentistry V* 3593(1):177-183.

Featherstone JD, Rodgers BE (1981). Effect of acetic, lactic and other organic acids on the formation of artificial carious lesions. *Caries Res* 15(5):377-85.

Featherstone JDB (1984). Diffusion Phenomena and Enamel Caries. In: Cariology Today. Int. Congr., Zurich 1983. Basel: Karger, pp. 259-269.

Featherstone JDB, O'Reilly MM, Shariati M, Brugler S (1986). Enhancement of remineralization in *vitro* and in *vivo*. In: Factors Relating to Demineralization and Remineralization of the Teeth. SA Leach editor. Oxford, U.K: IRL Press.

Featherstone JDB, Glana R, Shariati M, Shields CP (1990). Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. *J Dent Res* 69(Spec No):620-625.

Featherstone JDB (1999). Prevention and reversal of dental caries: role of low level fluoride. *Community Dent Oral Epidemiol* 27(1):31-40.

Fercher AF, Hitzenberger CK, Drexler W, Kamp G, Sattmann H (1993). In vivo optical coherence tomography. *Am J Ophthalmol* 116(1):113-4.

Fried D, Featherstone JDB, Glana RE, Seka W (1995). The nature of light scattering in dental enamel and dentin at visible and near-IR wavelengths. *Appl. Optics* 34(7):1278-1285.

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The concentration of the *Agrobacterium* suspension was 10⁶ cells/ml (○), 10⁷ cells/ml (□), 10⁸ cells/ml (△), and 10⁹ cells/ml (◇). The error bars represent the standard deviation of three independent experiments.

Fried D, Xie J, Shafi S, Featherstone JDB, Breunig T, Lee CQ (2002). Early detection of dental caries and lesion progression with polarization sensitive optical coherence tomography. *J. Biomed. Optics* 7(4):618-627.

Fujimoto JG, Boppart SA, Tearney GJ, Bouma BE, Pitris C, Brezinski ME (1999). High resolution in vivo intra-arterial imaging with optical coherence tomography. *Heart* 82(2):128-33.

Harris N, Garcia-Godoy F (1999). Primary Preventive Dentistry. 5th ed. Stamford: Appleton & Lange.

Hee MR, Huang D, Swanson EA, Fujimoto JG (1992). Polarization-Sensitive Low-Coherence Reflectometer for Birefringence Characterization and Ranging. *Journal of the Optical Society of America B-Optical Physics* 9(6):903-908.

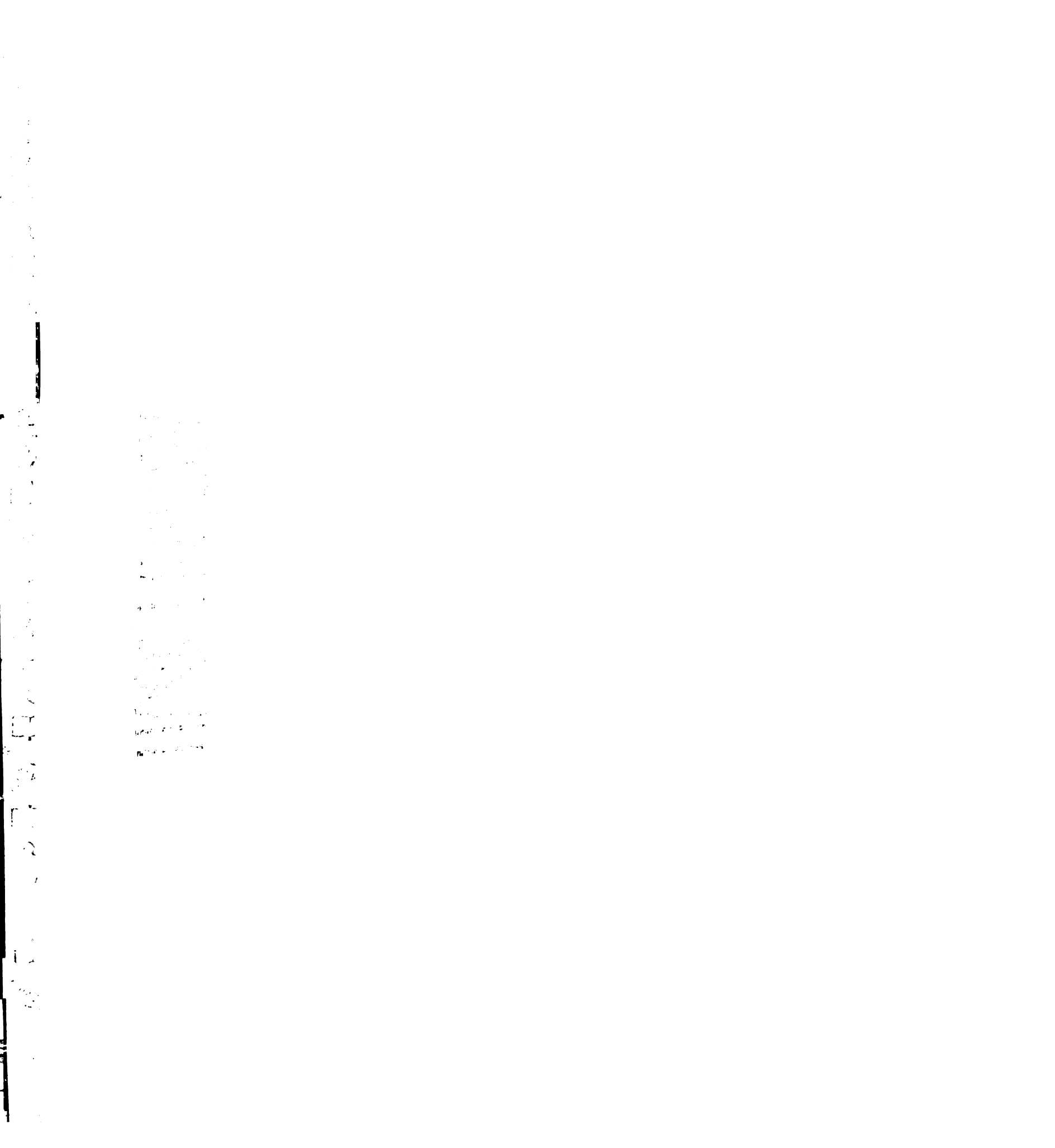
Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA, Fujimoto JG (1991). Optical Coherence Tomography. *Science* 254(1178-1181).

Huynh G, Darling CL, D. Fried (2004). Changes in the optical properties of dental enamel at 1310-nm after demineralization. *Proc of SPIE, Lasers in Dentistry X* 5313(1):118-124.

Issa AI, Preston KP, Preston AJ, Toumba KJ, Duggal MS (2003). A study investigating the formation of artificial sub-surface enamel caries-like lesions in deciduous and permanent teeth in the presence and absence of fluoride. *Arch Oral Biol* 48(8):567-71.

Izatt JA, Hee MR, Huang D, Fujimoto JG, Swanson EA, Lin CP, Schuman JS, Puliafito CA (1993). Optical Coherence Tomography for Medical Applications. In: Medical Optical Tomography: Functional Imaging and Monitoring. G Muller, B Chance, R Alfano, S Arridge, J Beuthan, E Gratton, M Kaschke, B Masters, S Svanberg and Pvd Zee editors. Bellingham, WA: SPIE, pp. 450-472.

Johnston TF, Sasnett MW (2004). Characterization of Laser Beams: The M^2 model. In: Handbook of Optical and Laser Scanning. GF Marshall editor. New York, Basel: Marcel Dekker, Inc, pp. 1-70.



Jones R, Fried D (2002). Attenuation of 1310 and 1550-nm Laser Light through Dental Enamel. *Proc of SPIE, Lasers in Dentistry VIII* 4610(1):187-190.

Jones RS, Huynh GD, Jones GC, Fried D (2003). Near-infrared transillumination at 1310-nm for the imaging of early dental decay. *Optics Express* 11(18):2259-2265.

Jones RS, Staninec M, Fried D (2004a). Imaging artificial caries under composite sealants and restorations. *J Biomed Opt* 9(6):1297-304.

Jones RS, Staninec M, Fried D (2004b). Imaging secondary caries under composite sealants and restorations. *Proc of SPIE, Lasers in Dentistry X* 5313(1):7-16.

Nieuw Amerongen AV, Oderkerk CH, Driessen AA (1987). Role of mucins from human whole saliva in the protection of tooth enamel against demineralization in vitro. *Caries Res* 21(4):297-309.

Poole DF, Shellis RP, Tyler JE (1981). Rates of formation in vitro of dental caries-like enamel lesions in man and some non-human primates. *Arch Oral Biol* 26(5):413-7.

Schmitt JM (1999). Optical coherence tomography (OCT): a review. *IEEE Journal of Selected Topics in Quantum Electronics* 5(4):1205 - 1215.

Tearey GJ, Brezinski ME, Bouma BE, Boppart SA, Pitris C, Southern JF, Fujimoto JG (1997). In Vivo endoscopic Optical Biopsy with Optical Coherence Tomography. *Science* 276(2037-2039).

Wang XJ, Milner TE, de Boer JF, Zhang Y, Pashley DH, Nelson JS (1999). Characterization of dentin and enamel by use of optical coherence tomography. *Applied Optics* 38(10):2092-2096.

White DJ, Featherstone JDB (1987). A longitudinal microhardness analysis of fluoride dentifrice effects on lesion progression *in vitro*. *Caries Res.* 21(6):502-512.

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CHAPTER V

THE EFFECT OF HIGH REFRACTIVE INDEX LIQUIDS ON PS-OCT IMAGING OF DENTAL CARIES

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1 INTRODUCTION

Previous chapters have determined that PS-OCT is not significantly affected by the varied occlusal tomography of posterior teeth in the same manner as fluorescence based imaging. Quantitative Light-induced Fluorescence (QLF), which relies on a ratio of fluorescence loss to that of sound enamel, is strongly affected by the thickness and orientation of the enamel (Ando *et al.*, 2003; Ando *et al.*, 2004). Although enamel thickness does not mitigate the imaging abilities of PS-OCT, surface roughness and the angle of the enamel during scanning influences imaging depths. Optimizing depth of field issues from the angles of the occlusal surface may be accomplished by recent advances in dynamic forward imaging (Liu *et al.*, 2004), where the system optics can change the focal point during imaging; however, forward imaging methods will not overcome surface scattering that limit the penetration of the incident beam and high angle occlusal slopes that can limit the path of the returning backscattered signal.

An objective for this chapter is to investigate methods to optimize PS-OCT imaging depth in sound enamel, while maintaining the high lesion contrast in carious teeth. Several biocompatible agents such as dextran, propylene glycol, and glycerol have been shown to reduce random scattering in soft tissue (skin and GI mucosa) and increase signal penetration in OCT imaging (Brezinski *et al.*, 2001; Vargas *et al.*, 1999; Wang and Elder, 2002). For dental hard tissue, the hypothesis of this study was that topical application of high refractive index agents will increase PS-OCT imaging depth in sound enamel and augment image contrast between sound and carious tissue.

2 MATERIALS AND METHODS

2.1 Artificial and Natural Occlusal Caries

10 sound human posterior teeth were mounted on 12-mm³ acrylic blocks after root resection. Teeth were sterilized using gamma irradiation and stored in a moist environment to preserve tissue hydration with 0.1% thymol added to prevent bacterial growth. Teeth were not chemically or mechanically pretreated before the study. An acid resistant varnish was applied on the teeth on all areas outside the 3x2 mm pit and fissure region, which was scanned using the PS-OCT system. Artificial occlusal lesions on the teeth were formed using a well-characterized pH cycling model replicating the cycle of demineralization and remineralization that takes place naturally in the oral environment (Featherstone *et al.*, 1986; White and Featherstone, 1987). Teeth were exposed for 14-days to a daily regimen of 6 hrs demineralization and 17 hrs remineralization (Featherstone *et al.*, 1990). Teeth were exposed 6 hours each day to a demineralizing solution of 40 mL aliquots of a buffer solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate and 0.075 mol/L acetate maintained at pH 4.3 and a temperature of 37°C. After demineralization, teeth were immersed for 17 hrs in a 20 mL remineralization solution of 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L KCl, 20 mmol/L cacodylate buffer maintained at pH 7.0 and 37°C. 2 additional teeth with natural occlusal pit and fissure caries were used in this study.

10

The map shows the northern Adriatic coastline of Italy and Slovenia. Sampling stations are marked with numbers 1 through 10. Station 1 is near the Italian coast, while stations 2-10 are further out in the sea. The map includes latitude lines (45°N, 46°N) and longitude lines (13°E, 14°E). A scale bar indicates distances up to 100 km.

1. *Pharmaceutical industry* – The pharmaceutical industry is a major source of funding for research in the field of aging. The industry has a vested interest in developing new drugs and treatments for age-related diseases, and it often funds research that is likely to lead to the development of such products.

2.2 Polarization Sensitive Optical Coherence Tomographic Imaging

Two-dimensional OCT *b*-scans were obtained by collecting a series of depth resolved signals by laterally scanning (buccal-lingual) the beam across the moist tooth. The details of the PS-OCT system are presented in Chapter I. The PS-OCT system used the 50-nm bandwidth 20-mW super luminescent diode (SLD), (COVEGA Jessup, MD). For this study, the total buccal-lingual dimension scanned was 12-mm. And one *b*-scan was collected from for every sample under each treatment conditions. In order to obtain comparable tomographic images, acrylic blocks attached to each tooth fit into a custom metal jig that allowed orientation and coordinates to be precisely replicated, within 50 μm , for every scan (Fig.1). A jig mounted on a hard tissue microtome was fabricated with a digital micrometer to allow the corresponding OCT scan region to be sectioned for histology.

2.3 Index Matched Liquids and Image Analysis

The ten teeth exposed to the acid demineralization were scanned with PS-OCT after 14 days of pH cycling. The teeth were scanned after immediate treatment of the five agents listed (Table 1). Between each of the applications of the liquid agents a thorough rinse and drying step was performed. The order of the four agents, after the scan of the dried tooth sample, was alternated to test the rinsing steps and reduce any residual effects of the agents on the final data analysis.

Image penetration depth and lesion contrast were measured for all ten samples under the different treatment conditions.

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When analyzing the imaging depths, a-scan profiles of the sound enamel which bordered the lesion were taken from the parallel axis images. The penetration depth analysis was standardized and defined as the signal depth from the surface to a region where the signal dropped below a 2-dB level above the noise floor. Lesion contrast was defined as:

$$\text{Lesion Contrast} = \text{abs}(I_L - I_E) / I_L \quad (1)$$

Lesion contrast is the absolute value of the difference between the mean lesion intensity, I_L , and mean sound enamel intensity, I_E , divided by the mean lesion intensity, I_L . Lesion contrast is a ratio that varies from 0 (lowest) to 1 (highest). The mean intensities of the lesion and sound enamel were calculated from a sampling method of the entire lesion and surrounding sound enamel in the perpendicular axis image. To compare the different treatment effects on the imaging depths and lesion contrast, a repeated measures analysis of variance (ANOVA) was performed with a multiple comparisons test (Tukey) to identify possible treatment groups. A few selected teeth were not only imaged immediately after the application of the liquid agent, but also after 5 minutes to determine if lesion contrast changed with time.

The two natural occlusal caries samples were imaged with PS-OCT under moist conditions and immediately following application of propylene glycol. After PS-OCT imaging, the corresponding section of the tooth was cut into a ~200 μm transverse section and imaged with high resolution digital microradiography

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(DM). The DM technique, described in Chapter IV, was used to calculate the volume percent mineral of the transverse section and quantify the occlusal lesion severity.

3 RESULTS

3.1 Artificial Occlusal Lesions

The results for the image penetration depth and the lesion contrast are summarized in Table 2. For imaging depth, the application of each of the liquid agents provided a 350-470 μm , or a 40-55% increase in the imaging depth over the dried samples ($p < 0.01$). No statistical difference between the different liquid agents was found between the imaging depths. For lesion contrast, the application of water and propylene glycol significantly reduced ($p < 0.01$) the lesion contrast versus the air dried, glycerol, and index matched liquid treated samples. Glycerol and the non-biocompatible index matched liquid improved the imaging depth markedly in the parallel-axis images and produced equivalent high lesion contrast in the perpendicular axis.

Figure 2 shows the results of one of the posterior tooth samples under the different treatments conditions. The PS-OCT images are shown in a false color scale, which is helpful in distinguishing the large change (high dynamic range) in reflectivity, with low values shown in black and higher values in white and blue. In Fig. 2A, the artificial lesion is created on the region of sound enamel exposed to the 14 day pH cycling, which is illustrated in the box. The lesion in the dried

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sample highly scatters and depolarizes the incident polarized light into the perpendicular axis causing a much higher signal than the sound enamel. The border of the lesion is clearly resolved in the perpendicular axis; however, the parallel axis shows such a strong reflection in the sound enamel surface that the lesion boundary is difficult to distinguish. In Fig. 2B-C, the water and propylene glycol treated teeth show an increase imaging penetration depth in the sound enamel with reduced surface reflection/scattering, but a significant reduction in the lesion contrast. Glycerol and the index matched fluid (Fig. 2D-E) show an increase imaging penetration depth when compared to the dried sample and an equivalent lesion contrast. The V-shaped artifacts above the occlusal surfaces that are seen in some of the images are coherence artifacts generated within the system fiber optics in response to both the strong surface reflection and lesion scattering.

The temporal effect of the liquid agents on PS-OCT imaging is illustrated in Fig. 3. Fig. 3 (top) shows the PS-OCT images of an artificial lesion immediately following the application of glycerol. After glycerol is left on the tooth for approximately 5 minutes, the PS-OCT images show a reduction in the overall scattering, evident in the parallel and perpendicular axis, and depolarization of the lesion into the perpendicular axis (Fig. 3-bottom).

3.2 Natural Occlusal Caries

PS-OCT images of a natural occlusal pit and fissure lesion under moist conditions are shown (Fig. 4-top). When looking at the parallel and

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perpendicular axes images, the moist sample shows imaging penetration depths and lesion contrast in the range between that seen in the dry and water immersed artificial lesion samples. The natural lesion is clearly evident in the perpendicular axis with high contrast versus the sound enamel. The application of propylene glycol increases the imaging depth in the sound enamel and virtually eliminates the scattering and depolarization of the natural lesion (Fig. 4-bottom). With propylene glycol, the lesion becomes less visible. To assess the lesion severity, the volume percent mineral was calculated from DM after the tooth was sectioned. The DM image of the lesion is presented in Fig. 5. The lesion is fairly advanced with over 30% of the mineral volume lost and contains a remaining volume between 45-55%. The sound enamel was calculated to be ~85% mineral volume. In the DM image, the dark region near the DEJ shows the lesion caries had reached the dentin.

In another tooth (Fig. 6-top), the occlusal lesion is imaged in both the parallel and perpendicular axis under moist conditions. The application of propylene glycol may increase the imaging depth in the sound enamel, but the lesion scattering and depolarization remains relatively the same (Fig. 6-bottom). DM reveals that the occlusal lesion has only lost 15-20% mineral volume and the lesion has an intact surface layer (Fig. 7).

4 DISCUSSION

The penetration depth in PS-OCT imaging increases substantially when a liquid agent is applied to the entire occlusal surface. This effect appears to be

caused by the wetting effect of the different agents. This surface wetting reduces the surface scattering from the surface roughness that attenuates the incident beam and limits penetration depth. The surface scattering has been found to be orders of magnitude greater than the backscattering of the bulk enamel tissue (Huynh *et al.*, 2004). The convoluted topography of the posterior occlusal surfaces increases the attenuation caused by Fresnel surface reflection. The percentage of light reflected from the enamel surface increases as the angle of incidence of the light increases away from normal incidence. The PS-OCT beam illuminates the posterior surfaces at large angles because of the dramatic occlusal slopes near the pit and fissures. Application of the liquid agents on the occlusal surfaces, fills the fissure region with the liquid, and reduces the initial angle of incidence at the liquid interface toward normal incidence. As the beam propagates, the reflective interface of the liquids to the enamel will be less affected from the high angle slopes because the indexes of refractions of the water, propylene glycol, and glycerol (1.33-1.45) are closer to enamel (1.63). After the incident beam is backscattered at various depths in the tooth enamel, the returning light is less likely to be internally reflected with the application of a liquid agent. Because of the high refractive index mismatch of enamel and air for a dry tooth, light will internally reflect at the enamel-air interface at any angle equal or greater than 37.8° , which is termed the critical angle. Application of a water layer in the occlusal surfaces increases this critical angle to 54.7° between the enamel-liquid interface, with is further increased with higher refractive index liquids. By decreasing the surface scattering and reflection of the incident beam

and increasing the critical angle with a liquid layer applied to the occlusal surfaces, more light penetrates and exits the tooth enamel. This produces deeper PS-OCT imaging depths. Images in the PS-OCT perpendicular axis represent the increase in scattering and depolarization of both artificial and natural caries. The scattering and depolarization of the lesion compared with sound enamel is quite pronounced when the lesion is dry or moist. The index of refraction mismatch between the air filled pores of the lesion and the enamel may cause the increase in backscattering. The reduction of the lesion scattering by liquid agents seems to be dependent on the viscosity of the liquid used in this study, while the effect from the differences of refractive index requires further work. Low viscosity agents such as water and propylene glycol immediately reduced the lesion scattering and depolarization by diffusing, or imbibing, into the lesion. This may reduce the index of refraction mismatch between the enamel and the air filled lesion pores. The liquid penetration is likely caused by a capillary effect from the lesion pores. The capillary effect has been observed in other studies examining fluorescent dye uptake by enamel lesions (Rawls and Owen, 1978; Van de Rijke and Ten Bosch, 1990), and dye penetration has been used to enhance caries detection in quantitative light-induced fluorescence (QLF) (Hall *et al.*, 1996). The high viscosity agent glycerol does not rapidly diffuse into the lesion; it takes a few minutes for the glycerol to reduce the lesion scattering and depolarization by imbibing into the lesion pores (Fig. 3).

Although the artificial lesions are shallow in depth, they are severely demineralized porous lesions. The artificial lesions and the natural lesion in Fig.

[illegible]

4 allow the diffusion of the liquids into the porosities. This reduces the lesion scattering and depolarization, which reduces the lesion contrast. However, the less severe natural lesion in Fig. 6&7, with only 15-20% mineral volume loss and an intact surface layer, is negligibly affected by the application of the low viscosity agent propylene glycol. The intact surface layer may prevent the diffusion of the liquid into the underlying demineralized lesion. This evidence suggests that the diffusion of liquid agents in PS-OCT can aid in determining the severity of a caries lesion. High viscosity agents, such as glycerol, improve the imaging depth in sound enamel with high lesion contrast and maybe beneficial in helping detect 'hidden' pit and fissure lesions. Since high viscosity agents do not diffuse rapidly into lesion pores, these liquids are limited in imaging the depth of larger lesions because the outermost surface of the decay will highly scatter and attenuate the light. Low viscosity agents, such as water and propylene glycol, may imbibe more readily into the pores of a larger lesion and allow the deeper structures to be imaged with PS-OCT by reducing the scattering of the outer parts of the lesion. The use of liquid agents with different viscosities in PS-OCT imaging may help determine the severity and depth of caries lesions in the occlusal pits and fissures.

5 FIGURES AND FIGURE LEGEND

Table 1 Treatments before each PS-OCT scans

Agent	Refractive index	Viscosity at 20°C (cP, centipoises)	Biocompatible
Air Dry (5 sec)	1.0	Not applicable	Yes
Water	1.33	1.00	Yes
Propylene Glycol (>99.5%)	1.43	45.66	Yes
Glycerol (>99%)	1.45	1194	Yes
Index Matched Cargille™ Fluid	1.63	2063 [†]	No

[†] viscosity at 25°C

Table 2 Imaging Depth and Lesion Contrast

Agent	Imaging Depth (optical depth μm)	Std Dev	Lesion Contrast	Std Dev
Air Dry (5 sec)	880	460	0.43 ^b	0.12
Water	1350 ^a	390	0.12 ^c	0.06
Propylene Glycol	1230 ^a	440	0.18 ^c	0.07
Glycerol	1290 ^a	435	0.45 ^b	0.12
Index Matched Cargille™ Fluid	1300 ^a	430	0.41 ^b	0.09

^{a,b,c} Values similarly marked were not significantly different (Tukey, >0.05)

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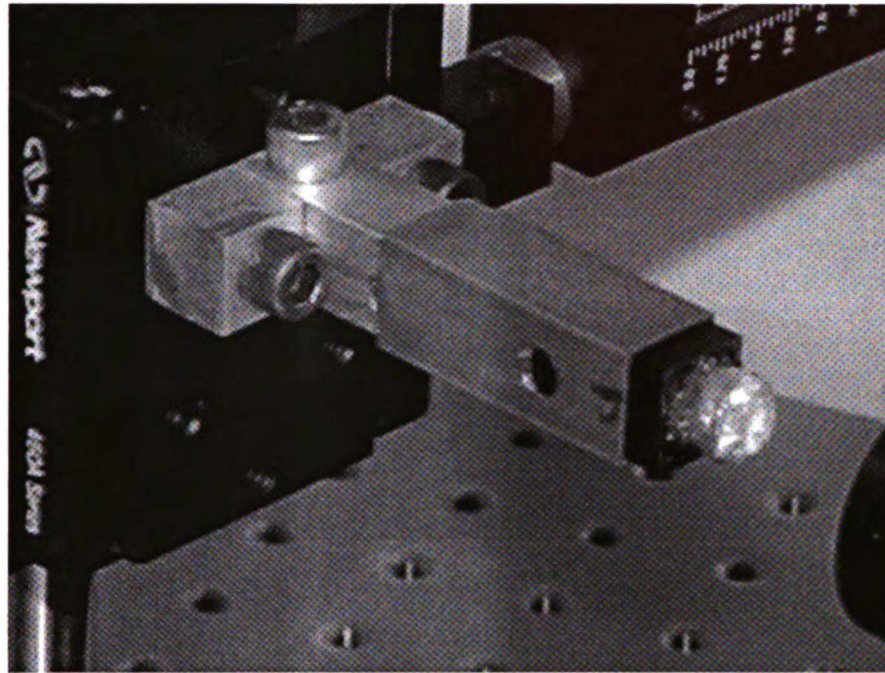


Fig. 1 Posterior teeth were mounted on 12-mm³ acrylic blocks after root resection. In order to obtain comparable tomographic images during repeated imaging with different topical agents, acrylic blocks attached to each tooth fit into a custom metal jig that allowed orientation and coordinates to be precisely replicated, within 50 μ m, for every scan. A similar jig mounted on a hard tissue microtome was fabricated with a digital micrometer to allow the corresponding OCT scan region to be sectioned for histology.

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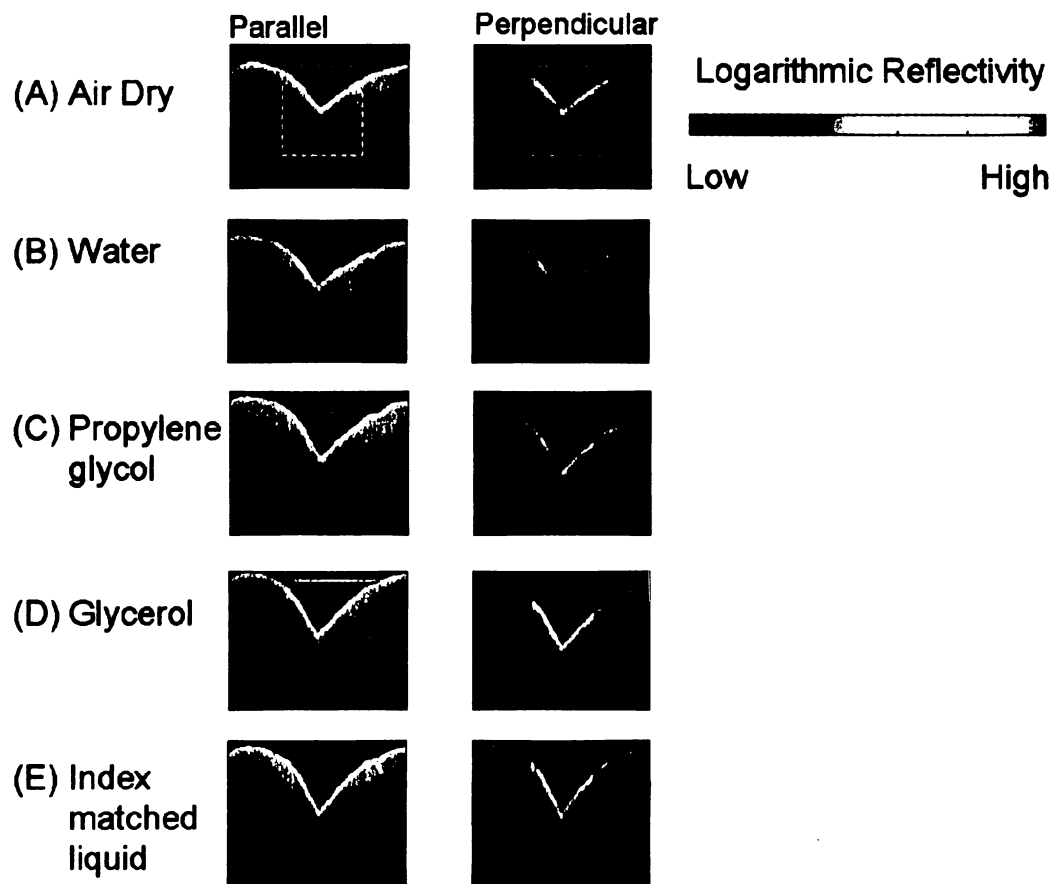


Fig. 2 PS-OCT parallel and perpendicular axis images of an artificial lesion on the occlusal surface of a posterior tooth are shown in a logarithmic false color scale. (A) Tooth imaged after a 5 sec air dry, with the box region highlighting where the lesion was created. The lesion highly scatters and depolarizes the incident light into the perpendicular axis. (B)-(C) Application of a water and propylene glycol layer increase imaging depths in the parallel axis, but reduced the lesion contrast in the perpendicular axis. (D)-(E) Glycerol and the Index matched liquid increase imaging depth compared to the dried tooth but maintain the high lesion contrast seen in the dried samples. The blue bar seen in the parallel axis in (A) is 1 mm of optical depth.

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The *Agrobacterium* strains were grown in the YEA medium for 24 h at 28°C. The cell concentration of the strains was adjusted to 1.0 × 10⁸ cells/ml. The cell suspension was mixed with the plant tissue and the transformation efficiency was determined. The results were expressed as the mean ± SD of three independent experiments. The asterisks indicate the significant difference between the strains at the same concentration of the cell suspension.

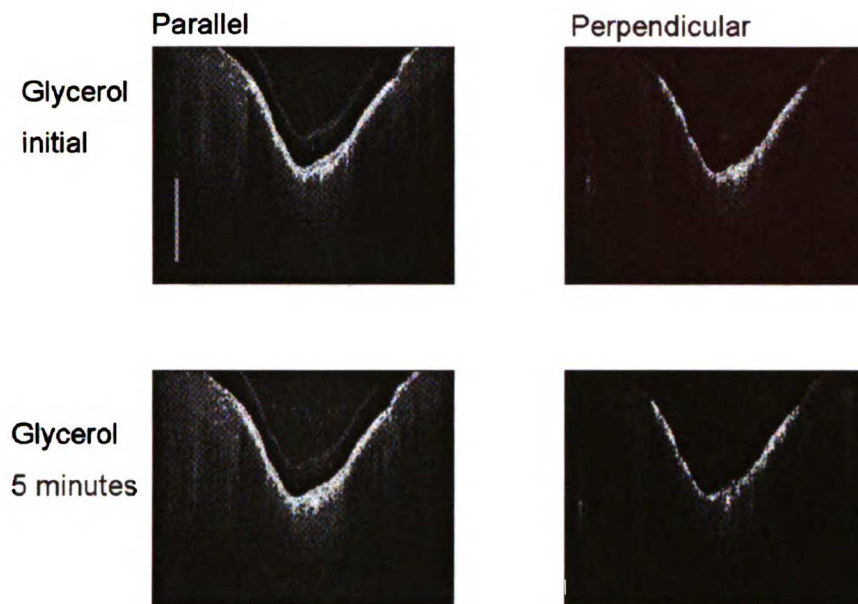


Fig. 3 PS-OCT images of the artificial occlusal caries in the two orthogonal polarization axes: (A) parallel and (B) perpendicular. Lesions are clearly represented in both axes and correspond to the transverse images of (C) Digital Microradiography (DM) and (D) polarized light (x25). Both PS-OCT and DM show the varying degree of severity of the artificial caries lesion, while polarized light only identifies the demineralized area.

the 1990s, the number of people in the world who are undernourished has declined from 1.1 billion to 800 million. The number of people who are malnourished has declined from 1.5 billion to 1 billion. The number of people who are obese has increased from 100 million to 300 million. The number of people who are overweight has increased from 100 million to 300 million. The number of people who are obese and overweight has increased from 100 million to 300 million. The number of people who are obese and overweight has increased from 100 million to 300 million.

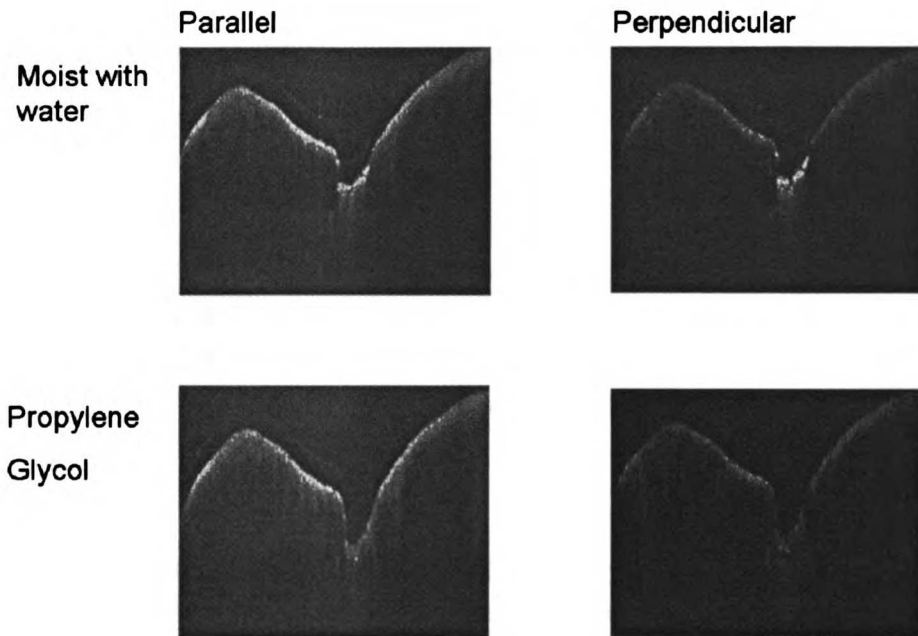


Fig. 4 Natural Occlusal Caries. (top) PS-OCT images show the high scattering and depolarization of a pit and fissure lesion under moist conditions. (bottom) The application of propylene glycol renders the lesion less visible by completely reducing the scattering of the demineralized lesion.

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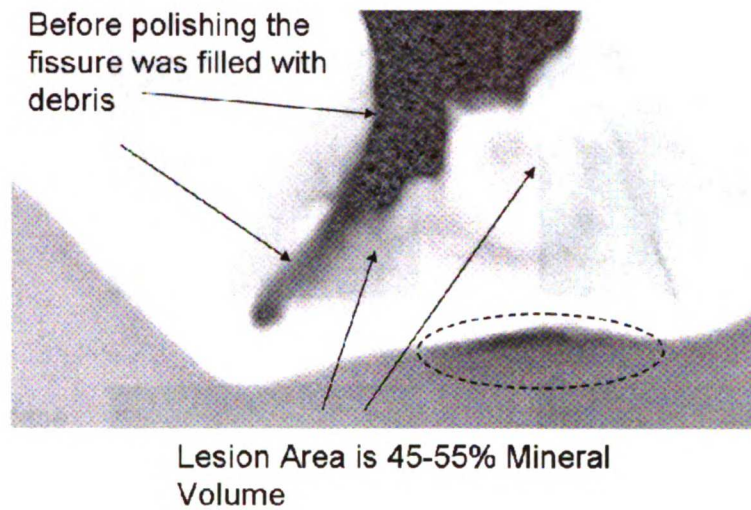


Fig. 5 Natural Occlusal Caries- After sectioning the tooth, digital microradiography was performed and the mineral volume % was calculated. DM shows high mineral volume in white and darker areas show a reduction in the volume. The lesion was quite advanced with a remaining mineral volume of 45-55%, and the dark area near the DEJ (marked) indicates that the decay had reached the dentin.

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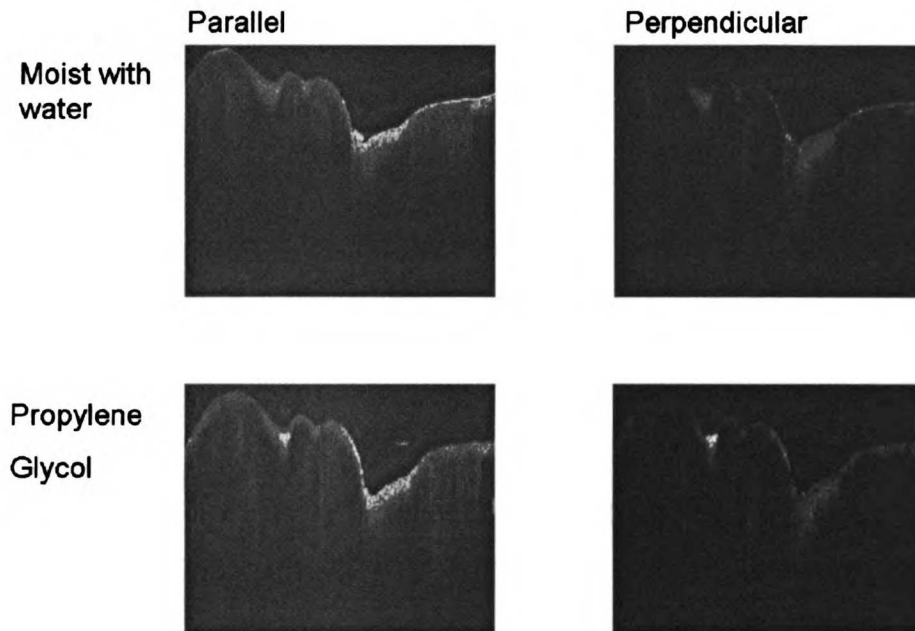


Fig. 6 Natural Occlusal Caries. (top) PS-OCT images show the higher scattering and depolarization of a pit and fissure lesion versus the sound enamel under moist conditions. (bottom) For this sample, the application of propylene glycol may increase the imaging depth but the lesion scattering and depolarization of the lesion appears unchanged.

1. *Chlorophyll a* (Chl *a*) is the primary photosynthetic pigment in most plants and algae. It is a green pigment that absorbs light energy in the blue and red regions of the visible spectrum.

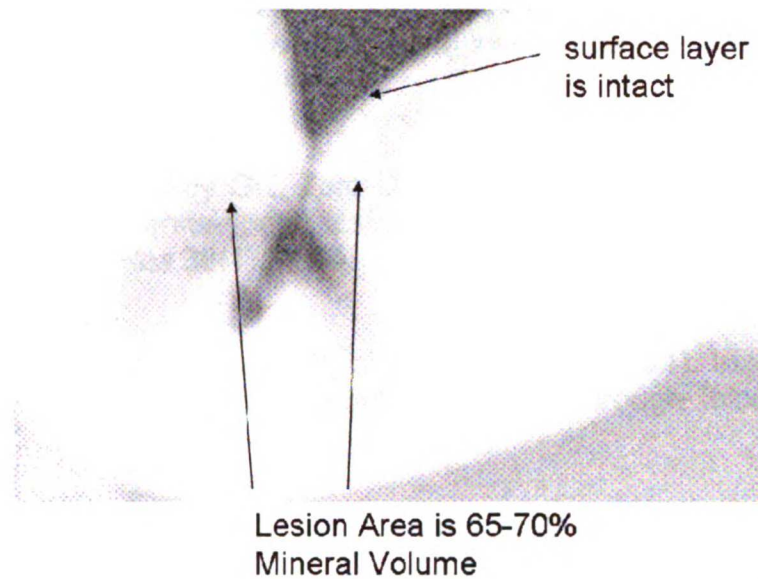


Fig. 7 Natural Occlusal Caries. Digital microradiography revealed that the lesion was less severe than the lesion seen in Fig. 5. The lesion had a remaining mineral volume of 65-70% and an intact surface layer. The sound enamel had a mineral volume of ~ 85%, and the dark mottled area within the fissure cavity is the background noise close to zero volume.

6 REFERENCES

Ando M, Schemehorn BR, Eckert GJ, Zero DT, Stookey GK (2003). Influence of enamel thickness on quantification of mineral loss in enamel using laser-induced fluorescence. *Caries Res* 37(1):24-8.

Ando M, Eckert G, Stookey GK, Zero D (2004). Effect of imaging geometry on evaluating natural white-spot lesions using quantitative light-induced fluorescence. *Caries Res* 38(1):39-44.

Brezinski M, Saunders K, Jesser C, Li X, Fujimoto J (2001). Index matching to improve optical coherence tomography imaging through blood. *Circulation* 103(15):1999-2003.

Featherstone JDB, O'Reilly MM, Shariati M, Brugler S (1986). Enhancement of remineralization in *vitro* and in *vivo*. In: Factors Relating to Demineralization and Remineralization of the Teeth. SA Leach editor. Oxford, U.K: IRL Press.

Featherstone JDB, Glena R, Shariati M, Shields CP (1990). Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. *J Dent Res* 69(Spec No):620-625.

Hall AF, Ando M, Schemehorn BR, Stookey GK (1996). Dye-Enhanced Laser Fluorescence Method. In: Early Detection of Dental Caries: proceedings of the 1st annual Indiana conference. GK Stookey editor. Indianapolis: Indiana University School of Dentistry, pp. 119-143.

Huynh G, Darling CL, D. Fried (2004). Changes in the optical properties of dental enamel at 1310-nm after demineralization. *Proc of SPIE, Lasers in Dentistry X* 5313(1):118-124.

Liu XM, Cobb MJ, Chen YC, Kimmey MB, Li XD (2004). Rapid-scanning forward-imaging miniature endoscope for real-time optical coherence tomography. *Optics Letters* 29(15):1763-1765.

Rawls HR, Owen WD (1978). Demonstration of dye-uptake as a potential aid in early diagnosis of incipient caries. *Caries Res* 12(2):69-75.

1. The first step is to identify the problem or question that needs to be answered. This involves understanding the context and the specific requirements of the task.

2. Next, it is important to gather relevant information and data. This can be done through research, consultation with experts, or by analyzing existing data sets.

3. Once the information is gathered, the next step is to analyze it. This involves identifying patterns, trends, and relationships that can help in understanding the problem.

4. After analysis, the next step is to develop a solution or answer. This may involve applying theoretical knowledge, using logical reasoning, or conducting experiments.

5. Finally, the solution should be tested and validated. This involves checking the results against the original problem and ensuring that the solution is accurate and reliable.

6. The final step is to communicate the results. This involves presenting the findings in a clear and concise manner, using appropriate language and visual aids.

7. It is also important to reflect on the process and the results. This involves evaluating the effectiveness of the approach used and identifying areas for improvement.

8. Finally, the results should be shared with others. This can be done through reports, presentations, or publications, allowing others to learn from the experience and apply the findings to their own work.

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CHAPTER VI

IMAGING ENAMEL REMINERALIZATION OF pH CYCLED ARTIFICIAL LESIONS WITH PS-OCT

1. *Pharmaceuticals* (1998) 10, 11.

1 INTRODUCTION

Remineralization of dental enamel is a physiological repair process that can reverse the damaging effects of mineral loss from early caries (dental decay). Topical fluoride applied to the tooth surface can enhance the effects of remineralization. The fluoride mediated remineralization produces fluorapatite or fluoridated hydroxyapatite crystals, from salivary calcium and phosphate ions, that are more acid resistant than the carbonated hydroxyapatite naturally found in enamel. Fluoride treatment, accompanied with antibacterial therapies, also can target the pathogenic bacteria that cause dental caries, which further enhances the tissue repair (Hamilton, 1990; van der Hoeven *et al.*, 1993). Since remineralization is a non-destructive process, opposed to invasive restorative procedures, it is the first therapeutic treatment option for early caries. However, it is imperative that caries lesions are longitudinally monitored during a period of remineralization treatment, since decay can rapidly progress instead of remineralize. Despite efforts to establish clinical diagnostic criteria to determine lesion status (ICDASII, 2005) and monitor lesion activity (Nyvad *et al.*, 2003), current detection methods and technologies, including clinical digital x-ray systems, have limited ability to safely and accurately monitor early enamel caries. For both clinical researchers and dentists, a reliable imaging modality is needed to help assess lesion severity and status and monitor caries lesion activity during non-surgical treatment.

Previous chapters have shown that PS-OCT can be used to quantify the severity of early artificial caries and has several advantages over conventional

OCT (Fried *et al.*, 2002; Jones *et al.*, 2004b). As discussed previously, conventional OCT is especially susceptible to the strong Fresnel surface reflection from the high refractive index enamel; however, PS-OCT uses linearly polarized light which does not depolarize from surface reflection near normal incidence. PS-OCT was able to quantify artificial lesions by a direct integration of the perpendicular axis since carious enamel depolarizes incident polarized light. This direct integration of the perpendicular axis quantifies the severity of caries compared with that of sound enamel; however, it is unclear how the scattering and depolarization is affected after remineralization and whether the integration of the perpendicular axis can quantify the remineralization of enamel caries. Several studies have demonstrated that remineralization has a significant effect on the mineral volume on the outer perimeter of the lesion, near the enamel surface (Cate and Arends, 1977; Iijima *et al.*, 1999; ten Cate and Arends, 1980; ten Cate *et al.*, 1981). Since the outer perimeter of the lesion, near the surface is usually obscured from the intense surface reflection seen in conventional OCT, PS-OCT may be more valuable in imaging the remineralization of caries lesions and discerning the subtle changes of the surface zone after remineralization.

In this chapter, the author used the well characterized pH cycled artificial lesions for testing PS-OCT ability to measure remineralization. Although natural caries and remineralized lesions could provide the strongest indication that PS-OCT can image enamel remineralization, it is difficult to longitudinally monitor remineralization since the lesion severity can only be confirmed by histology after destructively sectioning the tooth. Also, there isn't enough evidence to suggest

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that natural lesions can be reliably remineralized in vitro. Comparing 'caries' versus 'remineralized' natural lesions is complicated by the requirement to fully examine the entire mineral volume of the lesion because a well defined surface zone can be seen in active decay that may be advancing laterally across a tooth instead of pulpally.

In this work, we use an all fiber-based PS-OCT system operating at 1310 nm, where dental enamel has been shown to be nearly transparent (Fried *et al.*, 1995; Jones *et al.*, 2003), to image demineralized and fluoride-enhanced remineralized artificial lesions on smooth enamel surfaces. We postulated that PS-OCT was capable of measuring changes in the optical reflectivity and depolarization upon enamel remineralization. PS-OCT images of artificial lesions before and after remineralization were compared with the relative mineral loss, ΔZ (%vol x μm), obtained from high resolution Digital Microradiography (DM), and chemical composition changes measured with infrared spectroscopy.

2 MATERIALS AND METHODS

2.1 Artificial Smooth Surface Caries

Forty sound human posterior teeth were mounted on 12 mm³ acrylic blocks after root resection. Teeth were sterilized using gamma irradiation and stored in a moist environment to preserve tissue hydration with 0.1% thymol added to prevent bacterial growth. The outer enamel surface was polished to remove any previous remineralized enamel and expose normal sound enamel. An acid resistant varnish was applied on the teeth on all areas outside the 3x2 mm

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smooth surface test region, which was scanned using the PS-OCT system. Artificial, or *in vitro*, lesions on the teeth were formed using a well-characterized pH cycling model replicating the cycle of demineralization and remineralization that takes place naturally in the oral environment (Featherstone *et al.*, 1986; White and Featherstone, 1987). Teeth were exposed to either a 3 or 10 day (n=20) daily regimen of 6 hrs demineralization and 17 hrs remineralization (Featherstone *et al.*, 1990). Teeth exposed to the pH cycling for 10 days were intended to represent severe early caries, and the teeth exposed for 3 days represent a less advanced lesion in both depth and overall mineral loss. During demineralization, each tooth was exposed for 6 hours a day to a 40 mL aliquot buffer solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate and 0.075 mol/L acetate maintained at pH 4.3 and a temperature of 37°C. After demineralization, each tooth was immersed for 17 hrs in a 20 mL mineralizing solution of 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L KCl, 20 mmol/L cacodylate buffer maintained at pH 7.0 and 37°C.

Sample teeth from both the 3 day (n=10) and 10 day (n=10) regimens were subsequently exposed to a fluoride containing remineralization solution. This solution was identical in composition to the mineralizing solution used in the pH cycling during the formation of the artificial lesions, except that 2 ppm F⁻ in the form of NaF was added to the solution to enhance the remineralization effect. Teeth were then continuously exposed for 20 days to the fluoride remineralization solution. For histological evaluation, 200 µm thick transverse sections from the demineralized and remineralized samples were obtained with a

1. The first part of the report is a general introduction to the subject of the study. It discusses the importance of the study and the objectives of the research. It also mentions the scope of the study and the limitations of the study.

2. The second part of the report is a literature review. It discusses the previous studies on the subject of the study. It also mentions the gaps in the literature and the need for the study.

microtome and a digital micrometer, after embedding the samples in a resin polymer to preserve the lesion surface layer. Other tooth sections were also embedded in a resin polymer, polished to a 3 μm finish, so that the entire transverse lesion and underlying enamel was exposed for high resolution IR spectromicroscopic analysis.

2.2 Polarization Sensitive Optical Coherence Tomographic Imaging

PS-OCT system used in this study is described in detail in Chapter I. This PS-OCT system used a 20 mW broadband 1310 nm super luminescent diode (SLD), (COVEGA, Jessup, MD). The SLD source possessed a spectral bandwidth (FWHM) of 50 nm that produced within the system an axial resolution of 20 μm in air. The system axial resolution is increased to as much as 11 μm when imaging the higher refractive index enamel tissue ($n=1.63$). The lateral resolution of the system was 30 μm .

Two-dimensional OCT *b*-scans were obtained by collecting a series of depth resolved signals by laterally scanning the beam across 12 mm of the wet tooth. The acrylic blocks attached to each tooth was fitted into a custom metal jig that allowed orientation and coordinates to be precisely replicated, within 50 μm , for every scan (Jones *et al.*, 2004a). The fixed coordinate position and a reference focus point of sound enamel were necessary to ensure that both the demineralization and remineralization samples were placed into a fixed position.

The PS-OCT *b*-scan images were acquired at day 0 for the 40 teeth in the study. After either 3 or 10 days of pH cycling to create the artificial lesions, PS-

OCT images were again collected. Demineralized caries lesion in each group (n=10) were saved for histological evaluation, and the remaining teeth were again scanned with PS-OCT following 20 days of remineralization. A total of 8 serial *b*-scan images were acquired for each tooth at 400 μm intervals, which encompassed a 2.8 mm x 12 mm area of the exposed tooth surface. A series of line profile integrations over the depth of each lesion that encompassed the entire demineralized area were averaged and used to assess the overall lesion severity. This integration of the line profiles, a summation of the logarithmic reflectivity (dB), is presented in arbitrary units (a.u.). Using a single line profile of the lesion, the real lesion depth was determined by dividing the measured optical depth by the index of refraction of the enamel ($n = 1.63$).

2.3 Histological Analysis (Digital Microradiography)

Digital Microradiography (DM) of 200 μm thick transverse tooth sections was used to measure the mineral loss or gain from each of the four groups (n=6): the 3 and 10 day demineralized caries lesions, and the 3 and 10 day fluoride remineralized lesions. High-resolution microradiographs were using the DM method described in Chapter IV. From the DM technique, we obtained the quantitative mineral loss profiles (Angmar *et al.*, 1963). The relative mineral loss ($\text{vol}\% \times \mu\text{m}$), ΔZ , was calculated as the difference between the sound and carious profiles on the same sample. The hydrated tooth sections were examined under a polarized light microscope (Series 7, Westover Scientific, Seattle, WA; x400, NA = 0.25) interfaced to a high resolution digital camera with

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Image Pro Plus image analysis software (Media Cybernetics, Silver Spring, MD) to examine the demineralized and remineralized lesion.

2.4 Synchrotron Radiation Fourier Transform Infrared Spectroscopy (SR-FTIR)

High-resolution IR spectra of laser-irradiated surfaces were obtained using a Nicolet Magna 760 Nic-Plan IR Microscope (Nicolet, Madison, WI) interfaced to Beamline 1.4 at the Advanced Light Source at Lawrence Berkeley National Laboratory (McKinney *et al.*, 1997). The high brightness provided by the synchrotron radiation source allows IR spectra to be acquired with a spatial resolution of 10 μm . The specular reflectance spectra were acquired by scanning the 10 μm spot imaged by the FTIR microscope across the cross-sectional surfaces of selected remineralized lesions from each group.

3 RESULTS

3.1 Remineralization of Severe Early Artificial Caries

PS-OCT *b*-scan images, in both the parallel and perpendicular-axes, of the 10 day pH cycled demineralized caries lesions are presented in Fig. 2. The reflectivity in the parallel-axis shows the intense scattering of the demineralized lesion compared to the bordering sound enamel. In the perpendicular-axis, the caries lesion rapidly depolarizes the incident polarized light, and the signal is markedly greater than that of the signal in the sound enamel caused by the native birefringence. After exposure to the fluoride remineralization solution for 20 days, the lesion demonstrated an increase in both scattering and

depolarization. Integration of the perpendicular-axis line profiles of the lesion for both the demineralized samples and the samples exposed to the remineralization solution are presented in Table 1. The paired data, from identical samples, showed that the caries lesions exposed to 20 days of remineralization had a higher integrated reflectivity compared to the demineralized lesions (Repeated-measures analysis of variance, $p < 0.05$). The integrated reflectivity of the sound enamel in both groups was equal. The estimated real lesion depths, using the index of refraction of sound enamel, showed no difference in depth between the two groups.

The high resolution digital micrographs of the 10 day lesions, obtained after cross-sectioning, indicated that these teeth were severely demineralized with a large reduction in the volume percent mineral versus the sound samples (Fig. 3A-B). The quantitative mineral content profiles from the lesion and sound enamel were acquired (Fig. 3C-D). From these profiles, it is apparent that the artificial process of creating the 10 day lesions produced an intact 15 μm thick "surface zone" of higher mineral volume near the enamel surface. After the 20 day fluoride remineralization, samples showed higher volume percent mineral within the entire lesion (Fig 3B, D). The quantitative mineral content profile illustrated the increase in the mineral volume, with the most dramatic increase near the surface of the lesion. Within a 40 μm deep surface zone region, the volume percent mineral of the remineralized lesion was restored to close to that of sound enamel. Under polarized light microscopy, the surface zone and lesion body depolarize the light in both the demineralized and remineralized lesion (Fig.

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1). The relative mineral loss, ΔZ , was calculated from the mineral volume profiles to quantify lesion severity for each sample. The ΔZ and lesion depths from the 10 day lesions and the fluoride remineralized lesions are summarized in Table 2. The remineralized samples showed a statistically significant (Student's t test, $n=6$, $p<0.02$) reduction in the ΔZ value compared with the 10 day lesions. This indicated the samples remineralized with new mineral, and the lesion surface zone showed significant restoration of mineral volume. The lesion depth slightly decreased with remineralization (Student's t test, $n=6$, $p<0.05$) but only to a marginal degree.

3.2 Remineralization of Mild Early Artificial Caries

PS-OCT *b*-scan images of the 3 day demineralized caries lesions are presented (Fig. 4A-B). The images in both axes show that the 3 day lesions are much shallower in depth than the lesions created after 10 days of pH cycling. Before these lesion samples were placed in the fluoride remineralization solution, the bordering sound enamel, which is normally protected by a varnish, was deliberately exposed to the solution as well (Fig. 4D, arrows). After 20 days, it is evident (Fig. 4C-D) that a highly scattering and depolarizing outer layer of mineral approximately 30 μm thick is formed on the surface of the lesion and extended to the area of the exposed sound enamel. The boundary between this outer layer and the underlying lesion is clearly resolved in the perpendicular-axis, due to the minimal effects of surface reflection in this axis.

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The *Agrobacterium* strains were incubated with the plant explants for 2 weeks. The explants were then cultured on the selective medium. The number of explants transformed was counted. The results are shown as the mean $\pm$ SD of three independent experiments. * indicates a significant difference ($p < 0.05$) between the control and the treated explants.

After carefully sectioning the samples, the DM images of the remineralized samples (Fig. 5-top) showed a highly mineralized outer 35 μm thick layer with a mineral volume content equal to that of the sound enamel. The presence of this outer layer confounds an accurate lesion severity analysis since ΔZ is a product of both mineral volume and depth. The remineralized lesions have a larger depth than the demineralized lesions because of the addition of the outer surface layer. Synchrotron radiation IR spectroscopy was employed to identify the chemical composition of this outer layer. Spectra were acquired from the different regions of the remineralized 3 day lesion (Fig. 5-bottom). The first spectra (Fig. 6A) represents the normal sound enamel with the three characteristic peaks due to the phosphate group in carbonated hydroxyapatite near 1000 cm^{-1} and the two smaller peaks near 1400 cm^{-1} due to the carbonate group. The carbonate group is absent in the second spectra (Fig 6B) of the 3 day lesion body. The apatite phosphate bands are also altered from both the initial preferential dissolution of carbonated enamel crystals and the subsequent remineralization from the pH cycling mineralizing solution. The spectra of the remineralized outer layer (Fig 6C) displays the characteristic apatite bands, with an absence of the carbonate group, and the narrowness of the phosphate band indicates this layer has high crystallinity.

4 DISCUSSION

This present study determined that PS-OCT can non-destructively image the effects of fluoride-enhanced remineralization of mild and severe early artificial

caries. For the severe caries, using the 10 day pH cycled lesion model, an increase in the integrated reflectivity in the perpendicular-axis image was measured after exposure to a fluoride remineralization solution. Digital microradiography of the sectioned samples proved that the entire lesion body accumulated mineral volume from the remineralization, with a significant increase of mineral near the surface of the lesion. This observation is consistent with previous studies investigating caries remineralization models (Cate and Arends, 1977; Iijima *et al.*, 1999; ten Cate and Arends, 1980; ten Cate *et al.*, 1981). Those studies have shown that, as a caries lesion undergoes remineralization, the deeper porosities accumulate mineral, but to a more limited extent than the lesion surface zone. In fact, it is suggested that successful remineralization of the surface zone limits the continued diffusion of ions into the deeper regions of the lesion. This observation is in accord with *in vivo* remineralization studies of early caries (Arends and Gelhard, 1983), where the restoration of the surface zone with an acid resistant fluoride-containing apatite has a large effect on preventing decay in the subsurface lesion body. This is, due in part, to the fact that the pathogenic bacteria in early caries are populated above the enamel surface, dissolving subsurface enamel mineral through acid diffusion byproducts. Although the remineralized surface zone is only microns thick it prevents any further increase in mineral loss and lesion depth, and the lesion is termed *arrested*. Studies of artificial and natural caries have shown that the lesion depth is not radically decreased by remineralization, which may be caused by surface zone restoration (Iijima *et al.*, 1999). In this study, PS-OCT did not show any

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measurable change in depth, whereas the DM showed a minor change of 15 μm , which is near the PS-OCT system resolution. Our previous work has shown a strong correlation between lesion depth and lesion severity in DM and PS-OCT (Jones *et al.*, 2005; Ngaotheppitak *et al.*, 2005). In this study, it should be noted that the PS-OCT lesion depth comparison was a paired data set, and the final samples were then used for the lesion depth calculation of the remineralization DM images. Therefore, the lesion depth agreement was within 30 μm , despite the experimental difficulties in comparing the 20 μm tomographic slice thickness of PS-OCT with the DM sectional slices. Another possible source of error was using the refractive index of sound enamel ($n=1.63$) in the calculations to convert optical depth to real depth. It is likely that the refractive index of the lesions are different than sound enamel; if the refractive index decreased to a value similar to that of the less mineralized dentin tissue ($n=1.50$), the real lesion depth would be 10 μm thicker than the calculated depth using the sound enamel refractive index.

Lesion severity is determined by both the integration of the perpendicular-axis image and calculation of the lesion depth. In the case of remineralization of early caries, analyzing both these properties is important. This is because the integrated reflectivity has been shown to increase as a caries lesion progresses during pH cycling (Jones *et al.*, 2004b). This increase is accompanied with an increase in the depth of the lesion. Other artificial lesion studies have shown that as a lesion progresses, the mineral content at a specific depth does not significantly change after a certain volume, instead the diffusion of ions pass

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through the area without affecting the mineral volume (Arends *et al.*, 1997). In remineralization, mineral volume is increased by the growth of remaining crystals, the restoration of partially demineralized crystals, and the deposition of newly formed crystals (Yanagisawa and Miake, 2003). The influx of deposited crystals and restored crystals within a caries lesion can be a variety of sizes and irregular shapes, thus not perfectly emulating the organized rod-like structure of sound enamel (Miake *et al.*, 2003). The random orientation of crystallite mineral deposition during remineralization (ten Cate *et al.*, 1981), is expected to increase the number of crystal grain boundaries within the lesion, resulting in greater lesion scattering and depolarization. As seen under polarized light, the optical properties of the surface zone and lesion body were not restored to that of the sound enamel after remineralization. Therefore, the remineralization treatment did not cause an epitaxial repair of apatite, especially near the surface, that would behave similar to the organized and birefringent enamel. An increase in the integrated reflectivity without an increase in lesion depth may be indicative of *in vivo* caries remineralization under certain circumstances, although future work is necessary. A recent study of the remineralization of enamel erosion, which is the straight surface dissolution caused by exposure of gastric acids, found a reduction in the enamel reflectivity after a single 30-60 minute exposure to a remineralization solution (Wilder-Smith *et al.*, 2005). Although this study lacked histological evidence to support the increase in surface mineral volume, the findings may suggest that repairing surface roughness caused by straight demineralization may initially decrease reflectivity. In our recent study of the

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remineralization of *in vitro* caries lesions with porous surface zones, we measured the repair of a 35- μm thick surface zone of low reflectivity using PS-OCT within the lesion after exposure to a remineralization solution (Jones and Fried, 2006). This suggests that the remaining enamel crystal structure and lesion porosity of a caries lesion prior to remineralization strongly dictate the reflectivity and depolarization properties of the remineralized lesion.

The PS-OCT images of the remineralization of the 3 day lesions showed an outer growth layer on the surface of the lesion and the bordering sound enamel. From the DM images and FTIR analysis, this outer layer was found to have a mineral volume equal to that of sound enamel with a crystalline apatite structure. Previous studies have shown that the mineral volume increase, a change in the ΔZ , within a lesion during remineralization is correlated to the severity of a caries lesion (Iijima *et al.*, 1999). This indicates that the less severe 3 day caries lesions had a surface zone that rapidly remineralized. This remineralization prevented further mineral deposition into the lesion and caused the growth of an outer layer of highly crystalline phase resembling apatite. This outer layer, without the highly soluble carbonate group, is likely to be more resistant to acid dissolution. It is not clear from this study how integrated this outer layer is with the underlying sound enamel surface and whether this layer can withstand the mechanical forces that exist *in vivo*. Since the perpendicular PS-OCT axis image is minimally affected by surface reflection, the boundary and thickness of this outer caries resistance layer is resolved in the perpendicular-axis image. This is the first evidence, to the best of our knowledge, to show the

highly crystalline growth of the outer layer. Because this thin outer layer was detected by PS-OCT prior to sectioning, a resin embedding technique to preserve the integrity of the outer layer was used before DM analysis. We found that the outer layer was severely damaged if a high speed diamond grit cutting blade was used on the surface without embedding, which may explain the reason that this layer was not found during DM analysis in other studies.

In conclusion, this study demonstrated that PS-OCT can be used to quantify the remineralization of severe early artificial caries by measuring an increase in the perpendicular-axis integrated reflectivity with no accompanying change in lesion depth. For less severe lesions, PS-OCT imaged the highly scattering and depolarizing outer layer after fluoride-enhanced remineralization. The results of this work call for future studies investigating the ability of PS-OCT to image and analyze different synthetic (Yamagishi *et al.*, 2005) and *in vivo* enamel repair and remineralization treatments.

5 FIGURES AND FIGURE LEGEND

Table 1 Lesion Assessment with PS-OCT of the 10 day lesions

| | Lesion Depth
(μm) | Integrated Reflectivity of
Lesion (a.u.) | Integrated Reflectivity
of Sound Enamel (a.u.) |
|-------------------------|-----------------------------------|---|---|
| Demineralized
Lesion | 120 ± 20 | 214 ± 69 | 113 ± 49 |
| Remineralized
Lesion | 120 ± 15 | 316 ± 104 | 113 ± 61 |

Table 2 Lesion Assessment with Digital Microradiography of the 10 day lesions

| | Lesion Depth
(μm) | Relative mineral loss
compared to sound enamel
(ΔZ , vol% $\times \mu\text{m}$) | Surface zone (40 μm)
mineral loss compared
to sound enamel |
|-------------------------|-----------------------------------|---|--|
| Demineralized
Lesion | 163 ± 23 | 1728 ± 234 | 310 ± 63 |
| Remineralized
Lesion | 148 ± 22 | 1112 ± 489 | 173 ± 69 |

(continued)

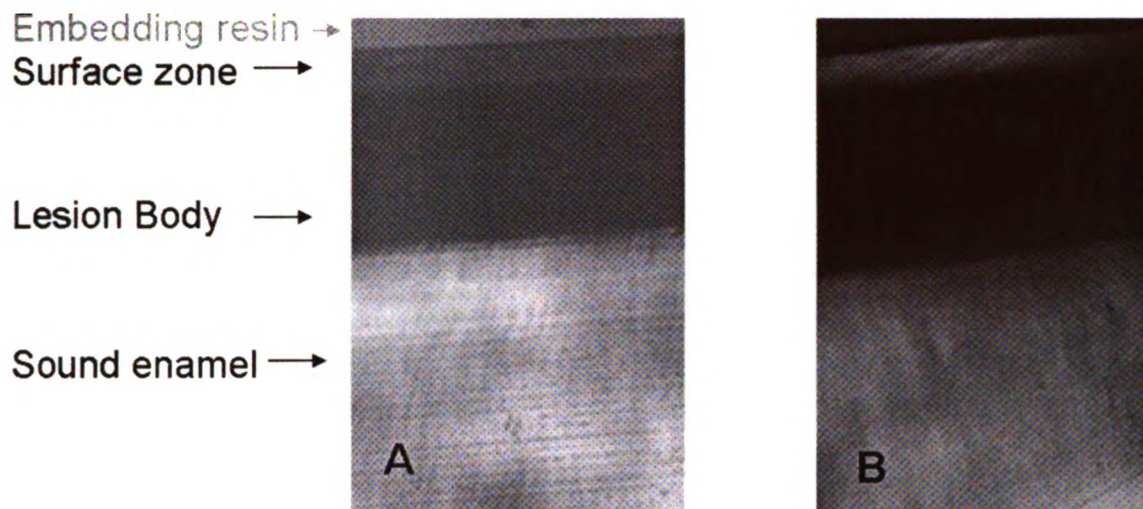


Fig. 1 Polarized light microscopy (400x) examined transverse sections of hydrated (A) 10 day pH cycled artificial enamel caries. There is an intact surface zone that is seen near the surface of the lesion that highly depolarizes the light but to a lesser degree than the lesion body. (B) A transverse slice of an enamel lesion after a 20 day regimen of fluoride remineralization. The overall lesion depth is similar to that of the demineralized enamel. The surface zone of the lesion is not markedly restored to the optical properties of the sound enamel.

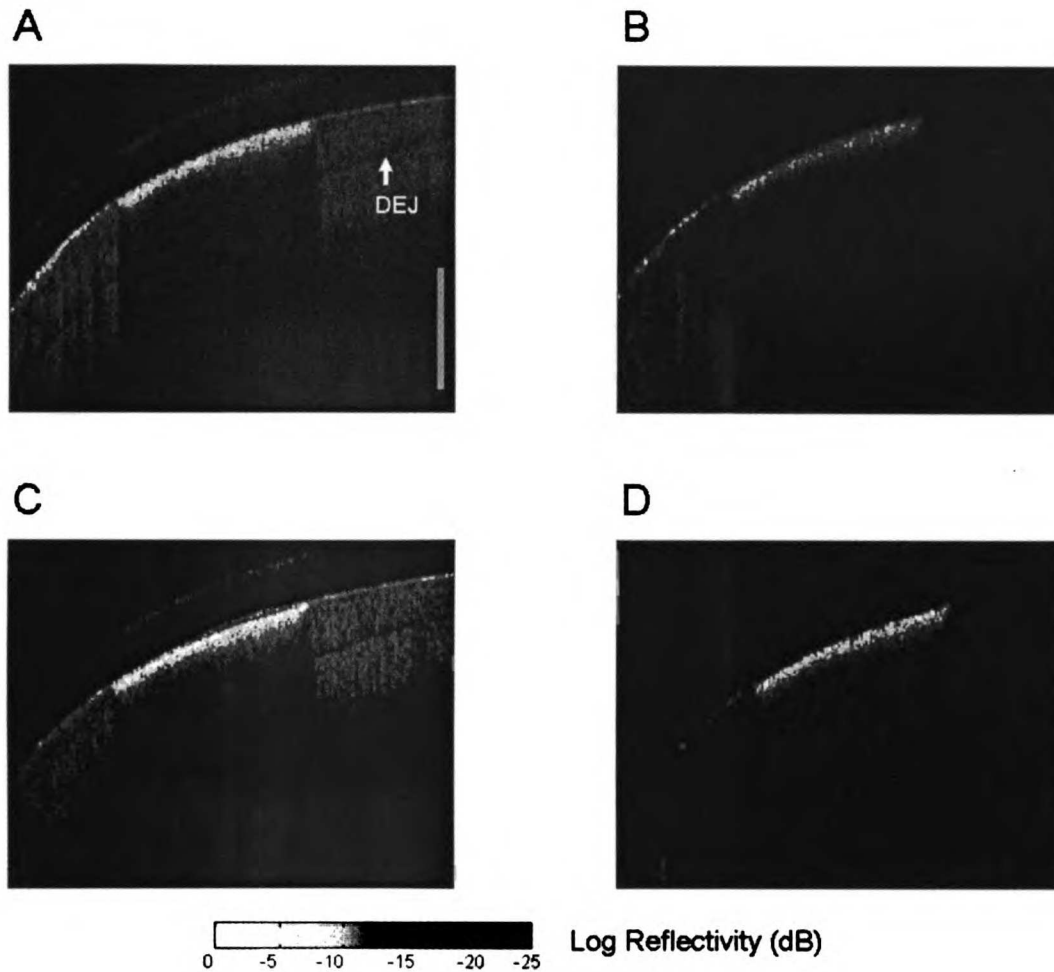


Fig. 2 PS-OCT *b*-scan images of a 10 day pH cycled artificial caries lesion before and after a 20 day regimen of fluoride remineralization. Images are displayed in a false-color scale (bottom). (A) The blue scale bar in is 1 mm of optical depth and the dentin-enamel junction (DEJ) is labeled. Parallel-axis images of the demineralized caries lesion (A) and the remineralized lesion (C) show an increase in backscattering of the lesion area after remineralization. Perpendicular-axis images of the demineralized (B) and remineralized lesion (D) show both the increase of backscattering and depolarization after remineralization. The reflectivity increase in the perpendicular-axis is quite evident near the surface of the lesion.

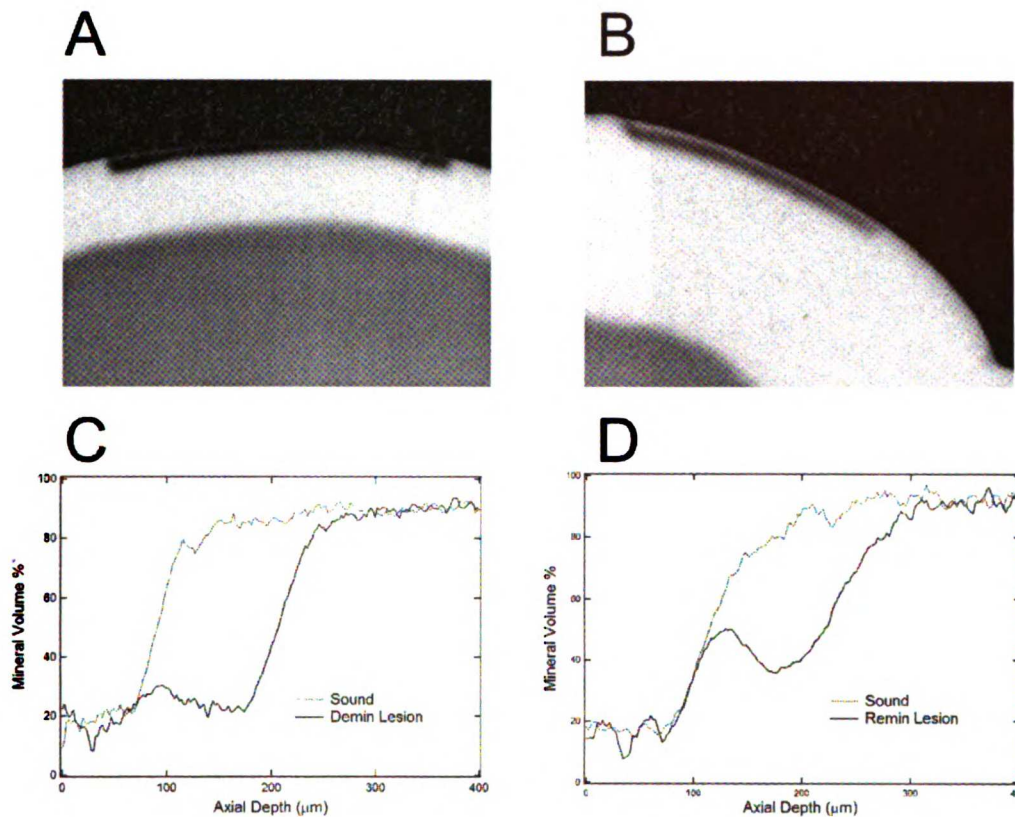


Fig. 3 High resolution digital micrographs of the 10 day pH cycled lesions (A) and the lesions after 20 day fluoride remineralization treatment (B) were obtained after tooth sectioning. The quantitative mineral content profiles from the demineralized lesion (C) and remineralized lesion (D) were acquired, in addition, to the sound enamel profiles. The relative mineral loss (vol% $\times$ μm), ΔZ , was calculated as the difference between the sound and lesion profiles on the same sample.

1. *Chlorophyll *a** and *Chlorophyll *b** were determined by the method of Arar and Collins (1971).

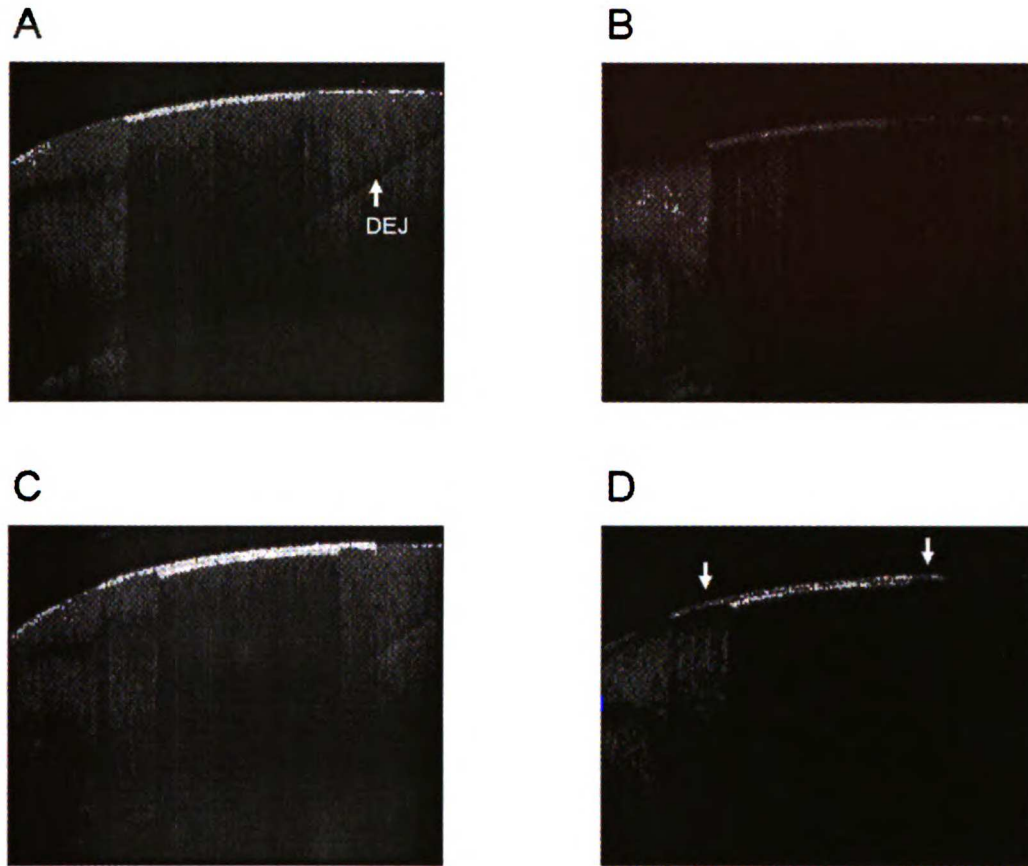


Fig. 4 PS-OCT *b*-scan images of the 3 day pH cycled artificial caries lesion are presented in the parallel (A) and perpendicular (B) axes. The lesions exposed to 20 days of fluoride remineralization are shown in the parallel (C) and perpendicular (D) axes. Before these lesions were remineralized, the bordering sound enamel, which is normally protected by a varnish, was also deliberately exposed to the solution (D, arrows). After remineralization, a highly scattering (C-D) and depolarizing (D) outer growth layer (30 μ m) is formed on the surface of the lesion and extended to the area of the exposed sound enamel.

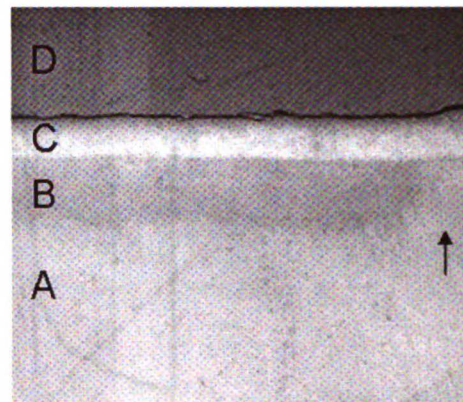
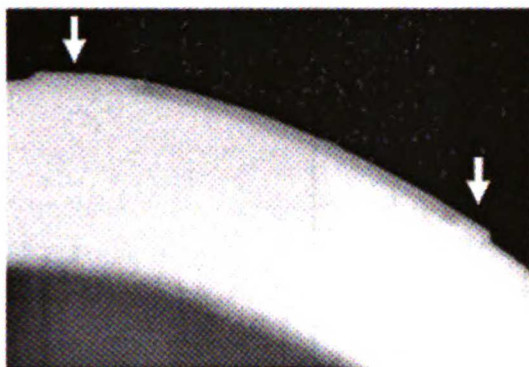


Fig. 5 (Left) A high resolution digital micrograph image, after transverse sectioning, of the 3 day pH cycled artificial lesion after the 20 days of fluoride-enhanced remineralization illustrates the high mineral volume of the outer growth layer (arrows). (Right) A 500x microscope image of the various regions of the remineralized lesion: (A) sound enamel, (B) lesion body, (C) outer remineralization layer, and (D) the embedding resin for sample analysis. The bordering sound enamel (arrow) near the lesion illustrates that the remineralization layer is formed above the surface.

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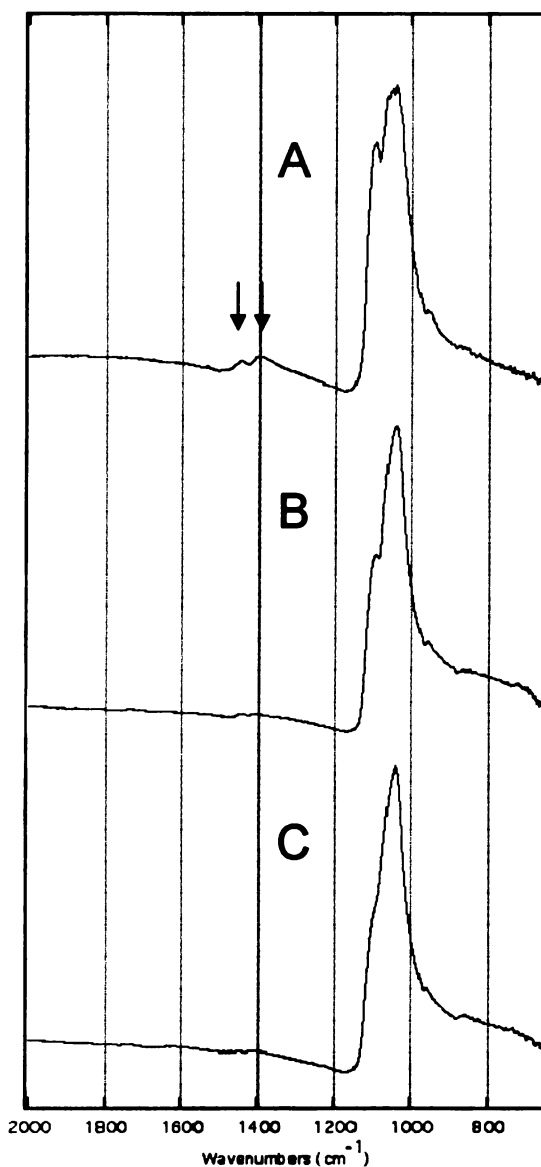


Fig. 6 Spectra by FTIR microscopy were acquired from the different regions of the remineralized 3 day lesion. (A) Sound enamel spectra with the characteristic peaks due to the phosphate group in carbonated hydroxyapatite near 1000 cm^{-1} and the two smaller peaks (arrows) near 1400 cm^{-1} due to the carbonate group. (B) Spectra of the lesion body shows that the carbonate group is absent, which is likely due to the preferential loss of carbonate during demineralization. (C) The outer remineralization layer displays narrow characteristic hydroxyapatite bands, indicating high crystallinity, with an absence of the higher solubility carbonate substitution group.

6 REFERENCES

Angmar B, Carlstrom D, Glas JE (1963). Studies on the ultrastructure of dental enamel. IV. The mineralization of normal human enamel. *J Ultrastruct Res* 8(12-23).

Arends J, Gelhard T (1983). *In vivo* remineralization of human enamel. In: Demineralization and remineralization of the teeth. S Leach and W Edgar editors. Oxford: IRL Press, pp. 1-16.

Arends J, Ruben JL, Inaba D (1997). Major topics in quantitative microradiography of enamel and dentin: R parameter, mineral distribution visualization, and hyper-remineralization. *Adv Dent Res* 11(4):403-14.

Baumgartner A, Hitzenberger CK, Dicht S, Sattmann H, Moritz A, Sperr W, Fercher AF (1998). Optical coherence tomography for dental structures. *Proc of SPIE, Lasers in Dentistry IV* 3248(1):130-136.

Baumgartner A, Dichtl S, Hitzenberger CK, Sattmann H, Robl B, Moritz A, Fercher AF, Sperr W (2000). Polarization-sensitive optical coherence tomography of dental structures. *Caries Res.* 34(1):59-69.

Cate JM, Arends J (1977). Remineralization of artificial enamel lesions in vitro. *Caries Res* 11(5):277-86.

Colston BW, Sathyam US, DaSilva LB, Everett MJ, Stroeve P, Otis LL (1998). Dental OCT. *Optics Express* 3(6):230-238.

de Boer J (2002). Polarization-Sensitive Optical Coherence Tomography: Introduction. In: Handbook of Optical Coherence Tomography. B Bouma and G Tearney editors. New York: Marcel Dekker AG, pp. 1-40.

de Boer JF, Milner TE, vanGemert MJC, Nelson JS (1997). Two-dimensional birefringence imaging in biological tissue by polarization-sensitive optical coherence tomography. *Optics Letters* 22(12):934-936.

Everett MJ, Colston BW, Sathyam US, Silva LBD, Fried D, Featherstone JDB (1999). Non-invasive diagnosis of early caries with polarization sensitive optical

coherence tomography (PS-OCT). *Proc of SPIE, Lasers in Dentistry V* 3593(1):177-183.

Featherstone JDB, O'Reilly MM, Shariati M, Brugler S (1986). Enhancement of remineralization in *vitro* and in *vivo*. In: Factors Relating to Demineralization and Remineralization of the Teeth. SA Leach editor. Oxford, U.K: IRL Press.

Featherstone JDB, Glana R, Shariati M, Shields CP (1990). Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. *J Dent Res* 69(Spec No):620-625.

Fercher AF, Hitzinger CK, Drexler W, Kamp G, Sattmann H (1993). In vivo optical coherence tomography. *Am J Ophthalmol* 116(1):113-4.

Fried D, Featherstone JDB, Glana RE, Saka W (1995). The nature of light scattering in dental enamel and dentin at visible and near-IR wavelengths. *Appl. Optics* 34(7):1278-1285.

Fried D, Xie J, Shafi S, Featherstone JDB, Breunig T, Lee CQ (2002). Early detection of dental caries and lesion progression with polarization sensitive optical coherence tomography. *J. Biomed. Optics* 7(4):618-627.

Fujimoto JG, Boppart SA, Tearney GJ, Bouma BE, Pitris C, Brezinski ME (1999). High resolution in vivo intra-arterial imaging with optical coherence tomography. *Heart* 82(2):128-33.

Hamilton IR (1990). Biochemical effects of fluoride on oral bacteria. *J Dent Res* 69 (Spec No):660-7; discussion 682-3.

Hee MR, Huang D, Swanson EA, Fujimoto JG (1992). Polarization-Sensitive Low-Coherence Reflectometer for Birefringence Characterization and Ranging. *Journal of the Optical Society of America B-Optical Physics* 9(6):903-908.

Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA, Fujimoto JG (1991). Optical Coherence Tomography. *Science* 254(1178-1181).

Huynh G, Darling CL, D. Fried (2004). Changes in the optical properties of dental enamel at 1310-nm after demineralization. *Proc of SPIE, Lasers in Dentistry X* 5313(1):118-124.

Iijima Y, Takagi O, Ruben J, Arends J (1999). In vitro remineralization of in vivo and in vitro formed enamel lesions. *Caries Res* 33(3):206-13.

Issa AI, Preston KP, Preston AJ, Tumba KJ, Duggal MS (2003). A study investigating the formation of artificial sub-surface enamel caries-like lesions in deciduous and permanent teeth in the presence and absence of fluoride. *Arch Oral Biol* 48(8):567-71.

Izatt JA, Hee MR, Huang D, Fujimoto JG, Swanson EA, Lin CP, Schuman JS, Puliafito CA (1993). Optical Coherence Tomography for Medical Applications. In: *Medical Optical Tomography: Functional Imaging and Monitoring*. G Muller, B Chance, R Alfano, S Arridge, J Beuthan, E Gratton, M Kaschke, B Masters, S Svanberg and Pvd Zee editors. Bellingham, WA: SPIE, pp. 450-472.

Johnston TF, Sasnett MW (2004). Characterization of Laser Beams: The M^2 model. In: *Handbook of Optical and Laser Scanning*. GF Marshall editor. New York, Basel: Marcel Dekker, Inc, pp. 1-70.

Jones RS, Huynh GD, Jones GC, Fried D (2003). Near-infrared transillumination at 1310-nm for the imaging of early dental decay. *Optics Express* 11(18):2259-2265.

Jones RS, Staninec M, Fried D (2004a). Imaging secondary caries under composite sealants and restorations. *Proc of SPIE, Lasers in Dentistry X* 5313(1):7-16.

Jones RS, Staninec M, Fried D (2004b). Imaging artificial caries under composite sealants and restorations. *J Biomed Opt* 9(6):1297-304.

Jones RS, DARLING CL, FEATHERSTONE JDB, Fried D (2005). Imaging Artificial Caries on the Occlusal Surfaces with Polarization Sensitive Optical Coherence Tomography. *Caries Res* 40(2).

Jones RS, Fried D (2006). Quantifying the Remineralization of Artificial Caries Lesions using PS-OCT. *Proc of SPIE, Lasers in Dentistry XII* 6137(1):in press.

1

Matheny ES, Hanna NM, Jung WG, Chen Z, Wilder-Smith P, Mina-Araghi R, Brenner M (2004). Optical coherence tomography of malignancy in hamster cheek pouches. *J Biomed Opt* 9(5):978-81.

McKinney WR, Hirschmugl CJ, Padmore HA, Lauritzen T, Andresen N, Andronaco G, Patton R, Fong M (1997). The First Infrared Beamline at the ALS: Design, Construction, and Initial Commissioning. *Accelerator-Based Infrared Sources and Applications*: SPIE.

Miake Y, Saeki Y, Takahashi M, Yanagisawa T (2003). Remineralization effects of xylitol on demineralized enamel. *J Electron Microsc (Tokyo)* 52(5):471-6.

Ngaotheppitak P, Darling CL, Fried D (2005). Measurement of the severity of natural smooth surface (interproximal) caries lesions with polarization sensitive optical coherence tomography. *Lasers Surg. Med.* 37(1):78-88.

Schmitt JM (1999). Optical coherence tomography (OCT): a review. *IEEE Journal of Selected Topics in Quantum Electronics* 5(4):1205 - 1215.

Tearney GJ, Brezinski ME, Bouma BE, Boppart SA, Pitris C, Southern JF, Fujimoto JG (1997). In Vivo endoscopic Optical Biopsy with Optical Coherence Tomography. *Science* 276(2037-2039).

ten Cate JM, Arends J (1980). Remineralization of artificial enamel lesions in vitro: III. A study of the deposition mechanism. *Caries Res* 14(6):351-8.

ten Cate JM, Jongebloed WL, Arends J (1981). Remineralization of artificial enamel lesions in vitro. IV. Influence of fluorides and diphosphonates on short- and long-term remineralization. *Caries Res* 15(1):60-9.

van der Hoeven JS, Cummins D, Schaeken MJ, van der Ouderaa FJ (1993). The effect of chlorhexidine and zinc/triclosan mouthrinses on the production of acids in dental plaque. *Caries Res* 27(4):298-302.

Wang XJ, Milner TE, de Boer JF, Zhang Y, Pashley DH, Nelson JS (1999). Characterization of dentin and enamel by use of optical coherence tomography. *Applied Optics* 38(10):2092-2096.

White DJ, Featherstone JDB (1987). A longitudinal microhardness analysis of fluoride dentifrice effects on lesion progression *in vitro*. *Caries Res.* 21(6):502-512.

Wilder-Smith P, Wilder-Smith C, Hammer-Wilson M, Nguyen V, Truong M, Jequinto G, Zhang J, Jung W, Chen Z (2005). Non-invasive quantification of enamel erosion and demineralization. *Lasers Surg Med* 36(S17):18.

Yamagishi K, Onuma K, Suzuki T, Okada F, Tagami J, Otsuki M, Senawangse P (2005). Materials chemistry: a synthetic enamel for rapid tooth repair. *Nature* 433(7028):819.

Yanagisawa T, Miake Y (2003). High-resolution electron microscopy of enamel-crystal demineralization and remineralization in carious lesions. *J Electron Microsc (Tokyo)* 52(6):605-13.

1. *Pharmaceuticals* (1998) 10, 1-12.
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CHAPTER VII

IMAGING ENAMEL REMINERALIZATION OF SURFACE SOFTENED ARTIFICIAL LESIONS WITH PS-OCT

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1 INTRODUCTION

Enamel caries have the potential to be arrested when the causal biofilm is removed, and the lesion can be restored with new mineral, especially with the aid of topical fluorides. Several studies have demonstrated that the process of remineralization restores mineral to a greater extent near the surface than the underlying lesion body (Cate and Arends, 1977; Iijima *et al.*, 1999; ten Cate and Arends, 1980; ten Cate *et al.*, 1981). It is well documented that caries can possess an intact surface zone that has similar histological appearances to sound enamel (Silverstone, 1973). When the surface zone is thick enough it is considered to be characteristic of an 'arrested' lesion, since the lower permeability may protect the underlying lesion from further progression and cavitation (Holmen *et al.*, 1985). The difficulty with assessing depth resolved changes within a remineralized lesion based on mineral volume, histology, or even mechanical properties is that it requires the destruction of the tooth and cannot be applied to understanding the process of remineralization *in vivo*. Clinical assessment of remineralized lesions, such as surface texture and appearance, are limited to the lesion surface. In contrast, directly measuring changes in the optical reflectivity within different layers of a remineralized lesion may prove to be valuable for clinical evaluation of lesion structure and severity. In this study, we hypothesize that the restoration of mineral volume will result in a measurable decrease in the reflectivity of polarized light from each layer of a lesion. The significance of this work is that optical reflectivity may provide

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diagnostic information, in ways similar to that of attenuation in radiography, by quantifying the effects of the remineralization of caries.

To test our hypothesis, optical changes were measured in artificial caries undergoing remineralization as a function of depth using Polarization Sensitive Optical Coherence Tomography (PS-OCT). Two dimensional images of the reflectivity of polarized light versus depth from the enamel surface were measured with depth resolution (layer thickness) of 11 μm .

In this study, optical reflectivity changes during remineralization will be measured on artificial lesions that are expected to have a porous surface, since previous PS-OCT work by our group determined that fluoride enhanced remineralization of *in vitro* artificial lesions created by pH cycling did not restore the optical properties of the enamel despite an increase in mineral volume (Jones *et al.*, 2006b). Those results suggest that the deposited mineral within demineralized pores during the pH cycling may have limited ion diffusion, restoration of partially demineralized crystals, and the growth of remaining crystals during the fluoride remineralization treatment.

2 MATERIALS AND METHODS

2.1 Artificial Smooth Surface Caries

Sound human posterior teeth (n=20) were mounted on 12 mm³ acrylic blocks after root resection. An acid resistant varnish was applied on the teeth on all areas outside the 3x2 mm smooth surface test region, which was scanned using

the PS-OCT system. Artificial lesions were formed by exposing the teeth for 9 days to a 40 mL aliquot acetate buffer solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate and 0.075 mol/L acetate maintained at pH 4.9 and a temperature of 37°C. After the lesions were created, half the samples (n=10) were exposed for 20 days to a 20 mL remineralizing solution of 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L KCl, 20 mmol/L cacodylate buffer maintained at pH 7.0 and 37°C. 2 ppm F⁻ in the form of NaF was added to the solution to enhance the remineralization effect.

2.2 Polarization Sensitive Optical Coherence Tomographic Imaging

PS-OCT system used in this study is described in detail in Chapter I with the same diode used in Chapter VI. Two-dimensional OCT *b*-scans were obtained, in less than one minute, by collecting a series of depth resolved signals by laterally scanning the beam across the mounted wet tooth. The *b*-scan images (n=20) were acquired at day 0 and day 9. Demineralized caries lesions (n=10) were saved for histological evaluation, and the remaining samples were scanned with PS-OCT following 20 days of remineralization. A total of 8 serial *b*-scan images were acquired for each tooth at 400 µm intervals, which encompassed a 2.8 mm x 12 mm area consisting of the exposed lesion and the bordering sound enamel. A series of line profile integrations over the depth of each lesion (ΔR , dB x µm) were averaged and used to assess the overall lesion severity. Using a single line profile of the perpendicular-axis image, the real lesion depth and

surface zone were determined by dividing the measured optical depth by the index of refraction of the enamel ($n = 1.63$).

2.3 Histological Analysis

High-resolution ($2.15\ \mu\text{m}$) digital transverse microradiography (TMR) of $150\ \mu\text{m}$ thick sections were used to measure the mineral loss or gain from the lesions before ($n=6$) and after ($n=6$) the remineralization treatment. From the TMR technique, we obtained the quantitative mineral loss profiles (Angmar *et al.*, 1963) taken normal to the outer enamel surface. The relative mineral loss ($\text{vol}\% \times \mu\text{m}$), ΔZ , was calculated as the difference between the sound and lesion profiles on the same sample. The hydrated tooth sections were examined under a polarized light microscope (Series 7, Westover Scientific, Seattle, WA; $\times 400$, $\text{NA} = 0.25$) to measure the thickness of the restored surface layer and overall lesion depth.

3 RESULTS

PS-OCT *b*-scan images, in both the parallel and perpendicular-axes, of the 9 day demineralized caries lesions are presented (Fig. 2C & D) along with the bordering sound enamel that was protected from dissolution (Fig. 2A & B). The parallel-axis image of the artificial caries (Fig. 2C) shows the intense reflectivity within each layer of the lesion compared with the reflectivity within regions of the sound enamel (Fig. 2A). In the perpendicular-axis (Fig. 2D), each distinct layer of the lesion depolarized the incident polarized light upon scattering. The integrated reflectivity in the perpendicular axis (ΔR) within the lesion was

markedly greater than that of the sound enamel (Fig. 2B) caused by the native birefringence. After exposure to the fluoride remineralization solution for 20 days, layers near the surface of the lesion had a reflectivity that was similar to sound enamel. Since the perpendicular-axis is less affected than the parallel axis by the confounding surface reflection of the incident polarized light, the reflectivity in that polarization state was used to quantify the properties of the enamel layers near the surface and determined the thickness of this surface zone. Enamel layers with high reflectivity under the surface zone remained after remineralization, which resulted in a minor change in the overall lesion depth.

The ΔR , relative mineral loss (ΔZ), lesion depth, and surface zone for the artificial lesions before and after exposure to the remineralization regimen are summarized (Table 1 & 2). The ΔR of the lesion decreased after the treatment (paired t-test, $p < 0.05$). This decrease was caused from the minimal reflectivity of the surface zone layers. There was no measurable reduction in the reflectivity of the underlying lesion body from that of the same region in the artificial caries. As a control in this experiment, there was no measurable difference in the ΔR of the bordering sound enamel before ($21 \pm 28 \text{ dB} \times \mu\text{m}$) and after ($19 \pm 20 \text{ dB} \times \mu\text{m}$) remineralization. Comparison of the TMR images of the two lesion groups (Fig. 3C & D) show that the ΔZ , was significantly less for the lesions after remineralization. The remineralization treatment restored mineral volume within the lesion. PS-OCT and PLM image analysis confirmed that the overall lesion depth did not significantly change after remineralization (Table 2). PLM revealed that the demineralized lesion samples possessed an intact enamel surface zone

of $10 \pm 4 \mu\text{m}$ (Fig. 3A). The thickness of the surface zone increased to $33 \pm 5 \mu\text{m}$ for the lesion samples exposed to the fluoride remineralization solution (Fig. 3B). Except for subtle differences in birefringence, the optical properties of the surface zone were restored to that of the sound enamel. Using the relative mineral loss of the most superficial $40 \mu\text{m}$ region of the lesion, the TMR images indicated that mineral volume near the surface of the lesion is substantially restored after remineralization (t-test, $p < 0.01$). The PS-OCT perpendicular-axis images did not identify a surface zone from the demineralized lesion, but did indicate a $35 \pm 7 \mu\text{m}$ enamel surface zone after exposure to the remineralization solution. This surface zone possessed significantly less reflectivity in the perpendicular axis image than the artificial lesion (paired t-test, $p < 0.001$).

4 DISCUSSION

An increase in mineral volume from the fluoride enhanced remineralization significantly decreased the optical reflectivity of artificial lesions within an enlarged surface zone; however, the reflectivity did not decrease significantly in the body of the underlying lesion after remineralization. It is important to consider that the lesion body did not remineralize to the same level as the surface zone, namely remineralization increased the center of the lesion body from 29 ± 7 to 48 ± 6 % mineral volume. Optical property experiments of enamel caries have shown that the change in scattering intensity depends on the mineral volume range (Darling *et al.*, 2006). There is a dramatic reduction in scattering between regions that are 70-86 % mineral volume, which is similar to the

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. If there is a significant difference, a problem is identified.

2. Once a problem is identified, the next step is to define the problem more precisely. This involves determining the scope of the problem, the resources available, and the constraints that may be affecting the problem.

3. The third step is to analyze the problem. This involves identifying the causes of the problem and determining the relationships between different factors. This step is often the most difficult, as it requires a deep understanding of the problem and the ability to think critically.

4. The fourth step is to develop a solution. This involves brainstorming different ideas and evaluating them based on their feasibility, effectiveness, and cost. The goal is to find a solution that addresses the problem in a way that is sustainable and meets the needs of all stakeholders.

5. The final step is to implement the solution. This involves putting the solution into action and monitoring its progress. It is important to have a plan for how to implement the solution and to have a way to measure its success. Once the solution is implemented, it is important to evaluate its effectiveness and make any necessary adjustments.

changes seen near the surface zone. On the other hand, demineralized enamel between 30 to 70% mineral volume does not have significantly different scattering intensities and explains the similar reflectivity within the lesion body after treatment. A possible mechanism is that the pore volume may have decreased in the lesion body by a reduction in the overall pore size but the number of pores remained high. The lesion body may also have not repaired by an organized crystal arrangement because of the substantial volume loss from the demineralization. Polarized light microscopy established that only the surface zone enamel was restored and repaired to a similar orientation and degree as that of sound enamel. It can be concluded from these results that the optical reflectivity is dependent on both the total volume of mineral and the directional nature of the repair.

The integrated reflectivity in the perpendicular axis (ΔR) measured by PS-OCT provided valuable information in identifying and quantifying the surface zone and lesion body of the remineralized lesion. These two structures had distinct optical reflectivity from each other and sound enamel. Since each layer of the repaired surface zone had a significantly reduced reflectivity, the surface zone thickness was able to be calculated and corresponded with the thickness measured by histology. Likewise, the overall lesion depth did not change after the remineralization regimen. The ΔR quantified the reduced lesion severity after remineralization. The demineralization and remineralization regimens used in this study should be treated as an artificial model that does not simulate the complex process that occurs *in vivo*. Despite the limitations with the model, the

histology in this study corresponds to remineralization that has been documented in other studies where there is an intact surface zone with an underlying lesion body (Iijima *et al.*, 1999; Larsen and Fejerskov, 1989). Lesion depth regression is considered difficult because the diffusion process is slow, and remineralization requires high ion concentrations deep inside the lesion to promote repair. Furthermore, the surface zone of the demineralized lesion may be accurately measured in the future with only a slight improvement to the current 11 μm axial resolution with a wider source bandwidth.

The ability to quantify the remineralized surface zone thickness and to identify the underlying lesion body, through differences in optical reflectivity, could be invaluable in lesion assessment and for caries diagnosis. Some authors have argued that the existence of a shiny even surface opposed to a dull rough surface can be used to differentiate an arrested lesion from active caries (Ekstrand *et al.*, 1997; Nyvad *et al.*, 1999). At least in the surface softened caries model, the demineralized and remineralized lesions had very similar visual appearances (Fig. 1), probably due to the predominant optical scattering of the lesion body. Both lesion types had an intact shiny surface upon visual observation, which may not always represent a clinical setting where mechanical abrasion can contribute to the changes in the visual appearance. The lack of noticeable visual change between the demineralized 'active' and remineralized 'arrested' lesion points out the need to study *in vivo* depth resolved changes to better diagnose caries activity. Depth resolved changes in optical reflectivity can be non-destructively measured by PS-OCT, and topography does not mitigate

1. The first part of the document is a list of the names of the persons who have been appointed to the various positions of the Board of Directors of the Corporation. The names are listed in alphabetical order, and each name is followed by the position to which he has been appointed. The names are as follows:

Mr. J. H. Smith, President
Mr. J. H. Smith, Vice President
Mr. J. H. Smith, Secretary
Mr. J. H. Smith, Treasurer
Mr. J. H. Smith, Chairman of the Board
Mr. J. H. Smith, Chairman of the Committee on Finance
Mr. J. H. Smith, Chairman of the Committee on Management
Mr. J. H. Smith, Chairman of the Committee on Public Relations
Mr. J. H. Smith, Chairman of the Committee on Research and Development
Mr. J. H. Smith, Chairman of the Committee on Sales and Marketing
Mr. J. H. Smith, Chairman of the Committee on Transportation and Logistics
Mr. J. H. Smith, Chairman of the Committee on Environmental Protection
Mr. J. H. Smith, Chairman of the Committee on Labor Relations
Mr. J. H. Smith, Chairman of the Committee on Safety and Health
Mr. J. H. Smith, Chairman of the Committee on Quality Control
Mr. J. H. Smith, Chairman of the Committee on Information Systems
Mr. J. H. Smith, Chairman of the Committee on Legal Affairs
Mr. J. H. Smith, Chairman of the Committee on Taxation
Mr. J. H. Smith, Chairman of the Committee on Accounting
Mr. J. H. Smith, Chairman of the Committee on Insurance
Mr. J. H. Smith, Chairman of the Committee on Real Estate
Mr. J. H. Smith, Chairman of the Committee on Intellectual Property
Mr. J. H. Smith, Chairman of the Committee on Government Relations
Mr. J. H. Smith, Chairman of the Committee on International Relations
Mr. J. H. Smith, Chairman of the Committee on Environmental Protection
Mr. J. H. Smith, Chairman of the Committee on Labor Relations
Mr. J. H. Smith, Chairman of the Committee on Safety and Health
Mr. J. H. Smith, Chairman of the Committee on Quality Control
Mr. J. H. Smith, Chairman of the Committee on Information Systems
Mr. J. H. Smith, Chairman of the Committee on Legal Affairs
Mr. J. H. Smith, Chairman of the Committee on Taxation
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Mr. J. H. Smith, Chairman of the Committee on Insurance
Mr. J. H. Smith, Chairman of the Committee on Real Estate
Mr. J. H. Smith, Chairman of the Committee on Intellectual Property
Mr. J. H. Smith, Chairman of the Committee on Government Relations
Mr. J. H. Smith, Chairman of the Committee on International Relations

the imaging ability (Fried *et al.*, 2002); therefore, the remineralization of occlusal caries can also potentially be assessed. Optical reflectivity measurements can be made by PS-OCT in comparable imaging and processing times as conventional radiography.

The results further demonstrate why it is necessary to investigate how optical reflectivity changes during *in vivo* remineralization and to determine the ability to monitor the remineralization of natural lesions. In that event, the potential for PS-OCT to accurately measure the surface zone thickness may aid in understanding the outcome of non-surgical therapy to arrest and remineralize smooth and occlusal early enamel caries.

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5 FIGURES AND FIGURE LEGEND

Table 1: Lesion Severity

| PS-OCT | Integrated Reflectivity of Lesion (ΔR , dB x μm) | Integrated Reflectivity of 40 μm Surface Zone (ΔR , dB x μm) |
|-------------------|---|--|
| Artificial Lesion | 114 $\pm$ 31 | 59 $\pm$ 22 |
| Remineralized | 79 $\pm$ 33 | 25 $\pm$ 14 |

| TMR | Relative mineral loss (ΔZ , vol% x μm) | Relative mineral loss of 40 μm Surface Zone (vol% x μm) |
|-------------------|---|--|
| Artificial Lesion | 2300 $\pm$ 479 | 212 $\pm$ 84 |
| Remineralized | 887 $\pm$ 394 | 64 $\pm$ 50 |

Table 2: Lesion Depth and Surface Zone

| PS-OCT | Lesion Depth (μm) | Surface zone (μm) |
|-------------------|--------------------------------|--------------------------------|
| Artificial Lesion | 115 $\pm$ 16 | N/A |
| Remineralized | 104 $\pm$ 12 | 35 $\pm$ 7 |

| PLM | Lesion Depth (μm) | Surface zone (μm) |
|-------------------|--------------------------------|--------------------------------|
| Artificial Lesion | 111 $\pm$ 26 | 10 $\pm$ 4 |
| Remineralized | 110 $\pm$ 21 | 33 $\pm$ 5 |

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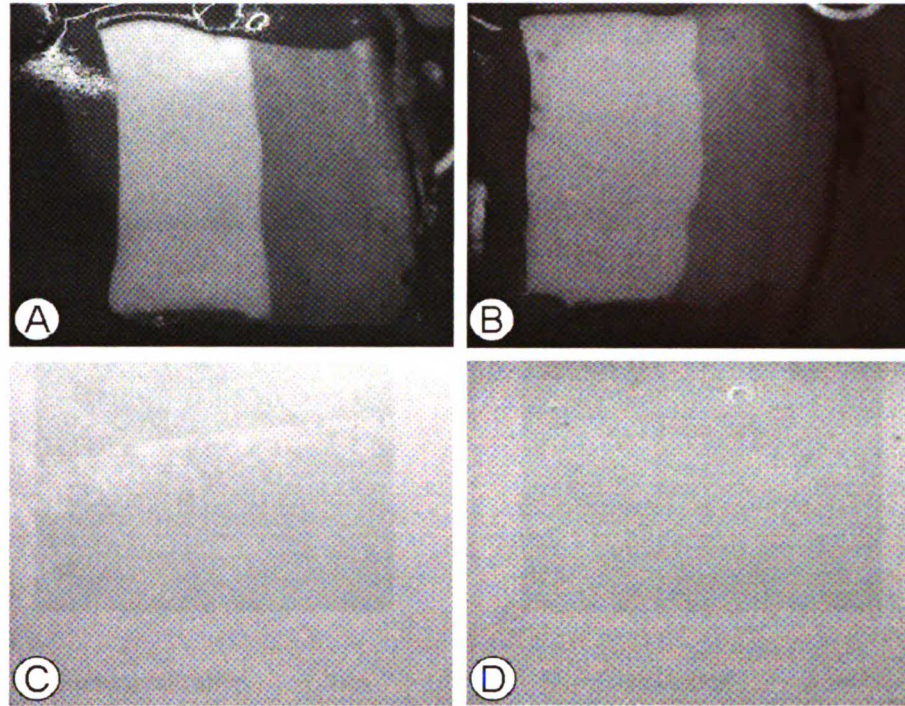


Fig. 1 Visual images of the artificial surface softened caries using a digital camera (2.5x) of the demineralized lesion (A) and after remineralization treatment (B). Despite the increase in mineral volume and surface zone thickness, the caries after remineralization have a similar visual appearance to the demineralized caries. Both lesions have shiny intact surfaces and are distinctly difference from the bordering sound enamel (right) where the varnish was removed. When the images are enlarged, beyond what is performed clinically, the demineralized lesions show the perikymata pattern (C) with the remineralized lesion having a slightly more uniform look (D).

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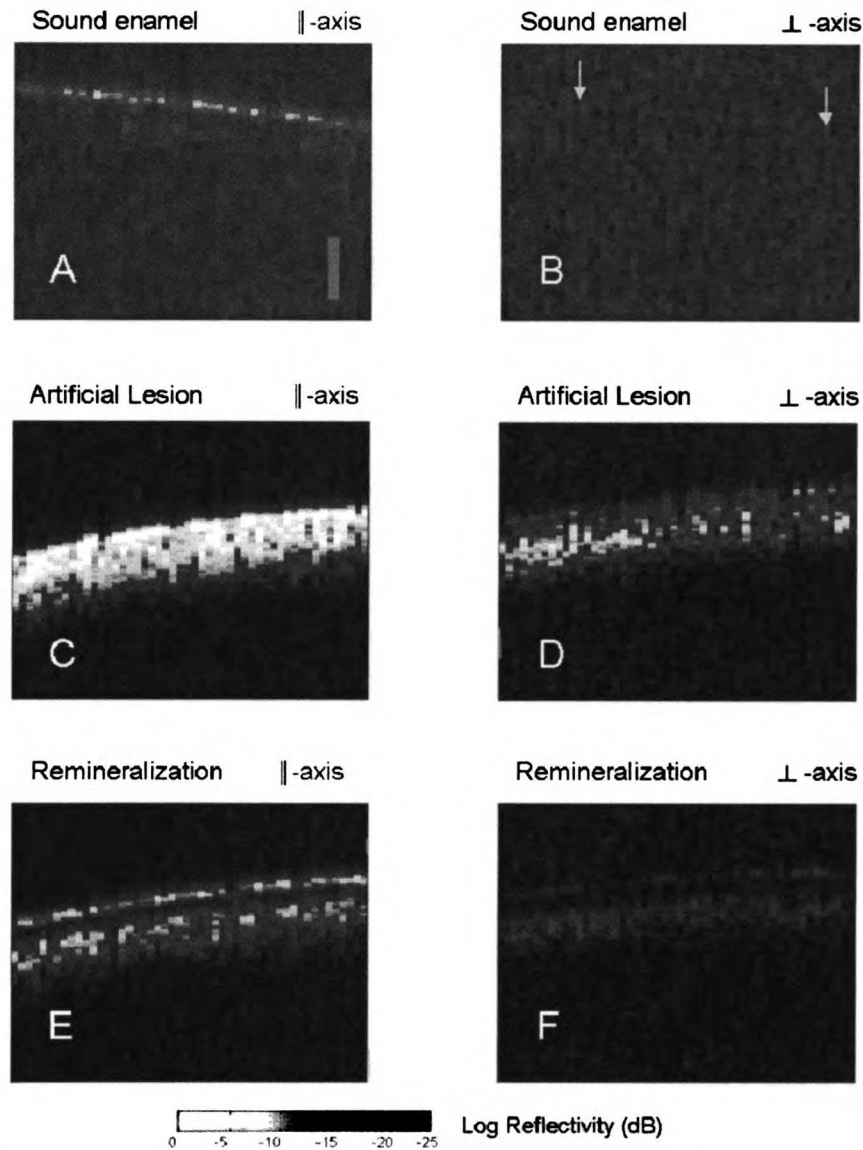


Fig. 2 PS-OCT b-scan images of bordering sound enamel in the parallel (A) and perpendicular (B) axes. Near the enamel surface (arrows), the signal in perpendicular-axis is minimal compared to the moderate backscattered intensity in the parallel-axis. Parallel-axis images of the 9 day artificial caries lesion (C) and after fluoride remineralization treatment (E) show the decrease in overall reflectivity with the existence of a surface zone of lower scattering after treatment. All of the parallel-axis images possess intense surface reflection that confounds analysis of the subsurface enamel and surface zone. Perpendicular-axis images of the artificial caries lesion (D) and the remineralized lesion (F) are shown. The perpendicular-axis image clearly resolves a surface zone, with similar optical properties as the sound enamel (B), and reveals that the overall reflectivity of the lesion decreases after remineralization. Images are displayed in a false-color scale (bottom), and the blue scale bar (A) is 200 μ m in real depth.

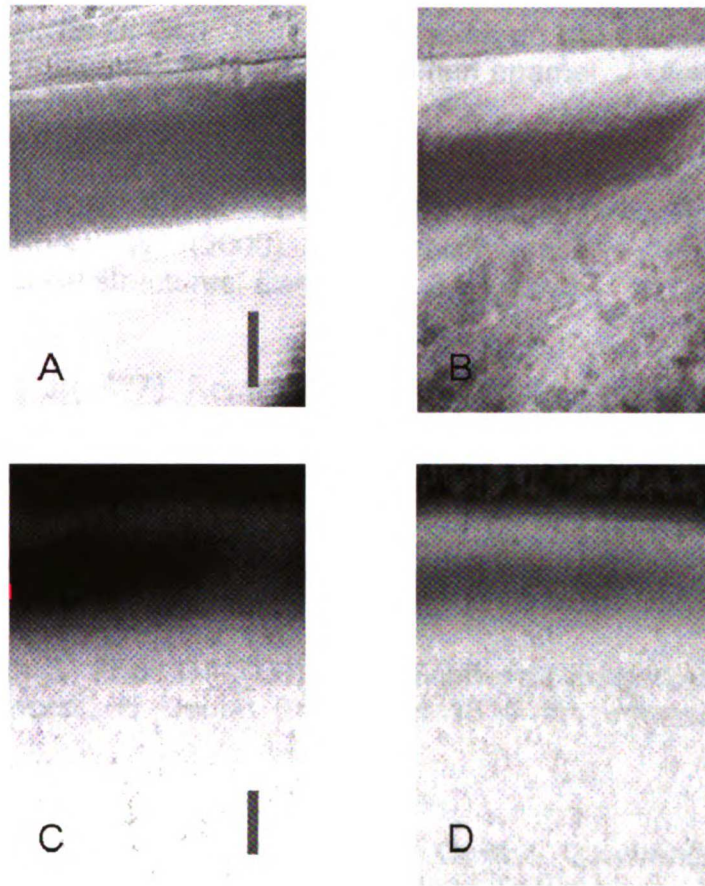


Fig. 3 Polarized light microscopy (400x) examined transverse sections of hydrated artificial enamel caries samples (A) and samples exposed to 20 days of fluoride remineralization solution (B). The artificial caries samples in (A) had an intact surface zone with a dark depolarizing lesion body. After remineralization, a zone of repaired enamel is evident over the remaining depolarizing lesion body. High resolution digital transverse microradiography (TMR) reveals the extensive mineral loss, with a higher mineral volume surface zone, after the 9 day exposure to the demineralization solution (C). After exposure to the remineralization solution, the mineral volume is increased in the lesion body and is completely restored near the surface zone of the artificial lesion. The blue scale bars are 50 μm .

6 REFERENCES

Angmar B, Carlstrom D, Glas JE (1963). Studies on the ultrastructure of dental enamel. IV. The mineralization of normal human enamel. *J Ultrastruct Res* 8(12-23).

Baumgartner A, Dichtl S, Hitzenberger CK, Sattmann H, Robl B, Moritz A, Fercher AF, Sperr W (2000). Polarization-sensitive optical coherence tomography of dental structures. *Caries Res*. 34(1):59-69.

Cate JM, Arends J (1977). Remineralization of artificial enamel lesions in vitro. *Caries Res* 11(5):277-86.

Colston BW, Sathyam US, DaSilva LB, Everett MJ, Stroeve P, Otis LL (1998). Dental OCT. *Optics Express* 3(6):230-238.

Darling C, Huynh G, Fried D (2006). Light Scattering Properties of Natural and Artificially Demineralized Dental Enamel at 1310-nm. *Journal of Biomedical Optics* in press

de Boer J (2002). Polarization-Sensitive Optical Coherence Tomography: Introduction. In: Handbook of Optical Coherence Tomography. B Bouma and G Tearney editors. New York: Marcel Dekker AG, pp. 1-40.

Ekstrand KR, Ricketts DN, Kidd EA (1997). Reproducibility and accuracy of three methods for assessment of demineralization depth of the occlusal surface: an in vitro examination. *Caries Res* 31(3):224-31.

Fercher AF, Hitzenberger CK, Drexler W, Kamp G, Sattmann H (1993). In vivo optical coherence tomography. *Am J Ophthalmol* 116(1):113-4.

Fried D, Featherstone JDB, Glena RE, Seka W (1995). The nature of light scattering in dental enamel and dentin at visible and near-IR wavelengths. *Appl. Optics* 34(7):1278-1285.

Fried D, Xie J, Shafi S, Featherstone JDB, Breunig T, Lee CQ (2002). Early detection of dental caries and lesion progression with polarization sensitive optical coherence tomography. *J. Biomed. Optics* 7(4):618-627.

Fujimoto JG, Boppart SA, Tearney GJ, Bouma BE, Pitris C, Brezinski ME (1999). High resolution in vivo intra-arterial imaging with optical coherence tomography. *Heart* 82(2):128-33.

Hee MR, Huang D, Swanson EA, Fujimoto JG (1992). Polarization-Sensitive Low-Coherence Reflectometer for Birefringence Characterization and Ranging. *Journal of the Optical Society of America B-Optical Physics* 9(6):903-908.

Holmen L, Thylstrup A, Ogaard B, Kragh F (1985). A polarized light microscopic study of progressive stages of enamel caries in vivo. *Caries Res* 19(4):348-54.

Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA, Fujimoto JG (1991). Optical Coherence Tomography. *Science* 254(1178-1181).

Iijima Y, Takagi O, Ruben J, Arends J (1999). In vitro remineralization of in vivo and in vitro formed enamel lesions. *Caries Res* 33(3):206-13.

Jones RS, Huynh GD, Jones GC, Fried D (2003). Near-infrared transillumination at 1310-nm for the imaging of early dental decay. *Optics Express* 11(18):2259-2265.

Jones RS, Staninec M, Fried D (2004). Imaging artificial caries under composite sealants and restorations. *J Biomed Opt* 9(6):1297-304.

Jones RS, Darling CL, Featherstone JDB, Fried D (2006a). Imaging Artificial Caries on the Occlusal Surfaces with Polarization Sensitive Optical Coherence Tomography. *Caries Res* 40(2):81-89.

Jones RS, Darling CL, Featherstone JDB, Fried D (2006b). Remineralization of in vitro Dental Caries Assessed with Polarization Sensitive Optical Coherence Tomography. *J Biomed Opt* 11(1):1-9.

Larsen MJ, Fejerskov O (1989). Chemical and structural challenges in remineralization of dental enamel lesions. *Scand J Dent Res* 97(4):285-96.

Matheny ES, Hanna NM, Jung WG, Chen Z, Wilder-Smith P, Mina-Araghi R, Brenner M (2004). Optical coherence tomography of malignancy in hamster cheek pouches. *J Biomed Opt* 9(5):978-81.

Nyvad B, Machiulskiene V, Baelum V (1999). Reliability of a new caries diagnostic system differentiating between active and inactive caries lesions. *Caries Res* 33(4):252-60.

Schmitt JM (1999). Optical coherence tomography (OCT): a review. *IEEE Journal of Selected Topics in Quantum Electronics* 5(4):1205 - 1215.

Silverstone LM (1973). Structure of carious enamel, including the early lesion. *Oral Sci Rev* 3(100-60).

Tearey GJ, Brezinski ME, Bouma BE, Boppart SA, Pitris C, Southern JF, Fujimoto JG (1997). In Vivo endoscopic Optical Biopsy with Optical Coherence Tomography. *Science* 276(2037-2039).

ten Cate JM, Arends J (1980). Remineralization of artificial enamel lesions in vitro: III. A study of the deposition mechanism. *Caries Res* 14(6):351-8.

ten Cate JM, Jongebloed WL, Arends J (1981). Remineralization of artificial enamel lesions in vitro. IV. Influence of fluorides and diphosphonates on short- and long-term remineralization. *Caries Res* 15(1):60-9.

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The concentration of the *Agrobacterium* suspension was 10⁶ cells/ml (○), 10⁷ cells/ml (□), 10⁸ cells/ml (△), and 10⁹ cells/ml (◇). The error bars represent the standard deviation of three independent experiments.

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CHAPTER VIII

SUMMARY AND CONCLUSION

the 1990s, the number of people in the world who are illiterate has increased from 1.2 billion to 1.5 billion. The number of illiterate people in the world is expected to reach 1.7 billion by the year 2015. The number of illiterate people in the world is expected to reach 1.7 billion by the year 2015. The number of illiterate people in the world is expected to reach 1.7 billion by the year 2015.

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1 SUMMARY

The potential of NIR transillumination and PS-OCT to improve upon the diagnostic abilities of existing technologies has been further demonstrated by the work using artificial and *in vitro* experiments presented in this thesis work. As mentioned in the first part of this work, new emerging technologies need to improve upon existing methods by (1) detecting enamel caries in earlier stages of development with high sensitivity and specificity, (2) allowing repeated longitudinal monitoring without an increase in health risk to a patient, (3) having minimal restrictions on surface type and morphology during imaging, (4) the ability to detect early recurrent decay under restorations, (5) imaging the depth resolved changes of the structure of a lesion, (6) quantifying lesion severity or overall mineral loss from caries demineralization and mineral gain after lesion remineralization, and (7) assessing the lesion activity, whether arrested or active, of early enamel caries.

1.1 NIR transillumination

According to the *in vitro* studies in this work, there are several important aspects where NIR transillumination improves upon existing diagnostic methods and technologies. NIR transillumination was shown to have significant advantages over visible light and x-rays to detect early enamel caries, with 1310 nm as an ideal wavelength for imaging due to the low scattering and absorption within enamel at this wavelength. Since NIR wavelengths do not pose health risks, NIR transillumination is designed as a real-time imaging system that can allow a clinician to reposition the tooth to acquire a series of images to better understand the extent of the decay. The

work in chapter 2 established for the first time that interproximal caries can be (1) detected at an earlier stage using NIR transillumination than visible light and x-rays. Concurrent studies, by Bühler et al, used a NIR system to detect occlusal caries with a set-up that involved acquiring images 90 degrees from planar focused light illuminating the cervical portion of a tooth (Bühler *et al.*, 2005). This occlusal NIR set-up, exploiting the low absorption of dentin to 1310-nm light and the multiple scattering in dentin to provide a diffuse illumination of the occlusal region, detected pit and fissure lesions that were unable to be identified with x-rays. Current work has used the 90 degree occlusal NIR set-up to detect interproximal decay from the occlusal region (D. Fried, unpublished). Since NIR transillumination is a projection image, a lesion that broadly extends buccal-lingual can appear deeper (mesial-distal) than reality, but by imaging the tooth interproximally and also from the occlusal, a clinician can determine if the lesion extends toward the dentin. Therefore, these concurrent studies along with this work demonstrate how a real-time imaging system can better detect and localize interproximal and occlusal lesions. NIR transillumination has the potential to (1) detect lesion *in vivo* earlier than x-rays, but future work is needed to determine the sensitivity, specificity, and predictive values. Also, more work is needed to study and optimize NIR system to have (3) minimal restrictions on surface type and morphology during imaging, especially through broad molar contacts. Future work is also needed to determine if NIR transillumination can (5) evaluate depth resolved changes and (2) monitor lesion progression of interproximal lesions. A caries lesion that continues to progress in depth during non-surgical management may need to be surgically restored to prevent excessive loss of tooth structure. NIR imaging is unable

to evaluate the depth resolved information of occlusal lesions and is unsuited for monitoring the activity of occlusal lesions during non-surgical therapy.

The ability of NIR transillumination to (4) detect early recurrent or secondary caries under restorations will depend on the type of material and NIR set-up. Much like bite-wing x-rays, the projection based NIR imaging is unable to detect early enamel lesions along the walls of occlusal and interproximal alloy restorations, since alloys greatly attenuate NIR light as they do x-rays. Porcelain restorations with metal oxides and glass ionomers with a high refractive index mismatch between the polyacid matrix and the glass powder will likely have similar limitations. Detecting early enamel lesions along the wall of interproximal composite restorations using NIR transillumination may be restricted by the optimized design to detect early enamel lesions. For NIR transillumination, cross polarizers are used to block out light that does not travel through the birefringent enamel tissue, which is necessary to prevent detector saturation from the intense illumination, and aids in detecting shallow enamel lesions. In Fig. 14 of Chapter 2, it is evident that the composite restoration appears dark like the enamel caries on the other side of the tooth. The caries highly scatters and attenuates the light producing the dark appearance on the NIR image. Composite materials without metal oxides do not significantly scatter or attenuate NIR light, as determined with PS-OCT studies of resin composites indicated in Fig. 5 & 9 of Chapter 3. In NIR transillumination, the composite material does not possess the birefringence that rotates the polarized illuminating light into the plane of the cross-polarizer (perpendicular) attached to the InGaAs FPA array. The light traveling through the composite restoration is blocked out from entering the lens of the camera

by the polarizer and appears dark. Identifying carious dark areas along the walls of the material would be very challenging with this set-up. A different NIR transillumination probe design, without polarization gating, that could locally scan the area of the composite may be needed in this specific circumstance.

It is unclear at this time if NIR transillumination has the potential to (6) quantify the severity or overall mineral loss of an interproximal lesion based on the level of overall attenuation of the NIR light. Present difficulties include calibrating the Indigo™ InGaAs focal plane array intensity values to a mineral loss value for a specific enamel thickness. In addition, the projection based imaging of NIR transillumination can not isolate the lesion from the entire enamel when recording the two dimensional images. Future studies are needed to determine if NIR transillumination can estimate the mineral loss level of interproximal lesions. Furthermore, it is yet to be determined if NIR transillumination can distinguish the subtle and micron changes near the surface of a lesion to (7) assess the lesion activity, whether arrested or active, of early enamel interproximal caries.

1.2 Polarization Sensitive Optical Coherence Tomography (PS-OCT)

PS-OCT significantly improves upon existing diagnostic methods and technologies to both detect and assess early enamel caries. Based on this *in vitro* work, occlusal lesions can be (1) detected at an earlier stage than conventional methods, such as bite-wing x-rays. The types of artificial lesions created in these studies are able to be detected by visual and tactile inspection, but as previously mentioned, these methods are unable to evaluate the depth of the lesion. PS-OCT is able (5) to evaluate the

depth resolved changes of an occlusal lesion and (6) quantifying the severity of the lesion by a simple integration of the perpendicular axis image.

Although the polarization sensitivity of PS-OCT adds additional complexity and cost to the system, a conventional OCT system is unable to quantify the severity of an early lesion because of the influence of surface reflection. Using linearly polarized light, PS-OCT is able to quantify the severity of caries lesions because the incident polarized light is depolarized into the perpendicular axis. PS-OCT systems that present intensity and phase information, and use circularly polarized light to eliminate the initial orientational effects, can measure enamel birefringence. However, the optical effects from enamel demineralization are not subtle; the magnitude of scattering and depolarization increases significantly after early demineralization (after only a few hours of pH cycling, Ch. 4, Fig. 2). The depolarization and scattering effects occur so rapidly, with only minimal mineral volume loss, that the polarization state of the light is almost entirely lost. Polarized Light Microscopy is often used in caries research studies to destructively image early enamel caries on tooth sections by recording the profound depolarization effects. The pathology of caries lesions does not gradually change the birefringence of the enamel; instead it causes an almost immediate increase in scattering and depolarization. Identifying the loss of polarization and phase does not detect caries lesions any earlier than recording the scattering and depolarization in a parallel and perpendicular axis image arrangement. With the perpendicular axis image, PS-OCT can quantify the decay unlike conventional OCT or the intensity and phase images of a PS-OCT system.

1. The first step in the process of the
2. is to determine the scope of the
3. project. This involves identifying the
4. objectives and the resources available.
5. The next step is to develop a plan of
6. action. This plan should outline the
7. steps to be taken and the timeline for
8. completion. It should also identify the
9. responsibilities of the individuals involved.
10. Once the plan is developed, the next
11. step is to implement it. This involves
12. carrying out the tasks outlined in the
13. plan. It is important to monitor progress
14. and make adjustments as necessary.
15. The final step is to evaluate the
16. results of the project. This involves
17. comparing the actual results with the
18. objectives and identifying any areas for
19. improvement. The evaluation should also
20. consider the overall impact of the project.

In this work, a high linear correlation ($r = 0.755$) was found between the increase in scattering and depolarization measured in the perpendicular-axis and a loss of mineral volume determined by Digital Microradiography (DM). As discussed in Chapter IV, this correlation applied to shallow artificial occlusal lesions. Ngaotheppitak et al. found a slightly lower correlation value ($r=0.68$) when examining natural shallow ($<500\text{-}\mu\text{m}$) white spot smooth surface lesions (Ngaotheppitak *et al.*, 2005). With even lower correlation values found for shallow pigmented lesions and a difficulty in precisely matching representative DM and PS-OCT images (Ngaotheppitak *et al.*, 2005), future *in vitro* work is needed with natural caries are needed to ascertain the relationship between mineral volume and a change in the perpendicular axis image. Initial studies with natural occlusal lesions have found strong linear correlations between the integrated reflectivity versus the integrated mineral loss to a depth of $500\text{-}\mu\text{m}$ (Ngaotheppitak *et al.*, 2006). A linear correlation may be found with natural occlusal lesions; however, this relationship may only apply up to a certain enamel depth. The exponential decay of the PS-OCT illuminating and backscattered signal as it penetrates through the tooth may need to be taken into account for deeper lesions. Nonetheless, these initial results to quantify the severity of a lesion with PS-OCT are encouraging, and work by our lab has shown that 'hidden' occlusal lesions can be detected and quantified with PS-OCT (Bühler *et al.*, 2005). In addition, artificial interproximal lesions scanned from the occlusal, through the marginal ridge, were detected with PS-OCT, demonstrating the PS-OCT can not only be used for occlusal surfaces, but interproximal surfaces as well (Ngaotheppitak *et al.*, 2006). These *in vitro* studies provide preliminary evidence that PS-OCT has (3) minimal restrictions on

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surface type and morphology during imaging. Future *in vivo* clinical studies will be conducted to determine the sensitivity, specificity, and predictive values of PS-OCT to detect early enamel lesions.

PS-OCT was shown in this work to have the ability to non-destructively (2) monitor enamel lesion progression on the occlusal surfaces. Before this *in vitro* work, PS-OCT was used to monitor the lesion progression on smooth enamel surfaces (Fried *et al.*, 2002). These studies showed that there was a definite increase in reflectivity measured in the perpendicular axis as the pH cycling process progressed over time. Future *in vivo* studies are planned to monitor enamel under plaque retentive environments to determine if early lesions can be detected and longitudinally monitored. Although the pH cycling *in vitro* lesions maybe different from early *in vivo* lesions, the *in vitro* progression studies need to be considered and also related to the remineralization studies in Chapters 6 and 7, because pH cycled lesions showed a high reflectivity after remineralization. The remineralized pH cycled lesions possessed a higher mineral volume. But polarized light microscopy showed that the surface zone and body of the remineralized lesions still highly depolarized light, and the optical properties were not restored (Ch. 6, Fig. 7). Under these circumstances, a lesion could progress over time and then remineralize, and be incorrectly assessed by the PS-OCT integrated reflectivity as progressing. However, PS-OCT was shown in this study to accurately measure lesion depth and showed that the remineralized lesions were not progressing in depth. Measuring lesion depth is an important adjunct with integrated reflectivity when assessing lesion severity and monitoring lesions over time.

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By measuring the surface zone remineralization and repair of surface softened lesions in Chapter 7, PS-OCT demonstrated the potential of (7) assessing the lesion activity, whether arrested or active, of early enamel caries. A well defined surface zone is indicative of an arrested lesion; however, more *in vivo* remineralization work is still needed. Interestingly, only a well defined non-depolarizing surface zone was formed when remineralizing the surface softened *in vitro* lesions; PS-OCT measured the thickness and decrease in reflectivity of this remineralized surface zone. As mentioned previously, the remineralization of the pH cycled lesions did not yield a restoration of the optical properties of the surface zone although mineral volume increased. PS-OCT measured an increase in the integrated reflectivity of these remineralized lesions. This work poses important questions that need to be answered in future work: What are the principle reasons the two lesions types had distinctly different outcomes after the remineralization treatment? Do the optical effects observed by PS-OCT after remineralization relate in anyway to the resistance of these lesions to future caries demineralization? Is epitaxial organized remineralization where the surface zone optical properties are restored a more protective repair? Future work is needed to investigate the differences in lesion porosity and analyze the remaining enamel crystal structure as possible reasons for the different remineralization effects. From this work, it appears that the differences in porosity may involve a shallow superficial layer, since the ΔZ values of the 40 μm surface zone were not vastly different between the two demineralized lesions types. Future work analyzing very thin lesion cross-sections ($\sim 80 \mu\text{m}$) will add additional precision when examining the first 5-10 μm surface regions by further reducing the edge effect in

microradiography. In addition, the use of index matching agents, such as glycerol and propylene glycol (Chapter V), to aid in assessing lesion activity based on the degree of porosity warrants further investigation.

An exciting prospect is that the surface softened artificial model should represent the *in vivo* enamel caries that should develop under plaque retentive environments, which will be tested in the near future. This would be an important step in clinically assessing the efficacy of anti-caries agents with PS-OCT during short term clinical trials.

PS-OCT was shown in this work to be able to (4) detect early recurrent or secondary decay in enamel under resin sealants and composite restorations. These artificial lesion studies will need to be followed-up with experiments examining natural lesions and *in vivo* studies. As mentioned with NIR transillumination, there will be challenges in examining secondary caries near amalgam, porcelain and glass ionomer and other ionomer restorations. By measuring the backscattering/reflected light, PS-OCT may be able to analyze the margins near these restorations much better than projection images.

2 CONCLUSION

The role of NIR transillumination is likely to be a detection system, capable of screening the dentition for early decay, with PS-OCT providing more valuable diagnostic information on lesion assessment, especially with occlusal lesions. As advances in non-surgical therapy develop, these two imaging systems will have complementary roles in detecting early enamel lesions and providing important

diagnostic information to aid a clinician in determining if non-surgical or minimally invasive restorative treatment is needed.

3 REFERENCES

Bühler C, Ngaotheppitak P, Fried D (2005). Imaging of occlusal dental caries (decay) with near-IR light at 1310-nm. *Opt. Express* 13(1):573-582

Fried D, Xie J, Shafi S, Featherstone JDB, Breunig T, Lee CQ (2002). Early detection of dental caries and lesion progression with polarization sensitive optical coherence tomography. *J. Biomed. Optics* 7(4):618-627.

Ngaotheppitak P, Darling CL, Fried D (2005). Measurement of the severity of natural smooth surface (interproximal) caries lesions with polarization sensitive optical coherence tomography. *Lasers Surg. Med.* 37(1):78-88.

Ngaotheppitak P, Darling CL, Fried D (2006). PS-OCT of occlusal and interproximal caries lesions viewed from occlusal surfaces. *Proc of SPIE, Lasers in Dentistry XII* 6137(1): in press.

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