SPECKLE REDUCTION IN AN ALL FIBER TIME DOMAIN COMMON PATH
OPTICAL COHERENCE TOMOGRAPHY BY FRAME AVERAGING

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SPECKLE REDUCTION IN AN ALL FIBER TIME DOMAIN COMMON PATH OPTICAL COHERENCE TOMOGRAPHY BY FRAME AVERAGING

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ABSTRACT

Optical Coherence Tomography (OCT) is a non-invasive, high resolution morphological imaging modality which has gained significance since its inception in 1992 due to its diversified areas of tissue diagnostics in medical imaging. Time Domain OCT (TDOCT) is one of its simplest commercially viable systems. Image noise in TDOCT has been fundamentally attributed to speckle and most of the commercial algorithms that tackle image noise are predominantly hardware related or utilize complex statistical procedures. Simple frame averaging is a cost effective, post processing algorithm whose effects on TDOCT systems have not been evaluated and hence there was an unmet need forming the basis of our investigation. To answer this need, the Niris® 1300e Imaging System, an all fiber common path TDOCT system was used. We investigated the efficacy of simple frame averaging using non-living and tissue phantoms containing various sizes and distributions of scatterers that give rise to random noise or speckle. Signal to noise ratio (SNR) was used to quantify the reduction in speckle noise for each averaged and un-averaged frames. The optical probe with an effective frame rate of 4 frames per second was used as part of our study. Our contention was to observe an increase in SNR for averaged frames hence providing an improvement when compared to un-averaged single frame. The second investigation was to observe the difference in SNR for 4, 8 and 12 averaged frames to depict that the improvement was dependent upon the number of frames averaged for analysis.
The four phantoms used for evaluation were- onion, skin from fingertip, oral mucosa and extracted teeth. We observed an improvement of upto 18% in SNR for 12 averaged frames, with the highest improvement in human fingertip frames. We also computed significant difference in 4, 8 and 12 averaged frames using ANOVA for evaluation. Thus, simple averaging was shown to be an effective speckle reduction algorithm in common path TDOCT.
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CHAPTER I

INTRODUCTION

Optical principles have been constantly utilized throughout history as a mode to generate reproducible diagnostic images addressing the development of non-radiological in vivo measurements. The continuous advancement in optical technologies, including lasers as a source of illumination and fiber optics aiding internal organ imaging, have been the driving force behind the development of multiple imaging modalities. These modalities take advantage of the characteristic strength of optical principles and technology to enrich the field of diagnostic imaging. Optical Coherence Tomography (OCT) has attracted attention of academia and industry experts working in the photonics field since it is the first diagnostic imaging technology which features coherent optics [1]. OCT, commercially born only a few decades ago, is an imaging modality that performs high resolution, cross sectional tomographic imaging of internal microstructure in materials and biological tissue, by measuring backscattered or back reflected light from an object depth. With a near infrared light source, image spatial resolution of 1-15μm can be achieved, performed both in real time and for in vitro measurements. The ability of OCT to provide higher resolution, albeit lower imaging depth, in comparison with high frequency ultrasound imaging has not stayed unnoticed [2].
The applications of OCT are ever expanding from its principal roles in ophthalmology to branching out as a viable platform for image guided surgery for other anatomical sites. OCT is also emerging as an invaluable tool for cancer diagnosis and treatment because of its ability to image superficial tissue changes in real-time at the point of care [3]. Its applications along with its relative simplicity and lower cost of hardware still face a number of technical problems, which might limit its ability to propagate as an established diagnostic imaging modality. One of the critical parameters that lower its clinical diagnostic capability is its susceptibility to the noise components of speckle.

All the coherent modalities, including OCT, are plagued by speckle. Since its first investigation as a laser speckle, studies have been focused on the origin of and algorithms that aid its suppression. The study regarding its origin shows that speckle can be both signal carrying and signal degrading. Hence, its suppression can often involve complicated methodologies [4]. Dealing with speckle as one of the major contributing factors to image noise has led to a myriad of algorithms and hardware manipulations across multiple domains [5]. The solutions proposed either perform sophisticated numerical procedures on the images using pre and/or post processing software, or manipulate the acquisition settings to get the desired level of speckle reduction [6].

Methods including spatial compounding[7], angular compounding[8], frequency compounding[9], strain compounding[10], and single B scan filtering[11] have been implemented on research and commercial platforms as viable solutions to speckle reduction under multiple domains in OCT. Although these methods have established their effectiveness, they rely on complex statistical models and hardware manipulations for speckle suppression. Software algorithms used for these purposes are primarily
focused on frame averaging and wavelet denoising [12]. The most commercially used method for speckle suppression in clinical OCT systems is frame averaging, since its implementation does not need specialized hardware and its effectiveness in speckle reduction is equivalent to, if not better than, the other methods mentioned above [5].

Simple frame averaging takes advantage of the fact that if the pixel values are averaged for consecutive frames, the background noise caused by random variation can be visibly diminished, assuming that the random variation arises due to speckle noise. Increasing the number of averaged frames decreases the random variability and hence decreases noise. Although, after a certain number of frames, it is difficult to see any further practical advantage in frame averaging since it tends to diminish edge sharpness by over averaging the pixels causing blurring or smearing effect. Signal to noise ratio (SNR) can be used to evaluate the effective image quality on the basis of pixel averaging and overall noise reduction. Successful application of simple frame averaging is contingent upon a number of factors, including frame acquisition rate which determines the variability across consecutive frames. Systems with high frame acquisition speed have an almost identical structural pattern across consecutive time frames leading to low variability in signal, since small motion between repeated frames can lead to relatively identical signal and speckle noise patterns. The fundamental attributes of speckle across multiple domains remains unchanged and hence its fundamental characteristics and physical attributes can be unified to understand the effect of frame averaging under different domains. Frame averaging has been evaluated for multiple OCT systems in the spectral domain, with changes applied along the frame acquisition axis to slow down the frame rate and hence manipulate the speckle pattern across different frames [5]. But, frame
averaging has not been evaluated for the common path TDOCT systems, which have significantly different image acquisition and processing parameters, albeit similar statistical attributes to speckle. The question remains if simple frame averaging can increase the SNR for a common path TDOCT.

1.1 Research hypotheses and objectives

The hypotheses addressing the questions raised above are the following:

1. Simple frame averaging shows an improvement in SNR upto 15% for the maximum number of averaged frames for a given phantom with perpendicular probe angle.

2. Improvement in SNR will depend upon the number of frames (ex: 4, 8 and 12) considered for simple frame averaging.

The overall objective of this research is to evaluate the aforementioned hypothesis for simple frame averaging. The specific objectives laid out for this research were the following:

1. Acquire frames from different phantoms ranging from non-living to tissues with varying constituents and matrices.

2. Analyze the SNR using different number of frames for frame averaging (ex: 4, 8 and 12).
1.2 Scope of the Research

The motivation behind this project was to understand the noise characteristics of an all fiber implementation of common path OCT system through simple post processing experiments. The experiments performed are aimed at recognizing speckle in OCT images and quantifying the efficacy of speckle reduction. The objective is to gain better insight in understanding the random variation caused by speckle and the extent to which these variations can be diminished by frame averaging. Different tissue types with varying scatters were used as a part of this research, so as to bolster the efficacy from a biomedical standpoint. Thus, if favorable results are observed, the scope of this project can be expanded into further applications and across domains.
CHAPTER II

LITERATURE REVIEW

2.1 Origin of Optical Coherence Tomography

Optical Coherence Domain Reflectometry (OCDR) is often attributed as the precursor to the principles behind the design and implementation of OCT. The telecommunication industry used OCDR techniques extensively to characterize an optical fiber by locating defects within the fiber cables and its network components [13]. The working principle involved measurement of back reflected light pulses, whose strength was integrated over time and this function was plotted to reveal the length of the fiber. This process also provided an estimate for attenuation through fiber losses and defect location. However, it was soon mainstreamed into the medical field for biological diagnostic purposes, due to its potential to provide high resolution images of internal microstructures in a highly scattering media. Its application in medicine was reported around 1991 with its first data reported by Dr. Huang in Science, demonstrating a coherence gating technique to enable ex vivo visualization of human retina in real time [1]. The first OCT system commercially introduced in 1996 was a time domain system. The main application of OCT still remains in the field of ophthalmology and burgeoning field of optical cancer diagnosis which in combination covers almost 90% of the current OCT market.
It acts as a strong diagnostic tool for eye diseases since ophthalmology demanded a non-invasive visualization system with high level of precision for corneal and retinal pathologies. The appeal of OCT lies in its simplistic usage by retinal specialists as a diagnostic tool for various macular diseases and retinal thickness assessment along with early onset of glaucoma [14]. OCT has proved its worth in cancer diagnosis due to its sensitivity to tissue structural changes caused by early stages of pre-cancer. Emerging OCT markets include image guided surgery and needle biopsy for in vivo cancer treatment and detection [15]. In contrast to taking a biopsy, it is possible to take a great number of measurements quickly and noninvasively, guiding surgery and other therapies and lowering the possibility of iatrogenic injury. Figure 2.1 shows the stance OCT takes with respect to other leading imaging modalities.

![Figure 2.1 Current medical imaging technologies and their corresponding depth of penetration and resolution](image)

There is one major fundamental limitation to OCT: depth of light penetration as shown by Figure 2.1. Human tissue contains many components that naturally reflect near
infrared light (NIR), thus limiting the ability of NIR to penetrate tissues to only ~1.5-3 mm. NIR-opaque structures and substances restrict depth penetration. NIR-transparent structures backscatter less and allow for greater depth penetration. The light penetration limitation does not preclude the visualization of superficial epithelial structures. Early epithelial cancers develop between the surface and 1.0 mm, well within the limits of NIR penetration depth. However, OCT has limited ability to discern invasion beyond the muscularis propria.

2.2 General Working Principles

Analogous to B mode Ultrasound imaging, Optical Coherence Tomography uses a light source to construct tomographic images by measuring axial distance or range information, essentially the echo time delay and intensity of backscattered or back reflected light [16]. Since echo time delay is measured to determine the distances within the tissue, ultrafast time resolution is required for image acquisition. Distance or spatial information may be determined from the time delay or reflected echoes according to the formula:

\[ z = 2\delta T \cdot v, \]

where \( \delta T \) is echo delay, \( z \) is the distance the echo travels and \( v \) is the velocity of light. This equation shows that for a resolution of 10 μm which is typical for OCT, we need an extremely fast time resolving system, of the order of femto seconds. Hence, correlation techniques are used that compare the backscattered light signal to a reference light signal travelling along a known path length.
Detection technique at the crux of OCT is low coherence interferometry, which measures the combined field of an optical beam based on path length difference [17]. Typical interferometry of this kind, a Michelson interferometer, is as shown in the schematic in Figure 2.2.

![Simple Michelson setup](image)

Figure 2.2 Simple Michelson setup

The broadband source used is typically a superluminescent diode (SLD) of a center wavelength of 830 or 1310nm, depending on the application of OCT. Typically, OCT uses 830nm as the center wavelength in ophthalmic applications which gives higher resolution consistent with the depth of penetration required for visualizing eye structures. Most of the eye tissue is transparent until the retina is reached. Non-ophthalmic OCT systems use 1310nm for better penetration into opaque tissue. Longer wavelengths than this are unsatisfactory because of water absorption in opaque tissues. During the scanning operation, the power of the diode is split using a beam splitter into two different arms - a reference arm and a sample arm. The light reflected by the sample (Is) and the reference
(Ir), which typically constitutes a moving mirror is collected by the beam splitter and focused onto a photo detector.

The advantage of using a finite coherence length source or a short coherence length source like an SLD is explained by coherence theory, which shows that interference patterns are observed only when the optical path length of the beams reflected by the sample and the reference mirror differ from less than the coherence length. Longitudinal or in depth cross sectional image is a function of the position of the mechanical movement of scanning mirror which is a typical “reference” in time domain OCT. This movement provides the optical path variation as dictated by the coherence theory. It is incrementally moved to measure the amount of backscattered light at corresponding depths throughout the sample. A cross correlation function between the two light fields yields the signal at the detector (Id), typically a photodetector. A single A-line is formed by in-depth scanning (1D) and a 2D image is formed by collecting and displaying multiple A-lines in a single plane. The light is confined by an optical lens system and focused in the region of interest [18].

The setup explained above defines a typical Michelson interferometer which uses the modulation of optical path length through the moving length of the reference arm. As explained by interference theory, phase modulation of the reference arm allows an interference pattern to be formed revealing reflections (data) at different depths within the sample. High speed frame acquisition of these depth resolved measurements require high speed interferometry. This can be achieved with the mechanical movement of a mirror or
rotating prism, but these are relatively expensive precision electrically driven optical mechanisms. Other methods like piezoelectric fiber stretchers and rapid scanning phase control delay lines have been designed for high speed applications like video rate OCT imaging. The Niris uses a fiber stretcher employing a piezoelectric material, typically a ceramic, to stretch and compress the optical fiber, hence changing optical path length. This method is mechanically stable but is susceptible to thermal instability [19-20].

The advantage of a common path interferometer or “autocorrelator” with an all fiber implementation is lack of a separate reference arm [21]. A refinement of common path is observed in Niris, where the reference signal is derived from the probe fiber tip (distal end). A small (< 2%) reflection here provides the reference. This has the added advantage of probes for endoscopy and any other application where probe flexibility is needed without dispersion and other artifacts interfering with the signal, such as fading with flexing of the probe. Further, this reduces probe production costs because the probe can be any length desired and there is no tolerance constraint in manufacturing.

2.3 Image Generation in OCT

Figure 2.3 depicts a typical OCT two dimensional (2D) acquisition or B-scan. To acquire a 2D cross sectional image, an incident optical beam is scanned laterally acquiring backscattering profiles (A-lines) from every axial position. Each A-line which is one dimensional represents the magnitude of reflection or backscattering of the optical beam as a function of depth in the tissue. A B-scan is made up of both axial (z-axis) and lateral (x-axis) measurements giving the acquired image a 2D representation. As shown in the
figure, the signal intensity varies as a function of imaging depth which represents a 2D signal with depth resolved components. Since the backscattered signal varies over five orders of magnitude, the logarithm of the signal is used to display the image, typically ranging from -50dB to -100dB, where the system approaches its sensitivity limit. This is accomplished by using a logarithmic amplifier at the processing stage after the backscatter generated photocurrent is detected by the photodetector. An analog to digital convertor (ADC) is used in order to digitize the signal and display the pixilated image as shown in the figure. The brightest white pixels in the image corresponds to the highest reflection or backscatter in the signal, while the black level depicts the weakest backreflection, or none.

Figure 2. 3 Image acquisition in common path time domain OCT

2.4 Characterizing Speckle Noise in an OCT System

Imaging modalities that primarily rely on coherence, e.g. radar, ultrasound, OCT, etc., suffer from an insidious form of noise called speckle. During the early discovery of
speckle, it was found that the reflection of a laser beam from a rough surface forms dark and bright spots giving it a mottled appearance, which change their pattern relative to the motion of the surface. Researchers declared this as an act of random interference between reflected waves that were mutually coherent. The size and temporal coherence of the light source used was an influential factor in speckle formation, along with multiple back scattering and phase aberrations of the beam propagating inside the surface [4]. These factors were also contributing to the speckle pattern observed in OCT.

2.4.1 Origin of Speckle: Optical Field Interaction

The variation in optical path of reference with respect to the sample which is placed at the focus of a converging lens generates the interferometry signal in OCT. This signal is essentially in the form of an ac current or a photocurrent, detected at the output of the photo-detector in an interferometer setup. This photocurrent, $i_d$, is proportional to the real part of the cross correlation product of the reference and sample optical field as:

$$i_d \sim \text{Re} \langle U_r U_s^* \rangle$$

where the optical reference field ($U_r$) and optical backscattered field ($U_s$), obtained from the sample, averaged over time and space which is denoted by brackets $\langle \rangle$. Note that the photocurrent is dependent on the optical field.

This photocurrent, $i_d$, depends upon both phase and amplitude of the cross correlation of the scattered and reference optical fields, $U_s$ and $U_r$, respectively. This dependency on phase is the root cause for the existence of speckle in OCT [4].
2.4.2 Origin of Speckle: Light Tissue Interaction

Figure 2.4 shows two forms of interaction that can take place when the penetrating light interacts with the tissue, as depicted by J. M Schmitt in Ref-4. In the figure, the sample volume represents the area of interest that needs to be imaged. Light has to propagate through multiple scatterers in the form of tissue constituents to finally reach the sample volume and propagate back to the detector with the relevant sample information. Hence, two forms of light propagation from the beam are observed; 1) forward propagation to the sample in the form of incoming wavefront and 2) back propagation from the sample eventually reaching the detector in the form of a returning wavefront. Since tissue interaction involves more than two scatterers, not necessarily from the sample volume, we are likely to see the following interactions; 1) random delay caused by multiple forward scatter distorting the incoming and returning wavefront and 2) multiple backscatter from the sample volume that distorts the returning wavefront. In both these cases, localized regions of constructive and destructive interference are observed before the returning wavefront is detected at the photodetector leading to speckle. The only condition that needs to be satisfied for scatterers to develop into speckle patterns is that two or more scatterers in the sample volume should backscatter waves that reach some point on the detector, out of phase within an interval of time less than the coherence time of the source.
Thus, speckle is characterized as noise because of the observed distortion of returning wavefront and reducing the correspondence between the local density of scattering particles and intensity variations in OCT images. This speckle noise degrades image quality by decreasing signal to noise ratio. Diagnostic capability of the system might be limited because low level signals, which result from low intensity backscatter, cannot be distinguished from speckle noise. Also, since speckle blurs the boundaries, segmentation becomes increasingly tricky. Thus, speckle suppression becomes vital to enhance diagnostic image quality.

2.5 Existing Solutions for Speckle Reduction

Based on the definition of speckle in section 2.4, reduction techniques can be divided into four primary categories; 1) spatial compounding, 2) frequency compounding, 3) strain compounding, and 4) image post processing techniques. Spatial or angular compounding averages the magnitude of the signal derived from the sample volume and slightly
displaced sample volume. This slight displacement is obtained by changing the illumination angle or angle of incidence to the sample [8]. Frequency compounding averages the speckled images recorded within varying optical frequency bands, which manifest reduced correlation from each other [9]. Strain compounding attempts to decorrelate different B scans by introducing the sample to varying strain conditions [10]. All the above mentioned techniques mandate a hardware design change for its successful implementation.

Image post processing techniques can either refer to all the methods that are applied after the image is formed or methods that are applied directly to the complex interference signal before the image is formed. The most popular ones are; 1) median filtering [22], 2) homomorphic Weiner filtering [23], 3) multi-resolution wavelet analysis [24], 4) adaptive smoothing [25] and 5) simple frame averaging. The first four methods are known to incorporate statistical models that distinguish the target from its background. Simple frame averaging has been implemented across multiple domains due its simplistic approach as a viable post processing technique [26-30]. The popularity of frame averaging lies in the fact that it attacks the random variation in pixels thereby decreasing the standard deviation, which quantifies the effective noise in an image. Simple frame averaging assumes speckle noise to follow a Gaussian distribution and hence its implementation has shown significant improvement in signal to noise ratio.
CHAPTER III
METHODOLOGY

The objective of this study was to evaluate simple frame averaging as a viable post processing algorithm for speckle reduction in common path TDOCT, along with investigating the number of frames to be averaged. Both the objectives were to be evaluated using a non-living (onion) and human tissues (e.g. skin, oral mucosa, and extracted teeth) available in vitro and in vivo which aids the demonstration of possible clinical implications.

The steps involved were

1. Acquire frames from a well calibrated system for four phantoms of interest and record the raw data generated during acquisition.

2. Process the raw data in order to compute simple frame averaging for 4, 8 and 12 averaged frames using a VB script for macros in Microsoft Excel 2003.

3. Color map the averaged data using ImageJ toolbox for visual evaluation.

4. Compute frame signal to noise ratio for base and the averaged frames.
3.1 Experimental Setup

The imaging system used for the purpose of this experiment was a common path TDOCT system, Niris 1300e (Imalux corporation Inc, OH USA), as shown in Figure 3.1. This system constituted a laptop computer, an imaging console and a long detachable probe.

The Imaging Console contains optical and electrical components that make up the image acquisition and interferometry system along with other system components, including a super luminescent diode (SLD) as the source for interferometry. The SLD provides Near Infrared light (NIR), which is directed from the console through the probe’s optical fiber tip to the sample of interest.

The optical light is backscattered from the sample, collected by the probe’s fiber and combined with an internal reference signal from the tip of the probe fiber. An in-depth profile (A-line) of the sample is generated by the mechanical movement of fiber stretchers. This combined with the lateral movement of the optical fiber using an electromagnetic device in the probe tip produces a high spatial resolution 2D image of the sample.

The fiber optic probe relays light between the sample and the console where this backscattered light is collected and analyzed by the interferometer in the console unit. This probe is reusable and has an external optic connector. Along with relaying of light, it also contains an electromagnetic mechanism to move the beam laterally back and forth.
The Niris 1300e system contains a light source that has a central wavelength of 1310 nm. The system frame rate is 8 frames per second (fps). This frame rate is achieved by driving the probe electromagnetic device at 4 Hz and acquiring frame during both sweep directions, up and down. However, in order to eliminate any errors that might be introduced by the non-linearity of the electromagnetic device, only frames acquired in the same sweep direction were used. This was accomplished by using every other frame acquired by the system, which effectively reduced the system frame rate to 4 fps.

The system is designed to provide the following scanning range; depth of 1.6 mm in tissue (2.2 mm in air) and a lateral range of 1.6 to 2.2 mm with a standard, forward viewing probe of 2.7 mm diameter. The flexible probe can be up to 4 meters long with
protective tubing for the optical fiber and electrical wires inside. The minimum bend radius of the probe is 2.5 cm. Optical power from the probe connector or probe tip is less than 15 mW. The axial resolution is between 10 to 20 μm. The beam width is directly governed by the numerical aperture (NA) of the probe lens which provides a lateral resolution of up to 25 μm in air.

3.2 Phantoms used for acquisition

The sections below explain the four types of phantoms used for our investigation; 1) onion, 2) extracted human teeth, 3) human fingertip, 4) oral mucosa/cheek tissue. The image acquisition and frame averaging algorithm have been explained in conjunction with all of the phantoms. The setup was identical and reproducible across all the phantoms. The same system was used for imaging all four phantoms. The system and probe were calibrated for operating consistencies.

3.2.1 Onion

The study was conducted using an onion as our phantom of interest, since its optical properties are well known. Also, since water is one the main constituents of onion, its visualization along with different structural components that make up its layers are easily distinguishable. Onion also fit our need to assess simple frame averaging on a relatively
3.2.2 Extracted Human Teeth (Dental)

OCT has proved to provide a safe and effective method for imaging dental microstructures for the evaluation of dental health from various clinical researchers. Dental tissues have been the subject of OCT investigations before [31]. This study attempted to investigate the feasibility of simple frame averaging for common path TDOCT in dentistry. The anatomy of a human tooth and connected soft tissues is depicted in Figure 3.2 below. Each tooth has a section that protrudes from the gum and has at least one root below its surface. The upper part corresponds to the crown (top of the tooth) which consists of enamel and dentin. The enamel is the hardest tissue of the body consisting of mostly calcium. The enamel is organized into hard rods or prisms that are 4-6 μm in diameter, with heavy inter-prismatic material in between. The rods begin upright on the surface of the dentin, with a heavy inclination to the side. Dentin is predominantly made up of collagen. The pulp at the neck is a highly vascular structure with an extensive nerve component. It is supported by loose connective tissue. Blood vessels and nerves enter through the root canal [32].

Soft material with higher penetrability to near infra red light.
Since dental tissues are dense and anisotropically oriented, the scattering distribution within these tissues is highly orientation dependent. This made for an interesting phantom for our investigation. We considered two teeth, incisor and a diseased molar, to be our dental tissues for this investigation.

3.2.3 Skin from Fingertip

Skin from human fingertip was used as the third phantom of interest. Positioning and acquisition simplicity of a human fingertip is the reason why this was chosen as a phantom for evaluation of simple frame averaging on living tissues. The optical properties of finger and its underlying components are well documented [33].
3.2.4 Oral Mucosa

The oral mucosal membrane covers most of the oral cavity [34]. Lining mucosa or buccal mucosa is associated with the lining of the cheeks. A previous study by Felix Feldchtein et al., published in 1998, provided the first record of in vivo OCT images of hard and soft tissues in the oral cavity. The study was focused on the different types of healthy oral mucosa and OCT’s ability to diagnostically differentiate structural information on pathological conditions inside the oral cavity. In this study, we focused on healthy oral mucosa and attempt to evaluate the efficacy of simple frame averaging on OCT images of mucosal layer.

3.3 Image acquisition setup

A simple setup was used for frame acquisition using the above four phantoms as shown in Figure 3.3 below. The setup shows the real time acquisition of human teeth in vitro. The tooth under investigation is carefully mounted on high precision positioner as shown on the right side of the image. After positioning the tooth, the probe is equivalently placed with the probe tip carefully secured in its place to avoid discrepancies in acquisition due to location changes. The tip of the probe should be in contact with the location of interest on the phantom. The connecter end of this probe is carefully plugged into the console as shown. Image acquisition is displayed by the laptop which shows the acquisition parameters on the left side of the screen and real time frame displayed on the right side of the screen.
Other phantoms (onion, oral mucosa and finger) were imaged without the aid of a positioner due to their relative stability and in vivo availability. The rest of the setup was reproducible from Figure 3.3.

Figure 3.3 System setup for frame acquisition of extracted teeth using Niris 1300e

An effective frame rate of 4 frames per second (fps) was maintained throughout the frame acquisition. The probe tip placed perpendicular to the surface of all the phantoms for frame acquisition. For all the phantoms, at least 12 frames were acquired for further processing.

Imaging of the diseased molar was done at a location where demineralization was apparent on its enamel surface that resulted in discoloration. These in vitro samples were stored in isotonic saline solution.
Data was acquired in two formats for processing, raw in comma separated value (or .csv) format and grayscale images in portable network graphic (or .png) format. The raw data contained 256 A-lines with 192 depth resolved measurements.

3.4 Image Assessment Parameter for Speckle Reduction

The system used for the investigation was previously calibrated and no instrumentation or system parameter changes were made or observed across the frame acquisition for all four phantoms. It can also be assured that the acquisition setup was not manipulated and no probe saturation (vertical black bands in the image) was observed during frame acquisition. The frames are identical and reproducible for their respective phantoms and no significant motion artifacts were encountered during acquisition.

The assumptions made to quantify the speckle reduction are based on the following:

a. Signal is almost identical across consecutive frames, providing the basis for frame averaging without losing spatial resolution. This is safe to assume since data is oversampled to provide consistency between consecutive A-lines in a single frame. If the data is assumed to be identical, the effective pixel mean across multiple averaged frames should stay consistently similar.

b. The random variation observed in A-line plots is now assumed to be from some form of speckle, which is spread randomly across the entire frame. Since this type of speckle is random, it is assumed to follow a Gaussian distribution which can be proved if frame averaging shows any difference across multiple averaged frames. Increasing the number of averaged frames decreases the random variability in
pixels and standard deviation is used to quantify the decrease.

The idea behind pixel by pixel frame averaging can be explained by a simple example below:

If $A = \begin{bmatrix} 1 & 0 & 1 & 0 \end{bmatrix}$, a 1x4 matrix, and $B = \begin{bmatrix} 0 & 1 & 0 & 1 \end{bmatrix}$ (another 1x4 matrix), then an average of matrices $A$ and $B$ represented by $C$ would be $\begin{bmatrix} 0.5 & 0.5 & 0.5 & 0.5 \end{bmatrix}$. Each element in matrix $C$ represents the average of corresponding elements across $A$ and $B$.

Simple frame averaging is based on the idea discussed in the example above. For our investigation we performed pixel to pixel averaging for frames with 256x192 data points (elements). Since data representing signal did not vary from frame to frame due to oversampling, it was assumed to be identical as shown in assumption "a" above. The effective mean of the pixels would stay relatively consistent across various averages for each individual phantom.

Standard deviation is a measure of variation between the pixels from the mean or expected value for the sample. A low standard deviation would imply that the pixels are closer to the mean and a high standard deviation implies that the pixels are far apart from the mean. This relative variation between the pixels is assumed to be due to random variation of speckle which can be deduced by standard deviation of pixels.

Hence, frame signal to noise ratio was the parameter of interest, chosen to evaluate speckle noise reduction. This was computed as:

$$\text{SNR} = \frac{\mu}{\sigma}$$
Where $\mu$ is the effective pixel mean of the entire image and $\sigma$ is the standard deviation of the pixels.

Each pixel in the image quantifies the backscatter intensity from the sample which is lowered by the amount of signal degrading speckle. SNR has always been the gold standard to quantify image quality.

For visual evaluation of speckle reduction, we investigated the following:

a) Visible reduction in background noise in averaged frames in comparison with its base frame.

b) Low level signal enhancement due to reduction in random noise.

In OCT images, a low level signal represents low intensity pixel which is not visibly distinguishable from speckle or random noise with relatively similar intensity level. This can cause blurring of structures and reduce diagnostic capability. We investigated the efficacy of simple frame averaging by observing a reduction in structural blurring and relative contrast enhancement.
In this study, a technique to reduce the speckle in common path TDOCT was successfully developed. The phantoms selected for the evaluation of the simple frame averaging as a viable speckle reduction technique ranged from non-living to human tissues with tissue matrix variability. This study categorized the efficacy of simple frame averaging across soft (human skin and oral mucosa) and hard (human teeth) tissues.

The following processing steps were followed for computation of signal to noise ratio:

1. Gathered raw pixel by pixel data (.csv files) along with image (.png files) during frame acquisition. The raw data required Microsoft Excel for processing.
2. Computed pixel by pixel averaging for 4, 8 and 12 frames using raw data from each phantom. The averaging was performed in Microsoft Excel 2003 using a customized VB script written as a macro.
3. Signal to noise ratio (defined earlier) was computed using effective pixel mean and standard deviation for the entire frame.
4. The averaged data was processed using ImageJ and color mapped to provide the visualization setup equivalent to the system.
5. Comparison of the results observed across different frame averaging for all the phantoms.
The raw data, which are generated in comma separated value (.csv) format, was used for our investigation because it consists of A-lines which were not color mapped or down-sampled for display purposes.

This data consisted pixel values between the range of 0-1024, since the analog to digital converter (ADC) used for image processing inside the console was 10 bit. These pixel values are down-sampled by 2 bits when image is displayed onto the screen, to get an 8 bit image. To avoid the effects of down-sampling, we decided to use the 10 bit raw data.

Microsoft Excel 2003 was used to analyze and process the raw data for the four phantoms of our investigation. We created a macro to automate the analysis and decrease computation complexity. This macro computed the frame averaging of different frames by averaging the pixels from their corresponding cells.

Single A-line plot was selected as the best graphical representation to depict signal to noise characteristics. Random noise corrupts this A-line by reducing the distinction between backscattered bright signals and the noise floor. This plot was consistently mapped across 4, 8 and 12 frames to show the effects of frame averaging on each individual A-line.

The averaged values were saved in text-tab delimited format (.txt) and imported onto ImageJ for display. Once the image was successfully imported, we adjusted the window level parameters by setting the center as 512 and width as 1024, the limits of 10 bit ADC in the system. Other image parameters were adjusted to mimic the display characteristics of the system.
The signal to noise ratio improved from the base frame as we increased the number of frames that underwent frame averaging. All the plots for A-line were acquired from 128th column of the raw data and plotted using Microsoft Excel data plot function.

Figures 4.1a-h represent the base frame of an onion, 4, 8, and 12 frames of onion subjected to frame averaging respectively. Figures 4.1e-h depicts the representative A-lines for the frames shown in Figures 4.1a-d respectively. The A-lines depict the variation in random noise that obscures the low level signal in the base frame and reduction of this random noise as the number of averaged frames increases from four to twelve.

Figures 4.2a-b show the base frame of human skin from a fingertip with no averaging and 12 frames of the same region subjected to frame averaging respectively. Figures 4.2c-d depicts the representative A-lines for the frames shown in Figures 4.2a-b respectively. These A-lines depict a significant reduction in random variation obscuring the signal as number of averaged frames increases. For images depicting 4 and 8 averaged frames, please refer appendix B.

Figures 4.3a-b represent the base frame of oral mucosa imaged in vivo with no averaging and 12 frames of the same region subjected to frame averaging respectively. Figures 4.3c-d depicts the representative A-lines for the frames shown in Figures 4.3a-b respectively. These A-lines showcase the reduction in random noise variation. For images depicting 4 and 8 averaged frames, please refer appendix B.

Figures 4.4a-b represent the base frame for human molar imaged in vitro with no averaging and 12 frames of the same region subject to averaging respectively. Figures 4.4c-d depicts the representative A-lines for the frames shown in Figures 4.4a-b respectively. For images depicting 4 and 8 averaged frames, please refer appendix B.
Figures 4.5a-b represent the base frame for human incisor imaged in vitro with no averaging and 12 averaged frames respectively. Figures 4.5c-d depicts the representative A-lines for the frames shown in Figures 4.5a-b respectively. These A-lines showcase the reduction in random noise variation. For images depicting 4 and 8 averaged frames, please refer appendix B

Table 4.1 shows the effective means and standard deviations for the base frame along with the averaged frames for each individual phantom with perpendicular probe orientation. The frame signal to noise ratio (SNR), used to demonstrate the deviation of low level noise from the effective mean which represents the high level signal in the frame, is taken as the ratio of effective mean to the standard deviation of the pixels (assuming Gaussian distribution). The improvement shown in percentage depicts the variability of averaged frame SNRs from the base frame for individual phantoms. As shown, there is a significant improvement in frame signal to noise ratio across all the phantoms. This improvement was consistently observed and reproducible. The highest percentage of improvement was shown by the human skin from a fingertip averaging, whereas, the lowest percentage was observed in the dental tissue averaging. Hence, a high level of speckle reduction was achieved.

Figure 6 shows the SNR plot for perpendicular data summarizing the tables from above.
4.1 Phantom 1: Onion

Figure 4.1 a (top-left) A single frame from Onion—the base frame for further averaging.

Figure 4.1 b (top-right) An average of 4 frames from Onion.

Figure 4.1 c (bottom-left) An average of 8 frames from Onion.

Figure 4.1 d (bottom-right) An average of 12 frames from Onion.
Figure 4.1 e (top-left) A single A-line from onion base frame representing the distorted low level signals.

Figure 4.1 f (top-right) A single A-line from the above 4 frame averaged raw data.

Figure 4.1 g (bottom-left) A single A-line from the above 8 frame averaged raw data.

Figure 4.1 h (bottom-right) A single A-line from the above 12 frame averaged raw data.
Figure 4.2 a (top-left) A single frame from fingertip-the base frame for further averaging

Figure 4.2 b(top-right) An average of 12 frames from fingertip

Figure 4.2 c(bottom-left) A single A-line from the above base frame raw data

Figure 4.2 d(bottom-right) A single A-line from the above 12 frame averaged raw data
4.3 Phantom 3: Oral Mucosa

Figure 4.3 a(top-left) A single frame from Oral mucosa -the base frame for further averaging

Figure 4.3 b(top-right) An average of 12 frames from oral mucosa

Figure 4.3 c(bottom-left) A single A-line from the above base frame

Figure 4.3 d(bottom-right) A single A-line from the above average of 12 frames
4.4 Phantom 4: Dental Tissue: Molar

Figure 4.4 a (top-left) A single frame from an extracted molar, imaged in vitro, used as a base frame.

Figure 4.4 b (top-right) An average of 12 frames from an extracted molar.

Figure 4.4 c (bottom-left) A single A-line from the above un-averaged base frame.

Figure 4.4 d (bottom-right) A single A-line from the above 12 frame averaged raw data.
4.5 Phantom 4: Dental Tissue: Incisor

Figure 4.5 a(top-left) A single frame from an extracted incisor, used as the base frame for further averaging

Figure 4.5 b(top-right) An average of 12 frames from an extracted incisor

Figure 4.5 c(bottom-left) A single A-line from the above single frame averaged raw data

Figure 4.5 d(bottom-right) A single A-line from the above 12 frame averaged raw data.
Table 4. Signal to noise ratio (SNR) for the four phantoms with perpendicular probe orientation. The % improvement depicts the increase in SNRs for the averaged frames compared to the base frame. It was calculated as the percentage difference between the SNRs of averaged frames to the SNR of base frame.

<table>
<thead>
<tr>
<th>SN #</th>
<th>PHANTOM TYPE</th>
<th>FRAME TYPE</th>
<th>EFFECTIVE PIXEL MEAN</th>
<th>STANDARD DEVIATION</th>
<th>SNR</th>
<th>% IMPROVEMENT IN SNR FROM BASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NON-CLINICAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>ONION</td>
<td>No Average-Base</td>
<td>243.44</td>
<td>78.87</td>
<td>3.09</td>
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<tr>
<td>3</td>
<td></td>
<td>Average of 4 frames</td>
<td>243.54</td>
<td>70.98</td>
<td>3.43</td>
<td>11.15</td>
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<tr>
<td>4</td>
<td></td>
<td>Average of 8 frames</td>
<td>243.47</td>
<td>69.53</td>
<td>3.5</td>
<td>13.44</td>
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<tr>
<td>5</td>
<td>HUMAN SKIN FROM FINGERTIP</td>
<td>No Average-Base</td>
<td>295.62</td>
<td>83.41</td>
<td>3.54</td>
<td>N/A</td>
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<tr>
<td>6</td>
<td></td>
<td>Average of 4 frames</td>
<td>295.21</td>
<td>73.58</td>
<td>4.01</td>
<td>13.20</td>
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<tr>
<td>7</td>
<td></td>
<td>Average of 8 frames</td>
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<td>71.67</td>
<td>4.11</td>
<td>16.06</td>
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<tr>
<td>8</td>
<td></td>
<td>Average of 12 frames</td>
<td>294.75</td>
<td>70.52</td>
<td>4.18</td>
<td>17.93</td>
</tr>
<tr>
<td>9</td>
<td>ORAL TISSUE</td>
<td>No Average-Base</td>
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<td>100.67</td>
<td>3.17</td>
<td>N/A</td>
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<td>Average of 4 frames</td>
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<td>90.53</td>
<td>3.51</td>
<td>10.87</td>
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<td>Average of 8 frames</td>
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<td>3.55</td>
<td>12.19</td>
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<tr>
<td>12</td>
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<td>3.54</td>
<td>11.73</td>
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<tr>
<td>13</td>
<td>DENTAL TISSUE</td>
<td>MOLAR</td>
<td>No Average-Base</td>
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<td>2.68 N/A</td>
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<td>99.59</td>
<td>2.86</td>
<td>6.61</td>
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<td>15</td>
<td></td>
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<td>96.94</td>
<td>2.93</td>
<td>9.51</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>Average of 12 frames</td>
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<td>95.93</td>
<td>2.96</td>
<td>10.66</td>
</tr>
<tr>
<td>17</td>
<td>INCISOR</td>
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<td>106.51</td>
<td>2.7</td>
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<td>100.28</td>
<td>2.8</td>
<td>6.26</td>
</tr>
<tr>
<td>19</td>
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<td>98.20</td>
<td>2.93</td>
<td>8.48</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>Average of 12 frames</td>
<td>287.63</td>
<td>97.27</td>
<td>2.96</td>
<td>9.51</td>
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ANOVA: Two-Factor without Replication

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<th>df</th>
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<td>0.000338</td>
<td>4.45897</td>
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</tbody>
</table>

Table 4. 2 Randomized Block ANOVA for 4, 8 and 12 averaged SNRs of all phantoms

![Comparison of % improvement in SNR](image)

Figure 4.6 a Comparison of % improvement of SNR from base, for all phantoms with perpendicular orientation of probe during acquisition.
CHAPTER V

DISCUSSION

This study represents the first systematic investigation of simple frame averaging as an effective speckle reduction algorithm for common path TDOCT systems. The present results support the first hypothesis of this study (Table 4.1). A visual representation of SNR improvement can also be observed across all the phantoms (Figures 4.1-4.5). The second part of the study was to investigate the variability between the averaged frames. This second hypothesis was supported by Table 4.2 which showed significant difference in the improvement among the averaged frames, with $p=0.000338<0.05$. The study included four different phantoms with varying tissue constituents, hence different textures. These phantoms successfully demonstrated the efficacy of frame averaging on all the phantoms taken into consideration.

5.1 Measurement Protocols and Acquisition Setup

The procedure involved acquisition of frames from a commercial common path TDOCT system, Niris 1300e. A survey of OCT literature indicated the need for a speckle reduction algorithm, which was relatively independent of tissue characteristics and not based on complex statistical models, as documented in chapter 2.
A study conducted by Tan et al documented the efficacy of frame averaging in spectral domain OCT using slow axis averaging [5]. The methodology followed was to reduce the acquisition speed along the frame axis and perform instantaneous averaging of the acquired frames by rigid image registration algorithm.

This was a promising study that systematically approached simple averaging using uncomplicated setup protocols and demonstrated clinically viable results. Our procedure attempted to exploit the frame acquisition speed in the Niris 1300e as the basis to simplify frame averaging. We have indicated previously that the frame variability played a decisive role in the efficacy of frame averaging.

Since the system under investigation demonstrated low frame variability for a calibrated system with an effective acquisition speed of 4 frames per second (fps), it was an ideal system to investigate the characteristics of simple frame averaging.

5.2 Frame Acquisition and Processing

The system was well calibrated in order to assure consistency in the acquisition parameters. The system provided the flexibility to acquire both raw data in .csv format and frames in .png format. We decided to proceed with our investigation using raw data since it indicated the direct backscatter intensity pixels without color mapping or thresholding to suit the display. This processing provided an ideal level of credibility to the experimentation accuracy, since it was independent of software processing for frame averaging.
The phantoms chosen for the study had varying scattering properties due to different compositions. A texture analysis study by Gossage et al indicated the variability in speckle characteristics based on the sample[35]. This study utilized various tissues to study the texture of each material with the hope to analyze speckle characteristics further. In our study, we attempted to study the efficacy of simple frame averaging as a speckle reduction algorithm using materials whose backscattering properties vary tremendously. These phantoms were selected in order to demonstrate the efficacy of simple frame averaging, irrespective of the tissue constituents. Dental tissue (human teeth) was one of the phantoms, which have a calcified dense composition, used for this purpose. They demonstrated a low, yet significant reduction in background noise of up to 10.66% for the entire frame (Figure 4.6a). Human skin from the fingertip showed the highest improvement in frame signal to noise ratio, 17.93%.

5.3 Significance of the Study

This study developed a proof of concept that demonstrated the effectiveness of simple frame averaging for time domain systems. The biggest contribution of simple frame averaging is increasing the visibility of low level signals. Non-living and human tissue phantoms of non-homogenous nature contain myriads of constituents with different level of interaction with light due to structural and chemical differences. Theses constituents backscatter light at different intensity, highest being a well defined structure with resolved boundaries for differentiation. Most of the medical diagnosis is contingent upon how well a structure is resolved and displayed in the form of an image. Speckle noise with relatively high intensity will not be well differentiated from a sample with similar
intensity of backscatter. This can obscure the low level signals amidst the speckle noise, leading to misdiagnosis at worst. Since most of the early cancers are epithelial in origin and difficult to differentiate, speckle noise can blur the boundaries and reduce its visibility. For example, observe the single frame from Onion in Figure 4.1a and notice the area circled in red. The boundaries of the cell are so obscured that its presence cannot be easily distinguished. Simple frame averaging averages the random variation in speckle noise, getting them closer to the expected mean of the pixel distribution, thereby suppressing the noise that caused the blurring of the low level signal as depicted in Figure 4.1d. Observe how the cell boundaries pop up amidst the suppressed noise, providing a diagnostically better image.

The health of medical diagnosis is fundamentally rooted in contrast to noise characteristics of the image. Simple frame averaging provides the much needed edge preservation for morphological imaging of high resolution as demonstrated by this study. Simple frame averaging does not alter the signal unlike other contrast enhancement algorithms like thresholding. Thresholding truncates the signal based on an adaptive mechanism to enhance the signal pixels in the image.

5.4 Clinical Significance of Dental Imaging-Dental Caries

The information available to dental clinicians for evaluating diseases in the oral cavity is currently inadequate, or utilizes antiquated methodologies which provide poor image quality and hence impairs early assessment of dental health degradation. We attempted to study the efficacy of our simple frame averaging technique with location specific defects and quantify our results to aid clinical diagnosis by image assessment. Dental caries is a chronic destruction of the tooth, characterized by demineralization, which can ultimately
lead to mastication malfunction. Caries are formed dynamically, which means that demineralization occurs over a period of time for caries lesion to develop. The tooth may undergo cycles of demineralization and remineralization. It is the net loss of minerals that ultimately determines the extent of caries. The earliest changes are dissolution of the enamel leading to pathways where diffusion can occur. If over a period of months to years the surface weakens sufficiently, then cavitations may result. Early lesions cannot be detected with current clinical techniques due to lack of resolution. Early detectable caries lesion is a white spot lesion where demineralization has progressed at least 300 to 500 μm [36]. Increasing the visibility of caries by reducing random variation caused by speckle noise will increase the accuracy of diagnosis.

5.5 Limitations

In the paper titled „Speckle in Optical Coherence Tomography: A review“, Joe Schmitt talks extensively about two different types of speckle that appear in OCT images and the dependent variables that contribute to them. The first is due to interference from multiple scattered photons. This type of „chance“ speckle is typically a single pixel wide, random, and can therefore be reduced by averaging, as successfully demonstrated by our study. Inherent speckle are much larger and are still present in the images, giving a limited improvement.

Simple frame averaging addresses this random nature of speckles and hence we see an improvement across varying averaged frames. Fully developed „chance“ speckle noise has the characteristic of multiplicative noise which has been successfully demonstrated by simple frame averaging. But it also explains why the contribution of simple frame
averaging is low, since only „chance” speckles have been eliminated. As the speckle
pattern does not change from shot to shot, the averaging of the images at one incident
angle merely reduces the „chance” speckle without affecting the „inherent” speckle [37].

Simple frame averaging addresses the random noise in images. Since most of the
diagnostic medical imaging is contingent upon visual perception by the human eye, it is
important to consider the image processing by human eye with respect to speckle
reduction. It is observed that integration time, the accumulation time of light energy
before relaying it to the brain is fast enough to integrate the movement observed in real
time to decipher the objects embedded in the image. This essentially means that the eye
reduces or eliminates speckle which is fundamentally documented in laser speckle
studies. For time domain systems which have an acquisition time ranging from 8-25 fps,
human eye does an excellent job of eliminating granular pattern that obscures the image
and final perception is a crisp diagnostic image.

Simple frame averaging finds its place in post diagnostic database of patients in case of
time domain OCT systems. Since static and dynamic frames have different perceptive
speckle pattern due to speckle reduction by human eye, a static image needs to undergo
speckle reduction to have the same image quality as deciphered by the human brain. A
static diagnostic image is a permanent part of patient history and frame averaged images
would increase the credibility of static images.
CHAPTER VI

CONCLUSIONS AND FUTURE WORK

This study successfully demonstrated the necessity of simple frame averaging in common path TDOCT systems. We used four different phantoms to investigate the efficacy of simple frame averaging, based on the tissue matrix and optical backscattering properties.

Subject to the limitations in the previous section, the following conclusions were drawn:

1. Simple frame averaging lead up to 18% improvement in SNR, thus supporting the second hypothesis of the study.

2. A significant difference was observed using ANOVA for frame averaging of 4, 8 and 12 frames thus supporting the second hypothesis of the study.

3. Visual representation of the averaged frames showed an enhancement of low level signals.

4. Simple frame averaging does not tackle „inherent” speckle that are coupled with the signal.
The application of simple frame averaging in TDOCT is still in its infancy. Future work includes coupling simple frame averaging with other forms of speckle reduction to evaluate its efficacy and provide better signal characteristics and reduced acquisition overhead. Most of the OCT market is heading towards Fourier Domain OCT (FDOCT), the discussion to which is beyond the scope of this thesis. Ref 38 talks about the operating principles and compares its performance with TDOCT. It is interesting to observe that speckle noise origin is similar although its manifestation on images would vary from TDOCT.

Hence, application of simple frame averaging in FDOCT in conjunction with other speckle reduction mechanisms would make an interesting addition to this study in the future.
REFERENCES


APPENDICES
APPENDIX A

SOURCE CODE USED FOR FRAME AVERAGING

Visual Basic script for frame averaging with macros- Excel 2003 Compatible

**Frame Averaging Module1- Workbook Code**

`Private Sub Workbook_Open()`

MsgBox "Please enter the number of frames to be averaged", vbInformation, "Frame Averaging"

End Sub

**Frame Averaging Module1- MyMacro Code**

`Public Sub MyMacro()`

FrameCount = Sheets("Averaged Frame").Cells(1, 2)

iCount = 1

For iCount = 1 To FrameCount

    MsgBox "Please Specify the Location for the Next Frame to Be Averaged", vbInformation, "Frame Averaging"

    Filename = Application.GetOpenFilename()

    If Filename <> False Then

        Workbooks.Open (Filename)

        Wkbkname = ActiveWorkbook.Name

        ActiveWorkbook.ActiveSheet.Copy
        After:=ThisWorkbook.Sheets(Sheets.Count)

    End If

Next iCount

End Sub
ActiveSheet.Name = "Frame" & iCount

    Application.Workbooks(Wkbkname).Close Saved = True

    End If

Next iCount
Sheets("Averaged Frame").Select
iCount = 1
For iCount = 1 To FrameCount
    jCount = 2
    kCount = 1
    For jCount = 2 To 193
        For kCount = 1 To 256
            Sheets("Averaged Frame").Cells(jCount, kCount) = Sheets("Averaged Frame").Cells(jCount, kCount) + Sheets(iCount + 1).Cells(jCount, kCount)
        Next kCount
    Next jCount
Next iCount

jCount = 2
kCount = 1
For jCount = 2 To 193
    For kCount = 1 To 256
        Sheets("Averaged Frame").Cells(jCount, kCount) = WorksheetFunction.Round(Sheets("Averaged Frame").Cells(jCount, kCount) / FrameCount, 0)
    Next kCount
Next jCount
Next iCount

iCount = FrameCount + 1
Application.DisplayAlerts = False
Do Until iCount = 1
    Sheets(iCount).Delete
    iCount = iCount - 1
Loop
Application.DisplayAlerts = True
End Sub
APPENDIX B

AVERAGED FRAMES FOR PHANTOMS CONTINUED FROM RESULTS AND
STATISTICAL TABLE

Phantom 2 - Skin on human fingertip

(Left) An average of 4 frames from fingertip
(Right) An average of 8 frames from fingertip

(Intensity (pixel) values)

800

600

400

200

0

1 31 61 91 121 151 181

Depth resolved pixels

(left) A single A-line from the above 4 frame averaged raw data

(right) A single A-line from the above 8 frame averaged raw data
Phantom 3- Oral Mucosa

(left) An average of 4 frames from oral mucosa

(right) An average of 8 frames from oral mucosa

(left) A single A-line from the above 4 frame averaged raw data

(right) A single A-line from the above 8 frame averaged raw data
Phantom 4- Dental tissue - Molar

(left) An average of 4 frames from extracted molar
(right) An average of 8 frames from an extracted molar

(left) A single A-line from the above 4 frame averaged raw data
(right) A single A-line from the above 8 frame averaged raw data
Phantom 4- Dental tissue- Incisor

(Left) An average of 4 frames from an extracted incisor

(Right) A single A-line from the above 4 frame averaged raw data

(Left) An average of 8 frames from an extracted incisor

(Right) A single A-line from the above 8 frame averaged raw data
Two factor ANOVA/ Randomized Block ANOVA

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<td>9.618</td>
<td>9.26737</td>
</tr>
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<td>12 FRAMES</td>
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<td>12.848</td>
<td>11.36152</td>
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<tr>
<td>8 Frames</td>
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<td>59.68</td>
<td>11.936</td>
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Total: 147.4084933 14

Note: Averaged frames (4, 8 and 12) are taken as rows and phantom types are taken as columns.