Doppler Optical Coherence Tomography for Microcirculation Studies

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Abstract for the thesis submitted by Donna Arthur for the degree of Doctor of Philosophy and entitled Doppler Coherence Tomography for Microcirculation Studies.

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This thesis forms part of an ongoing long-term project to investigate the suitability of Doppler optical coherence tomography (OCT) as a measurement tool to investigate skin thickness and blood flow in patients with systemic sclerosis.

There is a discussion of the characterisation of an electro-optic phase modulator for use in a Doppler OCT imaging system which is being built for the purpose of clinical studies. In addition to this the development of software for the same system is described. The work includes a comparison of two methods of obtaining Doppler information that were tested with the system; a phase resolved method and a correlation mapping method. Initial structural and Doppler images obtained using the system are presented.

In addition to this the development of semi-automated software to measure skin thickness from both OCT and high frequency ultrasound images is discussed. The results of a study, for which this software was developed, into skin thickness measurements using both techniques in both patients with systemic sclerosis and healthy controls are presented. Both OCT and high frequency ultrasound were able to measure a statistically significant difference in epidermal thickness at multiple locations on the body.

Finally, the modification of a freely available Monte Carlo simulation for light propagation in multi-layered tissue (MCML)\(^1\) to enable the simulation of structural and Doppler OCT images is covered. The simulation was able to extract the magnitude of the simulated flow accurately to within an order of magnitude, and after a simple filter was applied to eliminate fluctuations in the data the structure of the Doppler image closely matched what was modelled.

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Thanks must also go to my mum for her love and constant support. Getting this far would have been much more difficult if she hadn’t seen and encouraged my ability from when I was small.

This thesis is dedicated to my husband, James - I couldn’t have asked for a more patient and loving person to share this journey with.

“In most of my work, the laws of physics rarely seem to apply.” - Fox Mulder

“Life moves pretty fast. If you don’t stop and look around once in a while, you could miss it.” - Ferris Bueller

“When life gives you lemons, don’t make lemonade. Make life take the lemons back! Get mad! I don’t want your damn lemons, what am I supposed to do with these? Demand to see life’s manager! Make life rue the day it thought it could give Cave Johnson lemons! Do you know who I am? I’m the man who’s gonna burn your house down! With the lemons! I’m gonna get my engineers to invent a combustible lemon that burns your house down!” - Cave Johnson
Chapter 1

Introduction

1.1 Introduction

Optical coherence tomography (OCT) is a recently developed non-invasive method of imaging and is particularly suited to imaging optically scattering media such as biological tissue. It is capable of video rate imaging with a penetration depth of several millimetres in tissue and a resolution of a few microns. Images can be obtained in real time and can be repeated due to the non-destructive nature of the technique. OCT is perfectly suited for a wide range of clinical uses. Initial in-vivo applications were ophthalmological, but the technique has evolved to be successfully applied in clinical areas as diverse as cardiology, dermatology, and oncology. It has also found uses outside a clinical setting. An example of an OCT image of the forearm is shown in Figure 1.1.1.

Major advances have included the development of Doppler OCT, allowing the measurement of fluid motion within a sample while producing the structural image. Many human and animal in-vivo applications of Doppler OCT have been published in addition to imaging studies of flow phantoms using solutions which mimic the optical properties of tissue. Doppler OCT is discussed in more detail later in this chapter in Section 1.2.1.
Figure 1.1.1: An example OCT image of the forearm. Due to blood having a higher absorption coefficient than the surrounding tissue blood vessels appear as dark patches, as in the centre of this image. The downwards $y$ axis is increasing depth into tissue while the $x$ axis is approximately horizontal with the tissue surface. This image was obtained using a commercial system during the study discussed in Chapter 5.

Various studies have shown the usefulness of OCT in measuring skin thickness,[12, 13] and a natural progression from this point is to incorporate Doppler measurements to measure blood flow in vessels in the dermis of the skin in real time. There is limited literature on the application of Doppler OCT as a method of monitoring the condition of patients with specific skin diseases, and very little published work on its application to patients with a disease called systemic sclerosis, which affects both skin and blood flow.

1.2 Optical coherence tomography

There are several implementations of OCT; the earliest examples in the literature are applications of time-domain OCT, often using a Michelson-style interferometer. In its simplest implementation light from a single broadband source in the near infrared region is split and directed to two arms of the interferometer - Figure 1.2.1. One arm - the reference arm - reflects the light back using a mirror which moves linearly to adjust the path length of the arm. The other arm - the sample arm - contains the stationary sample. The light backscattered from both arms is recombined at the beamsplitter and directed towards the detector. OCT imaging uses a coherence gate; as a result of this
only light which has the same time of flight to within an amount limited by the bandwidth of the source causes interference. The motion of the mirror in the reference arm provides a moving coherence gate giving a way to measure the proportion of light reflected from each point within the depth of the sample as the mirror is moved. In order to achieve high resolution images the source needs a high spatial resolution in addition to a low temporal resolution. To produce a 2D image an additional mirror is placed in the sample arm - between the collimator and sample surface in Figure 1.2.1 - which moves the incident beam over the surface of the sample between A scans.

Figure 1.2.1: The simplest implementation of an optical coherence system using a Michelson interferometer. So: source. D: detector. BS: beamsplitter. C: collimator. M: mirror. S: sample.

Fourier domain OCT is a more recently developed technique which offers some benefits over time domain imaging including faster imaging speeds and a higher signal-to-noise ratio.[14] In this form of OCT the single detector is replaced by a diffraction grating and an array detector. This acts as a spectrometer allowing the measurement of the spectrum of the detected light rather than the intensity of the interferogram. Rather than taking a single measurement for each pixel within an A scan the spectrometer allows a single A scan to be obtained in a single measurement. Swept source OCT is a similar technique which produces a spectrum however it uses a swept source rather than a spectrometer. The spectrum is pieced together from individual measurements as the source sweeps through a range of wavelengths.

The resolution of OCT is dependent on the length of the coherence gate and thus the bandwidth of the source, for a given central wavelength. Using a higher bandwidth or a lower central wavelength can theoretically increase the resolution in a given OCT system. However the optical properties of a sample
must be considered when selecting the central wavelength as this may affect the penetration depth; generally absorption peaks of molecules within tissue in the near-infrared region (NIR), such as water and melanin, should be avoided. Typically sources in the NIR region are used, with 800 nm and 1300 nm being common choices.

1.2.1 Doppler optical coherence tomography

Doppler OCT is an extension to standard OCT which enables quantitative measurements of flow velocity within a sample. Flow images are generally produced from the same raw data as the structural images via additional processing, either in real-time or via post processing. Several methods have been developed to extract the required data including using a short-time Fourier transform to measure changes in a frequency spectrum,[15] a Hilbert transform to measure a change in phase,[16, 17, 18] looking for changes in speckle variation[19, 20] or via image cross-correlation methods.[21, 22] The first two methods rely on the Doppler effect; when light scatters from a moving particle its frequency is shifted by an amount proportional to the speed of the scatterer. This frequency shift is measured. The second two methods look for changes in the structural images themselves.

Velocity resolutions of a fraction of a millimetre per second have been achieved, high enough for in-vivo microcirculation imaging.[23, 24, 25] In addition correlation methods have been shown to be able to detect Brownian motion.[21, 22]

1.2.2 In-vivo uses of optical coherence tomography

Published in-vivo uses of OCT span many distinct disciplines, including ophthalmology, cardiology and dermatology. Its high resolution allows the distinction of different areas of tissue. OCT relies on changes in the amount of scattering and absorption of light within different areas of tissue to distinguish between them; differing amounts of light back scattering from each area lead to different intensities at the detector and hence different shades of grey on a greyscale image. Doppler flow images are often colour, rather than greyscale, with a scale ranging from blue for flow in one direction, which is perpendicular to the plane of the image, to red for flow in the opposite direction. The intensity of the colour is related to the velocity of the flow, with a brighter colour denoting a higher velocity and zero velocity appearing as black. This allows for the
distinction between both areas of flow and no flow, as well as differing flow
directions within the same sample.

Due to its non-invasive nature OCT is repeatable and does not damage the
sample being imaged. Initial uses were ophthalmological in nature, with OCT
now being a standard tool in the diagnosis and management of conditions such
as glaucoma, macular degeneration and diabetic retinopathy.\cite{26, 27, 28} It has
been used in dermatology as a measure of skin thickness,\cite{12, 29} to investigate
skin structure in psoriasis patients\cite{30} and even to image tattoos.\cite{31} It has
also found uses in oncology in identifying cancerous tissue.\cite{4, 32} OCT has been
shown to be a promising tool for skin imaging.

\section{1.3 Systemic sclerosis}

Systemic sclerosis is a multisystem disease which causes thickened fibrotic skin
(scleroderma) and changes in microvasculature function (ischaemia). While
there are childhood forms of scleroderma, systemic sclerosis is not normally
diagnosed until adulthood, sometimes several years after the first symptoms
develop. Abnormal microvasculature function can be studied using a variety of
techniques to investigate blood flow, while skin thickness can be measured by
palpitation of the skin as well as with imaging methods. There are also a range
of antibodies which may be present in blood which are indicative of the disease.

In a patient with systemic sclerosis skin has been shown to be affected by
thickening\cite{33, 34, 35} making it difficult for patients to fully open and close
their hands. The blood vessels in the dermis of the skin can become constricted,
reducing blood flow,\cite{36, 37} particularly in the hands and feet. This reduced
blood flow can make it particularly difficult for the skin to heal after injury.

There are 2 subgroups of systemic sclerosis; diffuse and limited cutaneous.
Patients with limited cutaneous systemic sclerosis are mainly affected by the
disease in their extremities - the hands, feet and face - while in patients with
the diffuse subtype the disease progresses to the upper limbs and the trunk.

Patients with this disease are often prone to a number of complications such
as telangiactases, sometimes known as spider veins, as well as the fingers and
toes being affected by ulcers. These ulcers can take several months to heal
due to the restricted blood flow. Internal organs may also be affected. It is
thought that the changes in skin reflect changes that are happening in internal
organs and that the understanding of fibrosis and the changes to blood vessels
is fundamental to understanding the disease. The first symptom to appear is generally Raynaud's phenomenon. In the extremities of the body, particularly the fingers and toes, the blood vessels become constricted leading to problems with blood flow and discolouration of the skin. Severe skin thickening in the extremities develops afterwards, which results in restricted movement.

1.3.1 Imaging methods

Several imaging methods are currently used for investigating skin structure and microvasculature structure and function. However, studies do not always focus on any specific disease or symptoms. High-frequency ultrasound has a higher resolution than traditional diagnostic ultrasound and has been a focal point of many research groups, primarily for measuring epidermal and dermal thickness. It has been shown to have good repeatability[38] and is a reliable method of measuring dermal thickness over 17 sites on the body.[33] However its resolution is limited to a few tens of microns. Confocal microscopy has also been studied as a method of measuring epidermal thickness, although it is not able to penetrate to the same depths as high frequency ultrasound or OCT. Its measuring ability is limited to a few hundred microns in depth[38] although it has been shown to be able to measure the thickness of the stratum corneum and epidermis, the upper layers of the skin.[39]

As well as the ability to measure skin thickness with a view to monitoring changes over time, clinicians are also interested in monitoring microvascular structure and function due to the nature of systemic sclerosis. As with skin structure there have been many studies assessing the ability of various imaging methods to investigate blood perfusion and vascular structure. Laser Doppler imaging has been shown to be able to detect differences in blood flow between healthy controls and patients with systemic sclerosis and has been shown to be able to determine between patients with Raynaud's phenomenon which is not caused by an underlying condition and patients with Raynaud's caused by systemic sclerosis.[40] However laser Doppler imaging is only able to show changes in blood perfusion over relatively large areas of tissue, rather than on the level of individual vessels. Patients with systemic sclerosis generally show a lower level of perfusion in the extremities. Nailfold capillary microscopy is able to image vascular structure and function in the nailbed, and it has been claimed that this is the most reliable method of looking for microvascular changes
in patients with connective tissue disorders.[41] This imaging method takes advantage of the fact that capillaries in the nail beds lie in a direction almost parallel to - and very close to - the skin surface, allowing high magnification images to be taken. With systemic sclerosis the capillaries are generally dilated, less dense and more disordered than the capillaries of someone without the disease. Due to systemic sclerosis affecting both microvasculature structure and function as well as skin thickness an ideal dual-imaging method would be non-invasive and be able to image structurally at a high resolution in addition to providing flow information at a spatial resolution greater than individual vessels. OCT fits these criteria suggesting it may potentially be a useful imaging tool for investigating the effects of systemic sclerosis. Although histology is the current gold standard for skin thickness measurement, OCT could potentially provide a non-invasive repeatable alternative for these measurements given that measurements of epidermal thickness have been shown to be strongly correlated with histology.[42]

1.4 Thesis outline

While work has previously been published where OCT has been used to investigate skin thickness or blood flow it has only recently been considered as an imaging tool in patients with systemic sclerosis.[43] As it is able to image both the structure of tissue as well as quantify flow, work is currently under way to assess the suitability of OCT for investigating and monitoring disease progression in patients with systemic sclerosis. The work presented in this thesis forms part of this ongoing project. Chapter 2 will cover theory and background of OCT, its functional extension Doppler OCT, and its application in clinical imaging. Chapter 3 discusses the characterisation of an electro-optic phase modulator. Extracting Doppler information in Doppler OCT using what is known as the phase resolved method generally requires a carrier frequency to be applied to the light in the reference arm of the interferometer. This is often achieved in the literature by applying a $\pi$ phase shift at a constant frequency using a phase modulator, with the resultant data demodulated around this frequency. A Pockels cell based modulator was purchased for use in a time domain OCT system, however the documentation only specified one voltage and frequency
combination which will produce a $\pi$ phase shift. To enable any voltage and frequency combination to be used the modulator had to be characterised; the results of this are presented in this chapter.

Chapter 4 will discuss the development of software for a custom built time domain OCT system. Both a phase resolved method, which uses the modulator discussed in Chapter 3, and a correlation mapping method were used to try to extract flow information from the interferogram. Preliminary structural and Doppler images as well as a discussion of the two flow calculating methods are given.

In Chapter 5 the development of semi-automated software to measure skin thickness from both OCT and high frequency ultrasound images is discussed. The software was used to measure images from patients with systemic sclerosis as well as healthy controls as part of a study to compare the two techniques. The results of this study are also presented.

Chapter 6 covers the adaptation of a freely available Monte Carlo simulation for light propagation in multi-layered tissue (MCML) to enable the simulation of structural and Doppler OCT images. The simulation is able to model the flow of a highly scattering liquid inside a straight capillary tube which can be surrounded by air or another scattering medium. The optical properties of the flowing liquid can be set by the user to enable simulation of blood flow in a vessel. The results of this simulation are compared to experimental data obtained using a flow phantom with similar geometry to the simulation.

Finally, Chapter 7 summarises the work presented in this thesis and discusses future work which could be undertaken to further develop OCT as a tool for monitoring disease progression in patients with systemic sclerosis.

REFERENCES


Chapter 2

Theory and literature review

2.1 Optical coherence tomography

Optical coherence tomography (OCT) is a recently developed non-invasive method of imaging using a broadband light source with an interferometer, and is particularly suited to imaging optically scattering material such as biological tissue. It is capable of achieving micron-resolution and video rate imaging at a depth of $1 - 2\text{mm}$ in human tissue. The light source used is generally in the near infrared region of the spectrum, usually with a power up to a few tens of milliWatts. Due to its non-invasive nature, optical coherence tomography is perfectly suited for a wide range of clinical uses, including ophthalmology,[1, 2] oncology[3] and dermatology.[4, 5] It has also found use in other areas of research and industry such as quality inspection of electronics,[6] art conservation[7, 8] and imaging laser induced damage to optical components.[9]

OCT is often compared to ultrasound due to similarities in the types of images they produce, however OCT is capable of imaging at much higher resolutions although it has a lower imaging depth. A 2D optical coherence tomography image is essentially a cross section of the sample being imaged. Figure 2.1.1 shows an OCT image of the palm side of the left ring finger. The image shows the structure of the top layers of the skin; the depth into the tissue is the $y$ axis. The skin surface itself appears as the thin bright white line near the top of the image.
Figure 2.1.1: An example of an optical coherence tomography image of the palm side of a human finger. This image was obtained during a study of non-invasive imaging techniques which will be discussed in Chapter 5.

The data to produce an image is recorded a single A scan (depth scan) at a time while moving the focus of the light laterally across the surface of the skin between each of these A scans - this produces the B scan as demonstrated in Figure 2.1.2. An example normalised intensity profile of a single A scan is shown in Figure 2.1.3 which was taken from the image in Figure 2.1.4. Multiple 2D images can be stacked to create a 3D image if the system incorporates some form of raster scanner to move the focus of the light over a 2D area on the surface of the skin. In general the A scans are squared and enveloped during post-processing to remove local variations in pixel intensity - 2.1.5.

Figure 2.1.2: A B scan is made by combining multiple A scans.
Figure 2.1.3: An unprocessed A scan from an image of a mirror. The large increase in the signal (indicated by the arrow) is due to reflection from the mirror.

Figure 2.1.4: An OCT image of a mirror, from which the raw A scan in Figure 2.1.3 is taken. The interferogram has not been enveloped resulting in slight oscillations of the pixel intensity at the surface of the mirror due to the oscillations in the interferogram.
Optical coherence tomography exploits the coherence properties of light using an interferometric technique i.e. measurements are made by interfering two light fields originating from the same source. One portion of the light backscatters from a sample in one arm of an interferometer with the other travelling a known distance in a reference arm. These two fields are recombined at the output of the interferometer. Interference fringes will only be seen if the optical path difference between the arms of the interferometer match to within the temporal coherence length of the source. If the two fields are coherent then they have a constant relative phase, and can interfere with one another. Outside of the coherence length the relationship between the phase becomes random and there is no longer a correlation between the phases of the fields.

Monochromatic light sources have a long coherence length, in the order of hundreds of meters. Broadband sources have a much shorter coherence length with sources used for OCT often reaching $< 10 \mu m$. The coherence length is dictated by the bandwidth and central wavelength of the source. For OCT imaging the coherence length of a source with Gaussian spectral profile is equal to the maximum theoretical resolution, $\Delta z$, through Equation 2.1.1 where $\lambda_0$ is the central wavelength and $\Delta \lambda$ is the bandwidth.

$$\Delta z = \frac{2 \ln 2 \lambda_0^2}{\pi \Delta \lambda} = 0.44 \frac{\lambda_0^2}{\Delta \lambda}$$  \hspace{1cm} (2.1.1)

By using a broadband source a coherence gating effect can be produced which rejects light that has a path length outside a very narrow range. This can also be thought of using coherence time, $\tau \approx \frac{\lambda_0^2}{c \Delta \lambda}$. Only if the time of flight of
the light from each arm of the interferometer is equal to within $\tau$ will there be interference when the beams are recombined. Outside of this range the beams are no longer coherent with each other and will be rejected by the coherence gate and not contribute to the interference.

OCT also places a requirement on the spatial coherence of the source. Spatial coherence is a measure of how similar the phase of the electric field is at different points across the wavefront, perpendicular to beam propagation. If a source with low spatial coherence was used for OCT imaging the differing beam profile would severely reduce the effectiveness of the time coherence gating, as there is no guarantee that the beams in each arm of the interferometer would be coherent and form interference fringes when recombined, even if their optical path length was equal. Hence light sources for OCT are required to be highly spatially coherent.\[10\]

### 2.1.1 History of optical coherence tomography

The first applications of OCT were published in the early 1990s,\[11, 12\] which in turn built on the earlier development of low coherence interferometry and femtosecond optical ranging in the late 1980s which had been used to measure thicknesses of parts of the human eye.\[13, 14\]. Similar techniques were also used in optical time domain reflectometry in order to test the performance of fibre optics. Since then the field has expanded to include various applications using a variety of light sources, interferometers and detection methods and the goal of higher resolution video rate images has inspired researchers to develop novel equipment set-ups. The first publications on OCT were applications of time domain OCT (TD-OCT). There was a gap in alternative imaging systems available at the time for a method that had a higher resolution than high-frequency ultrasound (limited to a few tens of microns\[15\]) but with a depth of penetration greater than that of confocal microscopy (limited to a few hundred microns in tissue \[16\]).

From Equation 2.1.1 the maximum theoretical axial resolution in a TD-OCT system is limited by the central wavelength and coherence length of the source; the shorter the coherence length, or lower the wavelength, the higher the resolution, so developments in this area in particular can significantly improve the highest resolution theoretically achievable by a system. Axial resolutions of a few microns or less can now be achieved.\[17, 18, 6\] The resolution in the sample or tissue being imaged can be calculated from the
free-space resolution, which itself is calculated using Equation 2.1.1, by dividing by the group velocity index of the sample.\[11\] For biological tissue, this is in the region of 1.35 to 1.5.\[19, 20\] The axial and transverse resolutions of OCT are independent of each other; the transverse resolution, $\Delta x$, Equation 2.1.2, is determined by the spot size, $d$, on a lens with focal length $f$ and can be increased by using a higher numerical aperture.\[21\] However, doing this will reduce the depth of focus within the sample.

$$\Delta x = 1.22\lambda\frac{f}{d} \quad (2.1.2)$$

Depth of penetration in tissue is typically up to $1 - 2$ mm. This is wavelength dependent since the two interactions affecting light attenuation in tissue - scattering and absorption - are both wavelength dependent themselves. The two most commonly used central wavelength regions for OCT imaging are around 800 and 1300 nm; 800 nm provides a higher resolution but a lower penetration depth than 1300 nm. The 1050 nm region is also occasionally used.\[2, 22\]

The first report of an optical coherence tomography imaging system in the literature was published in 1991\[11\] and used the Michelson interferometer based set-up shown in Figure 2.1.6.

In this basic Michelson set-up the light from the broadband low-coherence source is split using a 50:50 coupler, with half of the light directed to a reference arm and the other half directed to the sample. The reference arm contains a mirror which can be moved backwards and forwards parallel to the beam direction resulting in a change in the path length of that arm; it is essentially a variable delay line. A sample is placed in the other arm and backscattered
light collected and directed back to the interferometer. The sample arm allows for the point where the incident light reaches the tissue to be moved laterally so that a 2-dimensional image can be generated. The photons which are back-scattered from the sample arm and directed to the detector only contribute to an interference signal if their path length is the same as the path length of the reference arm, to within the coherence length of the source. The intensity of the interferometer output $I_0(t)$, oscillates as a function of path length difference, $\Delta l$, as in Equation 2.1.3. $E_R$ and $E_S$ are the electric fields in the reference and sample arms respectively, while $\lambda_0$ is the central wavelength of the source.

$$I_0(t) = \frac{1}{4}|E_R|^2 + \frac{1}{4}|E_S|^2 + \frac{1}{2}E_RE_S \cos \left( \frac{4\pi}{\lambda_0} \Delta l \right)$$ (2.1.3)

The oscillation seen in an interferogram is governed by the third term in Equation 2.1.3. This term is maximised for a zero path length difference ($\Delta l = 0$) between the two arms of the interferometer. As the mirror in the reference arm is moved to increase the path length in that arm the point where the photons from the sample arm which contribute to the interferogram back-scatter from appears to move deeper into the sample, generating a coherence gating effect. This results in a simple method of scanning through a depth of the sample. One major drawback from this style of set-up is the requirement for a moving mirror in the reference arm to provide the depth scan; the speed of the scan can only be as fast as the mirror can move, placing a limit on the imaging time for each frame. This can be minimised by using a delay line which does not rely on the linear translation of a mirror and instead using a rotating mirror in a rapid scanning optical delay line, which will be discussed later in Section 2.1.4. The magnitude of the interferometer signal at the detector gives the value which is used to determine what value of the grey scale that specific pixel will be shown as in the generated image.[23] which is often enveloped in post-processing to create a clearer image.

The first published images were generally of \textit{ex-vivo} biological samples using sources in both the 800 nm and 1300 nm regions with resolutions in the order of 10 to 20 microns.[11, 24] By the mid 1990s several groups had taken the step to image biological tissues \textit{in-vivo}.[12, 25] Ophthalmological imaging was particularly popular at the time due to the ease of imaging the interior of the eye. OCT images can be produced with optical powers low enough to be within safe limits for ocular exposure and sources in the 800 nm region in particular can easily penetrate the aqueous and vitreous humours of the eye in order to
reach the retina.

Another major development around this time was the introduction of Doppler flow measurements alongside the structural OCT images: Doppler optical coherence tomography.[26] This functional extension allows the generation of a flow velocity map and structural image simultaneously enabling measurement of velocities in individual vessels. In structural OCT images large blood vessels can sometimes be seen due to the decreased signal in that area as illustrated in Figure 2.1.7. However the velocity or direction of the flow can not be quantified using the structural image alone.

![Figure 2.1.7: An example of a structural OCT image where a blood vessel can be seen due to increased absorption of the incident light.](image)

Doppler OCT relies on the fact that light scattering from a moving object gains a Doppler shift proportional to the velocity of the scatterer. This Doppler shift can be extracted and used to determine the speed and direction of the flow from which it scattered. Due to the high resolution of OCT it is possible to measure flow in individual vessels, something which is not achievable with previous techniques such as laser Doppler interferometry or Doppler ultrasound. The first proof of principle was the measurement of flowing microspheres through a glass conduit,[26] the results of which correlated well with theoretical laminar flow. In-vivo animal model applications followed soon after,[27, 28] later leading to in-vivo imaging in humans.[29, 30, 31, 32] In early applications the Doppler shift was extracted by calculating the shift in the centroid of the detected power spectrum using a short time Fourier transform (STFT). However, when using this method the spatial resolution and velocity sensitivity are linked; a higher velocity sensitivity would result in a lower spatial resolution.[33] In addition, this calculation method is computationally
intensive. The development of a phase resolved method enabled spatial and velocity resolution to be decoupled, and imaging speed increased without loss of resolution, by using a Hilbert transform rather than a STFT.[29] Doppler OCT is discussed in more detail in Section 2.2.

The next major development which greatly improved imaging speed came with the introduction of Fourier domain OCT (FD-OCT) which removes the need for a moving mirror in the reference arm of the interferometer.[34] In this case the detector is replaced with a spectrometer or a diffraction grating and a linear array detector. An entire A scan requires just one simultaneous measurement of all of the camera pixels. The lack of mechanical parts means that the tissue can be scanned much faster[35] leading to a faster image rate (or a larger number of pixels per image produced at the same frame rate). Speeds of over 100 images per second have been achieved,[36] however this increase in imaging speed can lead to a decrease in sensitivity.[37] Swept source OCT (SS-OCT) is a similar method which detects a spectrum containing an entire A scan rather than sampling the interferograms to produce each pixel. Rather than a spectrometer at the interferometer exit a swept source is used in order to allow the detection of one part of the total spectrum at a time using a single detector, resulting in the full spectrum after one sweep through the source. FD-OCT and SS-OCT generally provide a higher sensitivity and signal-to-noise ratio when compared to TD-OCT.[38, 39, 40]

Today the uses of OCT are wide and varied, particularly in terms of clinical applications. Ophthalmological imaging has now advanced to the point where OCT is a standard diagnosis tool for conditions such as glaucoma, macular degeneration and diabetic retinopathy.[41, 42, 43] There have been many applications in dermatology from measurements of skin thickness,[44, 45] investigating skin structure in psoriasis patients[46, 4, 47] and even the imaging of tattoos.[48] It has also found uses in oncology in identifying cancerous tissue.[49, 50, 3, 51] Outside medicine OCT has been used to study security features of banknotes,[52] wall paintings[8] and damage to materials.[53, 9]

### 2.1.2 Light sources for optical coherence tomography

There are several requirements of sources used in OCT: emission in the near infrared (NIR) region, high spatial coherence, a short temporal coherence length and a high irradiance. The requirement for a NIR source results from the optical properties of tissue. The light needs to be able to penetrate the tissue
being imaged while being non-destructive and a proportion of the incident light also needs be reflected back into the interferometer to generate an image. This means that absorption of the light within tissue needs to be minimised where possible. Finally, the number of scattering events an average photon will undergo within the tissue needs to be considered. The more scattering events there are the less likely it is for that photon to contribute to the final signal.[54] Low angle multiple forward scattering can reduce the resolution of the image, as well as reducing the contrast between pixels creating a blurring effect in the image. Wide angle scattering also degrades the contrast but has a much smaller effect on the resolution.[55] Since OCT is a coherence gating technique photons scattered multiple times may be 'rejected' from the data of interest due to their different time of flight. Hence the proportion of photons which undergo a single backscatter should be as high as possible to improve the resulting image. NIR light best meets these criteria and several studies have shown the 1200 to 1800 nm region in particular to be suitable.[55, 56] Light from the 1300 nm region is capable of penetrating several mm into tissue due to the long scattering lengths and low absorption in this area of the spectrum. This depth is more than sufficient for in-vivo imaging of skin. High spatial coherence is needed to produce clear interference fringes whereas the requirement of a short temporal coherence length stems from the influence it has over the axial resolution. The need for high irradiance results from the low reflectance of tissue for the NIR wavelengths; the loss of light in the sample needs to be compensated for. However, care must be taken not to cause damage to the sample under investigation. A wide variety of broadband sources are available in the NIR region which can be used for OCT imaging. The decision to image using a source around the two most popular wavelengths, 800 nm and 1300 nm, means having to make a choice between a higher axial resolution for the 800 nm region due to its lower central wavelength, or the longer penetration depth provided by the 1300 nm region.

The dependence of the axial resolution on the bandwidth of the source has been studied at both 800 nm and 1300 nm;[57] a graph from one of these papers, Figure 2.1.8, presents this relationship for both wavelengths. If achievable resolution is the main priority, the advantage of using an 800 nm source is clear from this graph. To achieve the same resolution using a 1300 nm source as when a 800 nm source is used the bandwidth must be greatly increased to compensate for the higher central wavelength, as can also be seen from Equation 2.1.1. However the fact that sources in the 800 nm region suffer from a decreased
penetration depth must also be taken into account depending on the application. A 1300nm source can reach depths up to $2 - 3 \text{mm}$ in biological tissue,[15] allowing them to image microvasculature in the dermis which is at a depth of around $1 - 2 \text{mm}$.[58]

Figure 2.1.8: The relationship between the axial resolution and the source bandwidth for central wavelengths 800nm and 1300nm.[57]

Super luminescent diodes (SLDs) are available in both the 800nm and the 1300nm region, have a high irradiance and relatively low cost but can have a relatively long coherence length compared to other sources available. They are reportedly among the most commonly used sources for optical coherence tomography.[59] It has been suggested that the coherence length of an SLD-based system can be reduced by using multiple SLDs with differing central wavelengths.[55]

Femtosecond lasers are also used in OCT imaging and have been reported to give extremely high axial resolutions, in the order of $1 - 2 \mu\text{m}$[60, 61, 18] in tissue, although they can be expensive.[62] They can achieve much higher powers than other sources such as SLDs and have the advantage of being able to be focused to a spot size of a few microns[61] giving a high lateral resolution. However it has also been noted that a Ti:Sapphire laser in particular has excess amplitude noise, which can lead to a reduction in the signal to noise level, thus decreasing the system sensitivity if balanced detection is not used.[63, 15] Ti:Sapphire lasers can also be quite bulky, and as such may not be entirely suitable for some clinical uses which require portability. A direct comparison of the resolution
capabilities of a Ti:Sapphire and SLD source, both with a central wavelength of \( \sim 800\text{nm} \), has been made where the Ti:Sapphire laser had a higher achievable resolution and much clearer images.\cite{63} However, this paper was published in 2004 and SLDs are now available with spectral widths > 100\,nm providing a significant increase in resolution over the \( \Delta \lambda \simeq 30\text{nm} \) source used in this comparison. As of 2008 it was claimed that mode-locked fs solid-state lasers still gave the highest resolution in both FD-OCT and TD-OCT, whist enabling scanning at high speeds.\cite{61}

Photonic crystal fibre based supercontinuum sources have also been used in OCT imaging in recent years.\cite{64, 65, 6} Their usefulness stems from their broad bandwidth and their ability to achieve submicron resolution was demonstrated early on.\cite{64} They are generally pumped by a mode-locked laser, such as a femtosecond Ti:Sapphire, and are able to achieve bandwidths of several hundred nanometers in the NIR region with a power of several tens of milliwatts in the wavelength region used for imaging making them one of the highest resolution sources currently available. Sources with spectra which span both the 800\,nm and 1300\,nm regions have been used to simultaneously image at both wavelengths,\cite{65} generating two images and eliminating the previously mentioned problem of having to choose an appropriate wavelength region.

An alternative source which is commonly used in OCT is a swept source, where the source gradually cycles through its frequency range, which is usually around the same order of magnitude as the bandwidth of a SLD. These are used solely for SS-OCT and are several times more expensive than a SLD source. They have also been reported to introduce a depth-dependent phase error which can impact on Doppler measurements.\cite{66}

Overall the choice of source must be made with several key properties in mind: the output power, the spectral width and to a lesser extent the quality and the noise of the source. For clinical applications the cost and portability of a system should also be considered. It should also be remembered that the source power required to achieve a given SNR level increases with scanning speed.\cite{35} For the majority of standard resolution time and Fourier domain uses a SLD meets the requirements as they can achieve bandwidths of \( \sim 100\text{nm} \), power of a few tens of milliWatts and are relatively low cost, and fibre pigtailed SLDs in particular also have a high spatial resolution.\cite{10}
2.1.3 Interferometer styles

A variety of interferometer styles have appeared in publications since the invention of OCT over 20 years ago, although the majority are modified versions of either the Michelson or Mach-Zehnder interferometer which are shown in Figure 2.1.9. An OCT system requires an arm into which a sample can be placed as well as a reference arm which reflects enough light back in to the interferometer to balance the intensity returning from the sample arm. In the case of TD-OCT the should also be a method of generating a depth scan by varying the optical path length of the reference arm.

A basic Michelson-style interferometer (Figure 2.1.9a) has the potential to lose 75% of the optical power supplied by the source, with half of the power lost when the beams from the two arms are recombined since it is not directed towards the detector.[67, 68] Problems may also arise due to noise from the light in the reference arm of the interferometer adding to the interference signal, although this can be eliminated by using balanced detection.[55, 69] Optical circulators can be added to a Michelson-style interferometer to decrease the losses[67] - optical circulators are designed such that light incident on one of the ports is coupled to another so that the light is not directed back the way it entered, minimising the losses due to back reflections. However there are other choices of interferometer design which could be more suited depending on the requirements of the system.

Using a Mach-Zehnder style interferometer (Figure 2.1.9b) avoids much of the loss of light caused by a Michelson interferometer, particularly since none of the power is directed back towards the source by the beam splitter, and even more power can be saved if optical circulators are used in both the reference and sample arms. A Mach-Zehnder based interferometer also has an advantage in terms of the signal to noise ratio compared to a standard Michelson interferometer.[67]
The third type of interferometer which is occasionally used is based on a Fizeau interferometer - Figure 2.1.10. It has been suggested as a method of limiting any problems caused by changes in polarization in the separate arms of the interferometer due to changes in temperature or bending of the optical fibres[70] as there are no fibre-based separate sample and reference arms in the
interferometer. Any changes in polarization will affect the reference and sample signals equally. However, very little literature mentions this as a potential problem at all. A combined Fizeau-Michelson interferometer has been studied for use in OCT, however there are reportedly high losses associated with this style, potentially greater than when using a pure Michelson interferometer [71, 70]. It is also suggested that the SNR for a Fizeau interferometer is much less than that of a Michelson interferometer [71, 70].

A comparison of the Michelson and Fizeau interferometers, particularly with respect to the signal-to-noise ratio, has also been published [71]. The group calculated the theoretical SNR of various different implementations of the two interferometers; the highest SNR calculated was for a Michelson interferometer with an unbalanced beam-splitter and balanced detection, although the author does not state what splitting ratio was used, which also has an affect on the SNR. Another group studied a similar set of configurations which also showed that the Michelson interferometer with an unbalanced coupler and balanced detection had the highest SNR for a sample reflectivity of 1 [70].

The splitting ratio within the interferometer is also an important consideration, and can be optimised to increase the signal-to-noise ratio of the interferometers. The signal-to-noise ratio relating to photon detection in an optical coherence tomography imaging system is given by Equation 2.1.4. It is dependent on the power backscattered from the sample, \( P \), the photodetector efficiency, \( \eta \), the photon energy, \( E_p \), and the noise equivalent bandwidth of the detector, \( \text{NEB} \) [15].

\[
\text{SNR} = 10 \log \left( \frac{\eta P}{E_p \text{NEB}} \right) \tag{2.1.4}
\]
Since the SNR is dependent on the power which is backscattered from the sample and hence the optical source power illuminating the sample,[35] this power needs to be maximised while preventing damage to the sample. Also, to generate the clearest interferogram the optical power reaching the detector from each arm should be matched. The latter can be achieved by attenuating the reference arm of the interferometer. However it is advantageous to use an unbalanced coupler when splitting the light to the two arms, directing the unwanted reference arm light to the sample arm.[67] As a result of this, the splitting ratio determining the proportion of light being directed to each arm affects the overall SNR of the system.

The sensitivity of the imaging system for different splitting ratios, different interferometer designs and whether detection was balanced or unbalanced has been investigated in detail.[68, 67] Figure 2.1.11 is from one of these papers and shows the sensitivity for two interferometer designs, both in the balanced and unbalanced detection cases, with the sensitivity of a Michelson as a comparison. Balanced detection provides a significant improvement over unbalanced for all splitting ratios, and even more of an improvement when compared to a standard Michelson. However, the data used to generate this plot assumes properties of the noise which the authors state are only valid for a splitting ratio of $\alpha = 0.5$ (i.e. a 50 : 50 coupler). However, for the splitting ratio of where these assumptions are valid the difference in sensitivity between the Michelson and the two alternate interferometers is significant.

The authors suggest that the optimum splitting ratio for the set-up concerned is $\alpha = 0.12$,[68] therefore a 90 : 10 coupler should provide close to optimal SNR. Other groups have also suggested this splitting ratio to improve the sensitivity of a system.[72, 73] Figure 2.1.11 also shows that using balanced detection can improve the sensitivity of a system; in fact changing an unbalanced detection scheme to a balanced detection can result in a greater than 10 times increase of SNR.[74]
2.1.11: How the splitting ratio affects the sensitivity for 3 power conserving interferometer designs (i and ii indicate balanced and unbalanced detection respectively). Configuration B is a modified Mach-Zehnder interferometer, C is a modified Michelson utilising an optical circulator. It is clear that the modified Mach-Zehnder with balanced detection has the highest theoretical sensitivity from the designs considered by the authors.[68]

2.1.4 Delay lines

As mentioned previously TD-OCT requires a method of applying a varying time delay to light in the reference arm of the interferometer. Delay lines for use in OCT can be categorised into four groups, based on how the delay is produced:

1. Linearly translating mirrors
2. Rotating elements such as glass cubes
3. Physically stretching an optical fibre carrying the light
4. Applying a group delay in the Fourier domain.

No matter which method is used a high duty cycle is required to minimise scanning time ‘lost’ while data is not being recorded. The SNR, which needs to be as high as possible to increase sensitivity, is also proportional to the duty cycle.[75] High speed imaging is required in order to reduce movement artefacts which can occur when the sample being imaged moves during the imaging process. This is particularly important in in-vivo imaging. The speed at which the A scans can be produced ultimately imposes a maximum real time imaging speed on the system; for an imaging rate of 15 frames per second for an image with 512 A scans, 7680 A scans per second are required. The delay line
also needs to be able to produce an axial scan over a sufficient depth to be able to acquire data to generate an image with a depth many times the coherence length of the source.

Rapid scanning optical delay (RSOD) lines considered for use in OCT by various authors so far have included a galvanometer fitted with a retroreflector on a lever arm which allows for the path length to be scanned through as it is rotated through a small angle. However, repetition rates are only of the order of $\sim 100\text{Hz}$\cite{75} making it unsuitable for high speed imaging. Using a piezoelectric crystal to stretch the optical fibre presents problems with nonlinear fringe modulations due to hysteresis caused by the stretching itself\cite{75, 76}. A delay line using a rotating glass cube has also been suggested, and it is capable of achieving a high repetition rate. However, the delay produced by this is also non-linear which would require extra processing to account for this effect\cite{77}. Several advantages to using a grating-based RSOD have been noted by authors. They have a high scanning speed, high repetition rate and high duty cycle\cite{75}. Grating based RSODs can also be aligned such that they avoid introducing a phase modulation while scanning the group delay\cite{78, 79, 80} which is important when determining Doppler shifts of the light due to moving scatterers. An example of a grating based RSOD is shown in Figure 2.1.12. The light exits the collimator and is incident on the diffraction grating. From there it is focused by the lens onto a galvanometer mirror. The reflected light is collimated by the lens onto the grating and finally the mirror. The light repeats the path in reverse to arrive back at the collimator to be fed back into the interferometer. Double passing the light through the RSOD by using a mirror can avoid beam walk off due to the rotation\cite{75, 81}. As the only movement within the RSOD is a mirror undergoing small rotations, it is able to scan much faster than, for example, a linearly translating mirror moving along the direction of the beam propagation. Also, data collection can be synchronised with the portion of the scan where the rotational speed of the mirror can be considered to be linear to avoid problems due to the changing speed of the mirror while it is changing direction.
2.2 Doppler optical coherence tomography

Doppler optical coherence tomography (DOCT) is a functional adaptation to OCT allowing the measurement of flow within a sample with high precision.[37] In DOCT a structural image of the sample can be generated alongside a velocity flow map in real time. For clinical applications the resolution is such that individual capillaries can be distinguished and blood flow quantified. DOCT exploits the fact that if a photon scatters from a moving particle, such as a red blood cell in a blood vessel, then the photon gains a frequency shift which is dependent on the speed of the moving particle. This frequency shift can be extracted and the component of the velocity in one direction measured.

To aid in the detection of this frequency shift a carrier frequency is generally applied to the reference arm, for example using an electro-optic phase modulator (EOM). The moving particle produces a change in the phase which can be distinguished from this carrier frequency. Using a carrier frequency also helps to reduce low frequency noise.[82]

An EOM modulates light using the electro-optic effect and contains a birefringent material such as LiNbO$_3$, which is suitable for use with NIR or IR light. LiNbO$_3$ modulators make use of the Pockels effect, with phase modulators containing a single Pockel cell. The birefringence is induced by -
and proportional to - the local electric field. When a voltage, \( V_{RF} \), is applied across the EOM the refractive index of the crystal within the waveguide changes as they are linearly related according to Equation 2.2.1, where \( \Delta n \) is the change in refractive index, \( s \) is the coefficient indicating the strength of the electro-optic effect and \( E \) is the electric field caused by application of \( V_{RF} \). As the speed of light through a substance is related to its refractive index this change can cause the light to effectively slow down, thus producing a controllable phase delay for the light passing through.

\[
\Delta n = sE \quad (2.2.1)
\]

Modulators used in OCT are generally transverse; the voltage is applied perpendicular to the direction of light propagation through the cell, as shown in Figure 2.2.1. This avoids blocking the path of light in order to apply the voltage. As the crystal is birefringent in the presence of an electric field and has 2 axes - fast and slow - linear polarization of the light entering the EOM is important in order to prevent light receiving different phase shifts - and thus different delays - depending on the polarisation when it enters the crystal.

![Figure 2.2.1: A LiNbO₃ based electro-optic modulator. PM: phase-maintaining fibre. SM: Single mode fibre.][83]

If the voltage amplitude, \( V_{RF} \), required to produce a \( \pi \) degrees phase shift, \( V_{\pi} \), is known, then the voltage required to produce any phase \( \Delta \phi \) shift needed can be calculated through Equation 2.2.2.

\[
\Delta \phi = \pi \frac{V_{RF}}{V_{\pi}} \quad (2.2.2)
\]

If the reference beam interferes with light from the sample arm which has been back-scattered from a moving object then the detected temporal interference fringe, \( \Gamma_{ODT}(t) \), is given by Equation 2.2.3 where \( A(t) \) is the modulation of the
amplitude, $f_0$, is the phase modulation carrier frequency and $\phi(t)$ is a phase term dependent on the path length difference between the interferometer arms. $\Delta f_D$ is the Doppler frequency shift, which can be converted into the velocity of the scatterer by Equation 2.2.4 where $v_s$ is the velocity of the moving particle, $\nu_0$ is the central frequency of the source, $n_t$ is the local tissue refractive index and $\theta$ is the angle between the probe beam and the direction of motion of the scattering particle.

$$\Gamma_{ODT}(t) = A(t) \cos[2\pi(f_0 - \Delta f_D)t + \phi(t)]$$ (2.2.3)

$$f_D = \frac{2v_s \nu_0 n_t \cos \theta}{c}$$ (2.2.4)

One method of extracting information about the Doppler shift from the data is to use a short time Fourier transform (STFT) to calculate the centroid of the power spectrum. A shift in the centroid of the power spectrum due to a Doppler shift from moving scatterers can be measured and used to calculate the flow velocity at that location within the sample.\[^{26}\] Using this method the velocity is calculated from the difference between the centroid of the measured power spectrum and the carrier frequency provided by the EOM - $\Delta f_D$ - as shown in Equation 2.2.5.

$$v_{ODT} = \frac{\lambda_0 \Delta f_D}{2n \cos \theta}$$ (2.2.5)

One of the major drawbacks of this method is that the velocity resolution and the STFT time window are coupled by Equation 2.2.6, where $t_{STFT}$ is the STFT time window. To achieve a high velocity resolution a large time window must be used which leads to a decrease in imaging speed. The STFT method is also computationally intensive.

$$v_{min} = \frac{\lambda_0}{2n t_{STFT} \cos \theta}$$ (2.2.6)

From this equation the only other method to increase the velocity resolution would be to decrease the angle between the flow and the beam. However, as the transverse spatial resolution is also dependent on the STFT time window, as shown in Equation 2.2.7, where $\Delta t_p$ is the 1D scanning speed of the system, any increase in window size would decrease the transverse spatial resolution.
\[ \Delta x_p = v \Delta t_p \] (2.2.7)

Due to this coupling between the spatial and velocity resolutions another method of extracting the Doppler shift from the data is more favourable if both resolutions are important to the application. Rather than measure this frequency shift itself, the shift in the phase of the interferogram which it causes can be measured instead. A RSOD line creates a group delay in the light without affecting the phase delay if properly aligned. Therefore the only phase shifts applied to the light originate from either the EOM carrier frequency or a Doppler shift from a moving scatterer. This has led to the development of phase-resolved DOCT.

2.2.1 Phase-resolved Doppler optical coherence tomography

The phase-resolved method to obtain flow velocity information from a sample uses a Hilbert transform to extract the phase information from the detected interferogram. Extracting phase information directly from a real signal can be difficult. However, to extract the phase of a complex signal one can calculate the angle, or argument, using the real and imaginary portions of the signal. A Hilbert transform can be used to compute an imaginary value to complement the original interferogram which is used as the real value; this complex number is the analytical continuation of the original interferogram, which essentially applies a \( \frac{\pi}{2} \) phase shift to the signal. Both the structural and Doppler information can be obtained from an interferogram, with the structural information extracted from the real values and the Doppler information obtained from the analytical continuation as a whole. The Hilbert of a function \( \Gamma(t) \) is defined by Equation 2.2.8 where P.V. is the Cauchy principle value of the integral.[84]

\[
H(f(t)) = \hat{\Gamma}(t) = \frac{1}{\pi} \text{P.V.} \left( \int_{-\infty}^{\infty} \frac{\Gamma(t)}{\tau - t} \, d\tau \right) = \int_{-\infty}^{\infty} \left[ -i \text{sgn}(v) \right] f(v) \exp(2\pi i vt) \, dv
\] (2.2.8)

In practice a Hilbert transform can be applied to the interferogram by following steps 1-3 below.

1. Compute the (discrete) Fourier transform (FT) of the function \( \Gamma(t) \)
2. Multiply by \( i \times sgn(v) \), where \( sgn = \begin{cases} 1, & v > 0 \\ 0, & v = 0 \text{ is the sign function} \\ -1, & v < 0 \end{cases} \)

3. Compute the inverse Fourier transform (iFT)

To obtain the analytic continuation the Hilbert result is then multiplied by \( i \) and added to the original function, as in Equation 2.2.9.

\[
\hat{\Gamma} = \Gamma(t) + iH[\Gamma(t)]
\]  
(2.2.9)

The data for the structural image is the amplitude of this which can be low band passed to give an envelope of the original signal. The phase can be calculated via Equation 2.2.10, from which the velocity information can be extracted.[85, 33] The calculated phase - and hence the calculated velocity - is zero where the scattering particle is stationary. A non zero phase indicates motion.

\[
\phi = \arctan\left(\frac{\text{imag}(\Gamma(t) + iH[\Gamma(t)])}{\text{real}(\Gamma(t) + iH[\Gamma(t)])}\right)
\]  
(2.2.10)

Equation 2.2.11 is used to calculate the frequency shift from this phase, where \( \Delta f_D \) is the Doppler frequency shift, \( \Delta \phi \) is the change in phase between pixels and \( T \) is the time between each pixel. The change in phase is usually calculated between adjacent A scans, where \( T \) is the time between each A scan.[37] \( \Delta f_D \) can then be used in Equation 2.2.5 to calculate the flow velocity, with the sign of the phase change being used to determine the direction of the flow.[82]

\[
\Delta f_D = \frac{\Delta \phi}{2\pi T}
\]  
(2.2.11)

However, extracting the phase using the Equation 2.2.10 presents a problem when imaging fast flow velocities. Using this method the maximum phase shift that can be determined accurately is \( \pm \pi \), which corresponds to the Nyquist sampling frequency of the A scans.[33] Shifts greater than this are 'wrapped' and their exact frequency shift can not be determined and aliasing effects appear due to the loss of direction information. Techniques such as phase-unwrapping can be used to help determine the velocity if the frequency shift is greater than the Nyquist frequency.[85] Comparisons of the centroid method and the phase resolved method have shown that using the phase resolved method results in improved velocity resolution.[33]
2.3 Light-tissue interactions

The interactions between light and tissue can be complex. Overall attenuation within a tissue is generally described by three main types of interaction: absorption, scattering and reflection. The attenuation of light within tissue is governed by Beer’s law (Equation 2.3.1). $I_0$, $r$, $I(z)$ and $\mu_t$ are the initial irradiance, specular reflection, the attenuated beam at depth $z$ and total attenuation coefficient respectively. The attenuation coefficient is the probability that a photon will be absorbed or scattered when travelling unit distance.

$$I(z) = (1 - r) I_0 \exp(-\mu_t z) \quad (2.3.1)$$

2.3.1 Reflection

Reflection from tissue is generally diffuse and is most important to consider at the tissue interface. Light reflected from the surface can be calculated using Fresnel’s law, Equation 2.3.2, where $\alpha_i$ is the angle of incidence and $\alpha_r$ is the angle of refraction. If $\alpha_i$ is known $\alpha_r$ can be calculated using Snell’s law, Equation 2.3.3 where $n_1$ and $n_2$ are the refractive indices of the surrounding medium (e.g. air) and the tissue respectively. Light can also reflect from structures within the tissue. Liquids such as ultrasound gel or glycerol can be applied to the skin to reduce refractive index mismatch and improve image quality by reducing reflections from the surface of the sample.[86]

$$r = \frac{1}{2} \left[ \frac{\sin^2 (\alpha_i - \alpha_r)}{\sin^2 (\alpha_i + \alpha_r)} + \frac{\tan^2 (\alpha_i - \alpha_r)}{\tan^2 (\alpha_i + \alpha_r)} \right] \quad (2.3.2)$$

$$n_1 \sin \theta_i = n_2 \sin \theta_r \quad (2.3.3)$$

2.3.2 Absorption

To help achieve the greatest amount of backscattered light from a sample absorption should be minimised. At the wavelengths used for OCT imaging the molecules responsible for most absorption are water, melanin and haemoglobin with the exact level of absorption being wavelength dependent. The 600 nm to 1000 nm region is best for minimising absorption in tissue.[87, p39] with 800 nm being a common choice for OCT imaging. However the 1300 nm region is also
well placed for in-vivo imaging in terms of minimising absorption, as shown in Figure 2.3.1. The level of absorption is quantified by the absorption coefficient μₐ; the higher the absorption coefficient, the higher the probability of a photon being absorbed when it travels a given distance within the tissue.

![Absorption spectrum of water, melanin, and hemoglobin](image)

Figure 2.3.1: Absorption coefficient of water, melanin and haemoglobin.[88]

### 2.3.3 Scattering

In the range 500 nm to 1300 nm the scattering coefficient is much larger than the absorption coefficient,[89, p88] however the wavelength dependency is less than that of absorption. Photons which are scattered within the tissue can be split into two groups: singly scattered light and multiply scattered light. Ideally only singly scattered photons which backscatter towards the detector would contribute to the OCT signal as they would provide the most accurate depth information to produce an image. However, it is not possible to distinguish between these and multiply scattered photons which have a similar time of flight in the tissue. Multiply scattered photons are scattered many times within the sample meaning their path length can be much longer than their actual penetration depth. Figure 2.3.2 illustrates how they can contribute to the OCT signal despite their path length generally not being related to their penetration depth within the tissue.
Scattering in tissue is generally highly forward scattering with anisotropy, \( g \), in the region of 0.85 to 0.99.[87, p28] The value of \( g \) is calculated from the expected value of the cosine of the scattering angle using Equation 2.3.4, where \( p \) is the scattering pattern. \( p \) is often modelled by the Heney-Greenstein scattering function, Equation 2.3.5, in the case of small particle scattering, given that \( \int_{-1}^{1} p(\cos \theta) d(\cos \theta) = 1 \) and \( \int_{-1}^{1} p(\cos \theta) \cos \theta d(\cos \theta) = g \).

\[
\begin{align*}
g &= \frac{\int (\cos \theta) p(\cos \theta) d(\cos \theta)}{\int p(\cos \theta) d(\cos \theta)} \hspace{1cm} (2.3.4) \\
p(\cos \theta) &= \frac{1}{2} \frac{1 - g^2}{(1 + g^2 - 2g \cos \theta)} \hspace{1cm} (2.3.5)
\end{align*}
\]

Figure 2.3.3 shows the relationship of \( \theta \) to the direction of propagation before the scattering event, as well as the azimuthal angle, \( \psi \) which is also needed to determine the new direction of propagation. The azimuthal angle can be considered to be uniformly distributed over the region \( 0 < \psi < 2\pi \).[90]
Figure 2.3.3: How the deflection angle, $\theta$, and the azimuthal angle, $\psi$, are defined after a scattering event.

The scattering itself can be described by a combination of Rayleigh scattering, which is isotropic, and Mie scattering, which has a strong forward direction. In the region of $1300\text{nm}$ the scattering is typically due to collagen fibres in the skin, particularly in the dermis, and Mie scattering is several times stronger than Rayleigh, as shown in Figure 2.3.4.

The amount of scattering is quantified by the scattering coefficient $\mu_s$; the higher the coefficient, the higher the probability of a photon being scattered when travelling a given distance within the tissue. The reduced scattering, $\mu_t$, is calculated using Equation 2.3.6 using the scattering and absorption coefficients.

$$\mu_t = \mu_a + \mu_s (1 - g)$$ \hspace{1cm} (2.3.6)

The mean path length travelled by photons between interactions in tissue is given by $1/\mu_t$, which is $\sim 7\mu\text{m}$ in blood.[91]
Figure 2.3.4: The relationship between the reduced scattering coefficient as calculated by the Mie and Rayleigh theories, their sum and actual data from the dermis.[92]

2.4 Monte Carlo simulations of optical coherence tomography

Monte Carlo (MC) simulations are an extremely versatile method of simulating everything from protein folding to financial modelling. They were used to model light propagation in tissue before the development of optical coherence tomography, although the models were limited by the low memory and processing power of computers at the time. Monte Carlo simulations rely on the sampling of random numbers to simulate the expected value of a quantity to be studied. This expected value, $E(X)$, can be assumed to be equal to the actual value of the variable under consideration given that enough values have been simulated. As shown by Equation 2.4.1 each of the $N$ independent random variables, $X_i$ from the distribution $X$ are combined to give the expected value.

$$E(X) = \frac{1}{N} (X_1 + X_2 + \ldots + X_N) \quad (2.4.1)$$

In simulations of light-tissue interaction many 'photon packets' are usually propagated through the tissue independently of the other packets. Each packet is moved step by step, scattering along the way with the step size and deflection angles computed each time, with propagation continuing until the photon is
either absorbed or exits the tissue. The step size and scattering angle follow probability distributions which are sampled at each event. Reflection is also taken into account between boundaries within the tissue, for example where there is a change in refractive index, and also at the surface of the tissue. Each photon packet is moved individually meaning simulations of a large number of packets can be computationally intensive.

Source code for one simulation written in C - Monte Carlo for multi-layered media, or MCML - has been freely available since 1992[90] and has been adapted by many groups for their own use,[93, 94] including simulations of some aspects of OCT imaging. It allows the user to set the optical and dimensions of a 3 dimensional multi-layered sample at runtime, and outputs results to an output file for further analysis. In the original program these included reflectance and transmittance throughout the entire sample. The simulation treats the photon as a particle, rather than a wave and neglects properties such as polarisation.[90] In this simulation for each movement of each photon packet the probability density function of the free path - \( p(s) \), Equation 2.4.2 - is sampled to generate a step size, \( s \).[95]

\[
p(s) = \mu_t \exp(-\mu_t s)
\]

(2.4.2)

After the photon is moved through this distance in a direction given by the current direction of propagation the photon is either scattered or absorbed. This is determined by the weight of the photon packet, and the values of the absorption and scattering coefficients at that point in the tissue. The weight of the photon packet is unity when the photon is launched and reduces with each scatter by an amount depending on the properties of the surrounding tissue. The higher the absorption compared to the total attenuation coefficient, the greater the decrease in the weight. When the weight reaches \( \sim 0 \) the photon packet is completely absorbed and propagation of that photon ceases. This method can be thought of as a fraction of the photons in a packet being absorbed at each interaction point until they are all absorbed; the greater the absorption coefficient, the greater the proportion of photons absorbed at each step. If the packet is not absorbed after a particular step then it is scattered and a new sample of random numbers is used to determine the new direction of propagation. The Heney-Greenstein phase function is generally used to model scattering direction; the generating function is given in Equation 2.4.3, where \( P \) is a uniformly distributed computer generated pseudo-random number.
\[
\cos \theta = \begin{cases} 
\frac{1}{2g} \left( 1 + g^2 - \left[ \frac{1-g^2}{1-g^2-2gP} \right]^2 \right) & \text{if } g \neq 0 \\
2P - 1 & \text{if } g = 0
\end{cases}
\] (2.4.3)

The azimuthal angle is also based on another random number from a uniform distribution, and is given in Equation 2.4.4. The orientations of both angles are shown in Figure 2.3.3.

\[
\psi = 2\pi P
\] (2.4.4)

After a scattering interaction a new step size is generated, the photon packet is moved along this distance and then either scattered or absorbed once again. This continues until absorption or the remaining photon packet leaves the tissue. The process is then repeated with the rest of the packets, which is often in the order of \(10^6\) or \(10^7\) total packets for a single simulation.

From this style of simulation a wide variety of physical properties can be studied; fluence rates,[96] diffuse reflectance,[97] transmittance and absorption.[90] OCT images can be simulated by tracking the optical path lengths of each photon packet. A coherence gate can be simulated by comparing these path lengths with a theoretical reference arm path length. If these differ by more than the coherence length of the source then the packet does not contribute to the interference signal and thus is not used to generate the OCT image. It is possible to separate the photons with the best localisation information - i.e. the photons which have backscattered from the layer of interest - from the photons multiply scattered from other sections of the tissue, but which still contribute to the signal but to having the same path length.[98] In reality, though, these photons also contribute to the signal in a real OCT system as they cannot be distinguished and so separating them within a MC simulation is only useful for investigating the effect of noise due to multiply scattered photons.

Monte Carlo simulations have been used to investigate many aspects of OCT imaging, including the link between numerical aperture and probing depth,[93] noise[91] and the effect of multiple scattering events on the signal.[99] There have also been many simulations of the OCT signal itself however most of these focus on simulating a single A scan[98, 100, 101, 94], with few simulating a full 2D image.[102, 103] Simulations of a full 2D Doppler OCT signal are even rarer. The simulations themselves are often photon history generators, like the one described above, with the output of this simulation used to compute the
required physical quantities.\cite{91, 104, 105}

Through Monte Carlo simulations multiply scattered photons are known to affect the resolution and contrast of an OCT image\cite{106, 107} and they have confirmed that most of the localisation data required to produce an OCT image is from minimally, rather than multiply, scattered photons.\cite{104} In addition, it has been shown that the average number of scattering events undergone by a photon is proportional to the depth of penetration in the tissue, and so at large depths multiply scattered photons dominate the OCT signal, reducing the quality of the image.\cite{98, 106} Simulations such as these are essential in working out how the paths of the photons themselves affect the quality of the final image and to help increase SNR. They have also shown their ability to help optimise the equipment within the imaging system; using a small numerical aperture in the sample arm of the interferometer increases probing depth,\cite{93, 102} with each imaging system having a range of numerical apertures within which the clearest OCT signal can be obtained.\cite{54} It has been shown that this is because a larger NA leads to more multiply scattered photons being detected, but no increase in the least scattered photons required for the image.\cite{102}

Simulations have investigated one source of noise present only in Doppler OCT images - Doppler noise - which results in a flow signal appearing below the actual region of flow in the model. This is due to photons multiply scattering, particularly from moving scatterers, and having a path length long enough that the photon appears to have backscattered from below the vessel; this noise can be reduced by decreasing the angle between the incident beam and the direction of flow.\cite{91} The effect of increasing $\mu_s$ has been modelled\cite{108} which showed, as would be expected, that the average number of scattering events registered by detected photons increases as $\mu_s$ increases. However it also showed that increasing $\mu_s$ has a detrimental effect on the reconstructed velocity profiles, shifting them so that they appear deeper in the tissue and with a lower peak velocity; at higher values of $\mu_s$ multiply scattered photons begin to dominate the Doppler signal. This is also true for the depth of the flow in the sample; the deeper the flow, the lower the peak velocity and the more distorted the flow profile.\cite{109} Simulations of 2 vessels containing flow within a sample, with one positioned above the other, have shown that the quality of the Doppler signal at the depth of the second vessel is reduced due to Doppler noise.\cite{109}

From the results of published simulations it is clear that multiply scattering photons can have a detrimental effect on the Doppler signal, just as they can the structural signal. Monte Carlo simulations of Doppler OCT signals have
been compared to a similar experimental model in order to verify these results, for example multiple scattering lowering the SNR and hence image quality,[110] and to compare results of simulations of Doppler measurements to experimental results to validate the simulated model used.[111]

However, Monte Carlo simulations are somewhat limited in modelling real biological tissue. In real tissue there are often significant inhomogeneities in the refractive index as well as the scattering and absorption coefficient which are difficult to model precisely. Exact values of these at specific wavelengths can also be difficult to find in the literature, which also adds to the difficulty of comparing simulations between different groups as there are no standard values. Given that many of the functions used to compute the results of interactions are approximations this introduces another source of error. For example, despite being widely used in OCT Monte Carlo modelling the Heney-Greenstein phase function reportedly does not reflect the true angular distribution of light when scattered, particularly for anisotropic samples.[106, 112] Nevertheless these simulations have proved vital in understanding the interactions between light and tissue, in particular within an OCT system.

2.5 Discussion

This chapter has discussed the main background and theory of the many varieties of optical coherence tomography, its extension to Doppler measurements and its applications to imaging biological tissue. Different equipment set-ups, including interferometers and light sources, were compared. The functional extension Doppler OCT was discussed in detail and Monte Carlo simulations of photon propagation as applied to OCT have been introduced. This chapter has also briefly covered the interactions between light and tissue which are relevant to OCT imaging of tissue.
REFERENCES


Chapter 3

Characterisation of an electro-optic modulator for use in a Doppler optical coherence tomography system

3.1 Electro-optic modulators

The electro-optic modulator is an essential part of a phase resolved Doppler optical coherence tomography (OCT) set-up. It modulates the phase of the light in the reference arm of the interferometer which results in a carrier frequency which can be detected in the interferogram. If part of the sample being imaged is moving, for example blood flow in a vessel just below the surface of the skin, then the moving particles cause a small Doppler shift in the light which reflects from them. The purpose of the modulator and the carrier frequency is to make these Doppler shifts measurable such that they can be quantified at the same time as a structural OCT image is produced. The phase modulator discussed in this chapter is a LiNbO$_3$ based modulator (MPX1300PE-LN-0.1, Photline Technologies, Besançon, France), which takes advantage of the electro-optic effect and is suitable for use with near infrared or infrared light - Figure 3.1.1.
This chapter discusses the characterisation of the modulator, in particular the voltage required to produce a $\pi$ phase delay for a given frequency. This characterisation was required as the documentation accompanying the EOM only provided a value of $V_\pi$ for a frequency of 50kHz ($V = 3.2V$). However, for Doppler OCT imaging frequencies in the region of several hundred kHz are often used.[2, 3, 4]

### 3.1.1 Phase modulator theory

When a voltage, $V_{RF}$, is applied to the modulator the refractive index of the crystal within the waveguide changes linearly with the voltage. The speed of light through a substance is related to its refractive index; the higher the refractive index the lower the speed. An increase in refractive index on the phase modulator caused by an applied voltage will cause the light to travel at a slower speed than it would in a vacuum. This causes a phase delay in the light passing through. If the voltage required to produce a $\pi$ degrees phase shift, $V_\pi$, is known, then $V$, the voltage required to produce any other phase shift $\phi$, can be calculated through Equation 3.1.1.[1]

$$\Delta \phi = \pi \frac{V}{V_\pi}$$

(3.1.1)

For Doppler optical coherence tomography a $\pi$ phase shift is generally used, which corresponds to a half wavelength delay. This ensures that the DC and autocorrelation terms in the interferogram are eliminated and the cross-correlation terms, from which the Doppler signal is extracted, are
The measurement taken in OCT imaging is a cross-correlation of the light from the arms of the interferometer as a function of the path length difference between them. The autocorrelation terms in the interferogram can cause a ‘false’ element to the signal and are caused by the closeness of points of reflection within the sample.\[5, p64\] so it is advantageous to remove this signal as well as the DC signal wherever possible. Figure 3.1.2 illustrates the relationship between these three terms and the difference in path lengths between the points where the light is reflected in the sample and reference arms.

Figure 3.1.2: The intensity, \( i_D(z) \), of the A scan as a function of the difference in path length, \( Z \), between reflectors in the sample, \( S \), and reference, \( R \), arms. In OCT imaging the cross-correlation terms on the left is the signal of interest.\[5, p64\]

The voltage applied to the modulator is applied as a square wave with a constant frequency; the light passing through is then modulated with a carrier frequency of the same value. The resulting interferometer output is then demodulated around this frequency in order to measure the Doppler shift. The modulation frequency in the interferogram is shifted by \( f_s \), the Doppler frequency, if the light has scattered from a moving particle. Equation 3.1.2 allows the velocity of the scatterer, \( v_s \), to be calculated using this shift, provided that properties of the source are known as well as the orientation of the motion of the scatterer and the incident beam. This equation details the relationship between the Doppler shift in the sample arm light, \( f_s \), the velocity of the scatterer, \( v_s \), the central frequency of the source, \( v_0 \), the speed of light, \( c \), the local tissue refractive index, \( n_t \), and \( \theta \) the angle between the probe beam and the direction of motion of the scatterer.

\[
f_s = \frac{2v_s v_0 \cos (\theta) n_t}{c}
\]  
(3.1.2)
3.2 Experimental setup

The modulator discussed in this chapter, which is to be used in a phase resolved time domain optical coherence tomography system, was characterised to find $V_\pi$ for any given frequency value. To do this the fibre-coupled modulator is placed in a fibre-based Mach-Zehnder style interferometer, as shown in Figure 3.2.1, and the detector voltage recorded for different voltage amplitude and frequency combinations.

![Figure 3.2.1: Schematic of the Mach-Zehnder interferometer used for modulator characterisation.](image)

The source used to collect the data is a 1310nm laser diode, with a coherence length of $\sim$ 10cm. The air gap in the interferometer in Figure 3.1.1 was needed to ensure matching the arm lengths of the interferometer so that an interferogram can be seen at the detector; interference was only seen if the path lengths of the two arms are equal to within the coherence length of the source. The required distance for this air gap was calculated by measuring the lengths of the optical fibres in each arm and calculating the difference in path length. By using a laser diode, rather than the superluminescent diode source which is used in the imaging system itself, setting up the interferometer was simplified. The longer coherence length of the diode ensured that interference can be seen in the detector signal while the mirror is moved over a larger range. The mirror needed to be positioned so that as much of the beam was reflected back into the collimator as possible, while ensuring that the path length difference between the two arms of the interferometer was less than the coherence length. With the SLD having a much shorter coherence length of $\sim$ 11μm it would be more difficult to align the air gap while ensuring the correct air gap length.
The voltage generation and data recording was achieved using LabView visual interface (VI) with a data acquisition card (PCI 6115, National Instruments, Austin, Texas, USA) and connector block (BNC2110, also National Instruments). The voltage output of the connector block was connected directly to the modulator, while the detector output was connected to another port so that it could be simultaneously recorded using the same VI and exported to a file for later analysis.

3.2.1 The effect of modulation on the interferogram

An unmodulated interferogram is shown in Figure 3.2.2, and the Fourier transform of this is given in Figure 3.2.3. The interferogram shows low frequency oscillations, which can also be seen in the Fourier transform.

![Figure 3.2.2: The interferogram at the detector of the interferometer without a voltage applied to the electro-optic modulator. With the electro-optic modulator switched on modulations at the same frequency as the driving frequency will be seen in addition to the low frequency modulation seen here.](image-url)
Figure 3.2.3: The Fourier transform of the detector voltage with no voltage applied to the electro-optic modulator. The peaks around 50 Hz correspond to the frequency of the interference generated by the output of the interferogram.

Figure 3.2.4 compares the Fourier transforms of the interferograms with and without a voltage applied to the modulator i.e. with and without phase modulation in one arm of the interferometer. If the voltage is $V_\pi$ then a $\pi$ phase shift is applied to the light in the arm in which the EOM is placed. As the light in the other arm of the interferometer does not experience this shift, when the light recombines at the second beamsplitter the two beams are in anti-phase and interference is minimised, resulting in a reduction in the detector voltage. Due to the voltage being applied as a square-wave, this phase shift is essentially switched on and off at this frequency, meaning the interferometer output is also modulated; see Figure 3.2.4b.
Figure 3.2.4: A comparison of the Fourier transforms of the interferogram for (a) no voltage applied to the modulator and (b) an 8 V square-wave applied at a frequency of 10 kHz. Peaks can be seen at multiples of 10 kHz in (b), indicating measurable modulation in the interferogram.

3.2.2 Problems with frequency generation using a DAQ card

When testing the voltage generation element of the VI it was noticed that the voltage frequency applied to the modulator was not always equal to the frequency which was actually generated, due to the limitations of the data acquisition card in the computer. This effect was only noticeable for frequencies approaching 1 MHz. Figure 3.2.5 shows how the generated frequency compares...
to the measured output frequency for higher frequencies.

Figure 3.2.5: Comparison of the generated (red) and measured (black) frequencies of the voltage being applied to the modulator. While the generated frequency increases as expected the frequency measured at the point where it is applied to the EOM appears to jump in increasingly larger steps.

The National Instruments data acquisition card has a maximum source frequency of 20Msamples per second for outputting; this is the board clock rate. In order to output a square wave of 1MHz a minimum output sample rate of 4Msamples per second is required, since a minimum of 4 samples are needed to describe the square wave. However, due to the way the sample clock rate is calculated from the board clock rate, only discrete frequencies can be outputted. Other frequencies default to the next largest value which the board is capable of achieving. The relationship between the board clock rate and the square wave frequency is shown in Equation 3.2.1 where \( Y \) is an integer:[6]

\[
\text{Frequency} = \frac{\text{Actual clock rate}}{4} = \frac{\text{Board clock rate}}{4Y} \quad (3.2.1)
\]

Table 3.2.1 shows the square wave frequencies which the card can output approaching 1MHz. For microvascular imaging using DOCT a frequency of 800kHz applied to the modulator has been shown to be able to measure flow velocity.[2, 3]
<table>
<thead>
<tr>
<th>Board clock rate (Hz)</th>
<th>Y</th>
<th>Actual clock rate (Hz)</th>
<th>Frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 M</td>
<td>5</td>
<td>4000000</td>
<td>1000000</td>
</tr>
<tr>
<td>20 M</td>
<td>6</td>
<td>3333333</td>
<td>833333</td>
</tr>
<tr>
<td>20 M</td>
<td>7</td>
<td>2857143</td>
<td>714286</td>
</tr>
<tr>
<td>20 M</td>
<td>8</td>
<td>2500000</td>
<td>625000</td>
</tr>
<tr>
<td>20 M</td>
<td>9</td>
<td>2222222</td>
<td>555556</td>
</tr>
<tr>
<td>20 M</td>
<td>10</td>
<td>2000000</td>
<td>500000</td>
</tr>
<tr>
<td>20 M</td>
<td>11</td>
<td>1818182</td>
<td>454545</td>
</tr>
</tbody>
</table>

Table 3.2.1: Selection of square wave frequencies which can be outputted by the DAQ card.

As Table 3.2.1 shows there is not a linear increase between the frequency values which can be used. For high frequencies in particular there is a large difference from one frequency to the next and this limited the frequency values which can be used for characterisation.

### 3.2.3 Method of characterisation

The voltage which produces a $\pi$ phase shift at a given frequency can be determined by measuring the height of the peak of the modulation frequency in the Fourier transform of the interferogram. A sinusoidal curve would be expected when a wide range of voltage values are measured, with troughs corresponding to phase shifts which are odd multiples of $\pi$. In order to determine the voltage and frequency combinations which produce a $\pi$ phase shift, from which a general relationship can be obtained, a method of cycling through many combinations while recording an interferogram is needed.

The final design of the LabView program used to generate the driver voltage is able to cycle through the voltage amplitudes for a single user determined frequency. However, only frequencies which the card was capable of outputting to the modulator could be used. At the same time the detector signal was fed into the connector block where it was sampled at a rate of 4 Msamples per second while the modulation was applied, with the samples written to a file. Each file contained the samples recorded for one combination of voltage frequency and amplitude. Even for the highest frequency which could be applied to the modulator – 1MHz – the sample rate chosen was sufficient to allow the modulation to be seen in the detector signal.

The results presented below were taken from interferograms 800000 samples
long, corresponding to a 0.2 s sample of the detector signal. The signal was Fourier transformed using Matlab to enable analysis of the individual frequency components of the signal and the amplitude of the peak at the modulation frequency measured. A plot of one of these Fourier transforms is shown in Figure 3.2.6.

![Fourier Transform Plot](image)

**Figure 3.2.6:** The Fourier transform of the detector voltage. A voltage of 0.345 V at a frequency of 500 kHz was applied to the electro-optic modulator. The peaks at 1 MHz and 1.5 MHz are harmonics of the 500 kHz peak.

One advantage of extracting the modulation information from the interferogram through the use of a Fourier transform is that any outside interference only affects the results if it is at the same frequency as the modulation. Since this is highly unlikely, it can be assumed that the amplitudes of the peaks in the Fourier transform which correspond to the modulation frequency are not subject to errors due to interference.

### 3.2.4 Measuring amplitudes of frequency components

The amplitude of the peak in the Fourier transform corresponding to the modulation frequency varied as the voltage applied to the modulator is scanned over the range of 0.25 to 10 V for each specific modulation frequency. A plot of this - such as in Figure 3.2.7 - indicates which voltages result in a higher level of modulation appearing in the detector signal for a given modulation frequency. A minimum, one of which occurs at around 4 V in Figure 3.2.7, corresponds to the combination which provides a $\pi$ phase shift.
Figure 3.2.7: Amplitude of the 833 kHz peak in the Fourier transform of the detector voltage when a voltage of varying amplitude between 0.25 to 4.98 V was applied at 833 kHz. The amplitude varies as the voltage is changed due to the changing phase delay being applied to one arm of the interferometer.

The voltage amplitude at which the minimum occurs can be plotted against frequency to give an indication of how varying the frequency applied to the modulator affects the amplitude required to apply a $\pi$ phase shift to the light, giving a method to calculate the voltage required to produce the correct phase delay at a specific modulation frequency.

The trend in Figure 3.2.7 - a sinusoidal oscillation - continued past the 5 V point illustrated in the plot. This was due to higher voltages producing increasingly large phase shifts which are a multiple of $\pi$; odd multiples of $\pi$ correspond to minima. This trend repeated for every frequency tested but with the location of the minima increasing as the frequency increases.

### 3.3 Finding $V_\pi$

To find the voltage value corresponding to the first minimum for each the modulation frequency a sinusoidal curve was fitted to the plot of the amplitude of the peak; the curve was fitted using Origin 8 Pro (ver 8.0891). An example of the resulting fit is shown in Figure 3.3.1. The minimum of this curve was taken to be the voltage which produces a $\pi$ phase shift for that specific frequency value. 200 voltage amplitudes were plotted for each frequency, from 0.25 to 4.98 V in steps of 0.024 V.
Figure 3.3.1: The amplitude of the 714kHz peak in the Fourier transform for an electro-optic modulator voltage of 0.25 to 4.98 V. The trough at 3.79 V indicates the voltage which produces a \( \pi \) phase shift for that frequency.

The minima for each frequency and voltage combination were then plotted to show the frequency and voltage combinations which gave a \( \pi \) phase shift - Figure 3.3.2.

Figure 3.3.2: The voltage amplitudes which correspond to the peaks in the amplitude-voltage plots for each modulation frequency.
The linear fit in Figure 3.3.2 is given by Equation 3.3.1, providing a method of calculating the required modulator voltage amplitude, \( V \), for a given frequency \( f \), in kHz which results in a phase shift of \( \pi \) to light passing through.

\[
V = 9.34 \times 10^{-4} f + 3.20
\]  

(3.3.1)

The equation gives a voltage of 3.950 V for a frequency value of 800 kHz. The documentation accompanying the EOM states that the voltage to produce a \( \pi \) phase shift at a modulation frequency of 50 kHz is \( V = 3.2 \text{ V} \). Using the equation above gives a calculated value of \( V = 9.34 \times 10^{-4} \times 50 + 3.20 = 3.24 \text{ V} \). Equation 3.3.1 can be used to find the required voltage for any frequency in order to obtain a half wavelength delay of the light travelling through the modulator. In combination with Equation 3.1.2 the voltage amplitude and frequency combinations to generate any phase shift from the 0 to 2\( \pi \) region can be calculated.

### 3.4 Discussion and conclusion

This chapter discusses the characterisation of a LiNbO\(_3\) based electro-optic phase modulator. By investigating how changing the frequency and voltage amplitude applied to the modulator changes the interferogram recorded at the output of an interferometer a general relationship between the frequency and voltage has been found. This allows the calculation of \( V_{\pi} \) for a given value of modulation frequency, \( f \). Using this in combination with Equation 3.1.1, which links the phase change with the voltage, the voltage required to produce any phase change can be calculated for any required frequency.

This phase modulator was to be used in a custom build Doppler OCT system to enable flow measurements. The development of the software for this system - and the use of the modulator - is discussed in Chapter 4.
REFERENCES


Chapter 4

Software for a Doppler optical coherence tomography system

4.1 Introduction

This chapter discusses the development of software for a time domain Doppler optical coherence tomography system, the synchronisation of the hardware and software as well as two methods for generating a Doppler flow image which were tested. These methods are:

- Phase resolved Doppler OCT where the Doppler flow information is calculated from the change in phase between adjacent pixels, which can be extracted from the interferogram using a Hilbert transform.

- Correlation mapping Doppler OCT a correlation map between two images captured close in time is calculated, with areas of low correlation signalling flow and high correlation signalling a stationary sample.

The optical system itself is discussed in more detail in Section 4.1.1. Figure 4.1.1 shows the processes the software must include in order to produce both a structural and velocity image from the raw detector data. The analogue to digital conversion of the detector signal is achieved using a high speed data acquisition card controlled by the same software which processes the data and renders the image to the screen.
Figure 4.1.1: Overall flow of the data from the optical system to the software. The interferometer is connected to a fibre-coupled balanced amplified detector. The signal from the detector is collected by a data acquisition card which carries out the analogue to digital conversion. The signal is then processed to generate a structural image of the sample, as well as an image showing areas of flow.

The software was written for 32-bit Windows in C++, using Open Graphics Library (OpenGL) to provide hardware accelerated image rendering as well as a number of additional 3rd party libraries to produce the user interface and to process the data. One high speed data acquisition card (ATS660, Alazar Technologies Inc, Quebec, Canada) was used to capture data from the detector and transfer it to the application using the software development kit accompanying the card. This card was also connected to the analogue signals used to synchronise the hardware and data capture, including those from the driver board of the galvanometer mirror in the delay line. Another data acquisition card (PCI 6115, National Instruments, Austin, Texas, USA) was used together with a BNC connector block (BNC2110, also National Instruments) to control a raster scanner on the hand probe to produce a B scan.

4.1.1 The OCT system

The OCT system which this software was designed to obtain raw data and display the resulting images from is a rapid scanning optical delay (RSOD) based time domain system. It uses a fibre based Mach-Zehnder style interferometer - Figure 4.1.3 - with a diffraction grating and galvanometer mirror in the RSOD line to produce the depth scan. A fibre-coupled SLD (SLD1325hp-32, Thorlabs, New Jersey, USA) with a central wavelength of 1321nm, 110nm spectral width and 25mW maximum power (Figure 4.1.2) is used as a source and an AC-coupled balanced amplified InGaAs detector is used to detect
the interferogram at the interferometer output. Graham Dinsdale, a research associate, designed and built the optical system in Figure 4.1.3. The housing for the main section of the interferometer and the RSOD, in addition to the handprobe, were designed and built by the School of Physics and Astronomy workshop, University of Manchester.

![Figure 4.1.2: The normalised spectrum of the superluminescent diode, as measured with a spectrometer (NIRQuest-512, Ocean Optics, Ocean Optics, Dunedin, Florida, USA).](image)

An electro-optic phase modulator (EOM) (MPX1300PE-LN-0.1, Photline Technologies, Besançon, France) is used to create a stable reference phase in the reference arm of the interferometer when the phase resolved method is being used. Light from the source is split using a beamsplitter and directed through the sample and reference arms with a 50:50 beamsplitter recombining them before the detector. The splitting ratio of the initial beamsplitter is chosen to ensure the output power from each arm of the interferometer is matched as close as possible. Circulators are used to ensure that the majority of light reflected from each of the sample and reference arms is directed towards the second beamsplitter to be recombined. The detector is a dual balanced amplified detector with 2 inputs from the second beamsplitter and is connected directly to the fast DAQ card in the computer. The system is designed to generate 256 by 512 pixel images at a maximum rate of approximately 30 Hz, which is dictated by the A scan speed of the RSOD.
The only moving part in the RSOD - Figure 4.1.4 - is the galvanometer mirror (Counting Rotating Scanner (CRS), GSI Lumonics, Massachusetts, USA) which is driven by a driver board. This driver board also outputs analogue signals which can be used to synchronise hardware and software; these signals and their usage are discussed further in Section 4.3.1. The RSOD is aligned so that no phase delay is generated, leaving the EOM as the sole source of phase modulation.

For correlation mapping OCT the phase modulator is not required and can be replaced with a patch cable.

Figure 4.1.4: The grating based rapid scanning optical delay line. The light enters the delay line via a fibre and is launched from a collimator (A). It is then diffracted from a grating (B) and focused by a lens (C) onto a galvanometer mirror (D, inside a sealed chamber). This reflects the light back to the grating and onto a stationary mirror (E) from where it is reflected back to the galvanometer mirror and then to the collimator to be sent back to the interferometer. Part of the driver electronics for the mirror are just visible (F).

The optical components at the end of the sample arm are mounted on a handprobe, as pictured in Figure 4.1.5. This includes a raster scanner to enable 3D imaging; currently the system only moves one of the mirrors to generate a lateral scan for a 2D image. The light is focused on the sample using an
achromatic lens. The lengths of the two arms of the interferometer are such that the focal point of the lens is within the depth scan.

Figure 4.1.5: The hand probe. Light from a collimator (A) is incident on a set of $x$-$y$ scanning mirrors (B) and then focused by an achromatic lens (C) onto the sample, in this case a mirror (D). Light reflected from the sample is collected by the lens and reflected back to the collimator by the mirrors.

Modifications were made to the system to help increase the amount of light received from each of the interferometer arms:

- The collimators in each arm were changed as it was discovered they were not the correct type for the fibre being used - the fibre being APC (angled physical contact) while the collimators were designed for PC (physical contact) fibres.

- The lens in the hand probe of the sample arm was also replaced with an achromatic lens with a higher numerical aperture, enabling light to be more accurately focused where it is needed.

- The galvanometer mirror in the RSOD is kept in a sealed container which has been flushed with a gas to remove any dust which could damage the mirror while it is rotating at high speed. The window into this chamber through which the light passes was also replaced with a piece of glass with broadband anti-reflective coating to increase transmission.
4.2 Hardware and external libraries

The software was developed for use on a Windows XP 32-bit desktop computer with the following hardware:

- Alazar ATS660 16-bit 125 MS/s 2 channel PCI digitiser
- National Instruments PCI 6115 12-bit 10 MS/s data acquisition card
- National Instruments BNC2110 connector block
- NVIDIA GeForce 7900GS graphics card

The graphics card used supports OpenGL version 2.1 with the latest drivers installed. While this is not the most recent version of this graphics library it was sufficient to allow hardware accelerated rendering of the images. The high speed Alazar card is used solely for the data capture process; 3 of its inputs are used for timing signals, and one of the data input channels is connected to the detector. The slower speed NI DAQ and accompanying connector block are used to generate a trigger input to the Alazar card to signal the start of a frame as well as control a set of mirrors on the hand probe which creates a B scan. The generation of these signals was achieved by using a shared library generated from a LabView visual interface (VI); the VI on which this is based was originally written in LabView by research associate Graham Dinsdale. The adapted code was exported from LabView as a DLL and is loaded by the software at runtime. Both cards rely on the signals generated by the driver board of the RSOD galvanometer mirror to enable synchronisation of the A and B scans of the image as well as the analogue to digital conversion of the data. In the case of the signals from the VI a signal from the RSOD is used as a time base to produce signals with lower frequencies. These signals and their application are described in more detail in Section 4.3; how each signal is connected to each of the DAQ cards and their oscilloscope traces are specifically discussed in Section 4.3.2 and Section 4.3.3 respectively.

The following libraries were also used:

- Alazar software development kit [version 6.0.3, June 2011] for data capture using the Alazar ATS660 digitiser
- wxWidgets [version 2.9.2, July 2011] for the graphical user interface (GUI)
• Open Graphics Library (OpenGL) [version 2.1, July 2006] OpenGL
Extension Wrangler (GLEW) [version 1.5.8, January 2011] for image
rendering

• Fastest Fourier Transform in the West (FFTW) [version 3.3, July 2011]
to implement the Hilbert transform[1, 2]

• Signal Processing Using C++ (SPUC) [version 2.3.1, July 2009] to apply
a filter

• BOOST [version 1.47, July 2011] for simple multi-threading

4.2.1 wxWidgets
wx-DevC++ is an integrated development environment (IDE) which allows user
interfaces to be created using the wxWidgets library; C++ as a programming
language does not have a standard library to create GUIs. Using this IDE
the GUI can be designed and appropriate functions linked to widgets such as
buttons, scroll bars and drop down menus.

4.2.2 OpenGL
OpenGL is a multi-platform application programming interface for
hardware-accelerated rendering of graphics supported by all modern graphics
cards. OpenGL and GLEW are used for real time rendering of the structural
and Doppler images. The data for each image is used to generate a texture
object, with this texture then applied to a rectangle called a primitive quad.
This quad is the exact size of the image to be rendered - 256 pixels wide by 512
pixels deep. As a new set of data is captured the next image is produced by
substituting the current texture with a new one generated from the new data.
The next texture appears on the quad when the screen is refreshed.

4.2.3 Fastest Fourier Transform in the West (FFTW)
library
The Fourier transform and its inverse - required to extract phase information
to generate a flow image for phase resolved Doppler OCT - are implemented
using the FFTW library. This library provides tools to automatically select
the most efficient method of computing a FT or iFT for a given data size on a
specific computer reducing the impact of the computationally intensive Hilbert transform on the imaging speed. The plan or method of applying the FFT - known as wisdom - can be saved to a file and imported again later to recreate the previous plan, saving time if the same size FT is used continuously as is the case with this software.

The software was initially written to provide structural images from the OCT system, with the Doppler extension added once the system could produce OCT images at an acceptable speed. Changes to the software included applying a Hilbert transform using the FFTW library in order to extract phase information from the interferogram.

The Hilbert transform $H[f(t)]$ of the function $f(t)$ is defined by the following 3 steps:

1. Compute the (discrete) Fourier transform (FT)
2. Multiply by $i \times sgn(w)$, where $sgn(w) = \begin{cases} 1, & w > 0 \\ 0, & w = 0 \\ -1, & w < 0 \end{cases}$ is the sign, function
3. Compute the inverse Fourier transform (iFT)

Following on from this, the result is multiplied by $i$, and added to the original signal to give the analytical continuation, $f(t) + iH[f(t)]$. The amplitude of this can be filtered to give the envelope of the original signal, which is used to generate the structural image. The imaginary term gives the phase information which is required for the velocity information to be extracted.

### 4.2.4 Signal Processing Using C (SPUC)

The Signal Processing Using C++ (SPUC) library is used to generate and apply a Butterworth IIR low pass filter. This filter is used on each A scan to produce an envelope of the interferogram. Without an envelope any long interferogram would appear as bands of black and white as the interferogram oscillates. The filter must be designed so that the structural information is preserved, but that this oscillation is significantly reduced.

### 4.2.5 BOOST

Boost is a collection of libraries which builds on the basic functionality of standard C++ libraries. It was used for simple multi-threading so that the
software could capture data while a LabView VI - which is run as a DLL from within the software - runs. Without running each of these processes on a separate thread the software would wait for the VI to complete running before capturing data, missing the frame trigger signal in the process.

4.3 Structure of the software

4.3.1 Timing of data capture

There are 3 timing signals generated by hardware signal which together trigger the analogue to digital conversion of the current detector voltage by the fast DAQ card. These signals, which originate from 2 sources, are explained here. Figure 4.3.1 and Figure 4.3.2 show how they are used in relation to the DAQ cards in the computer. Oscilloscope traces of the signals themselves are shown later in this section in Figure 4.3.3, Figure 4.3.4 and Figure 4.3.6.

1. The frame trigger, where the rising edge signals that the mirror on the hand probe is starting a B scan. This signal originates from the LabView VI via the BNC connector block and has a maximum frequency of $\sim 30$ Hz. This signal is high for 256 pulses of the line trigger and then low for 2, during which the raster scanner moves back to its original position. Its exact frequency depends on how often the function which leads to the generation of this signal is called, which in turn depends on how fast the software can process each frame of data.

2. The line trigger, also known as the pixel enable signal. This originates from the driver electronics of the rotating mirror in the RSOD line. This is high only when the mirror is at a point during its scan where the mirror is in the central 66% of its scan; this avoids the portions of the scan where the mirror is changing direction and so the depth scan is not linear. It has a frequency of $7.91$ kHz.

3. The pixel trigger, also known as the pixel clock, also originates from the RSOD driver electronics. This is a varying signal, with a frequency in the region of $7 - 9$ MHz, which triggers the capture of a single data value from the detector by the high speed DAQ provided that the previous triggers have been received. The signal varies in frequency so that pixel data is obtained at equal points in the depth scan.
4.3.2 Connections to the DAQ cards

Figure 4.3.1 details the inputs to the fast DAQ card. The 7.9 kHz pixel enable signal and MHz pixel clock signal originate from the RSOD galvanometer driver electronics and act as a trigger for an A scan and individual pixels respectively. The rising edge of the Hz frame clock from the slow DAQ card via the connector block triggers the start of a frame. This card also outputs a voltage ramp connected to the raster scanner on the hand probe to move a mirror which moves the focus of the light laterally along the sample surface; this produces the B scan. It uses the kHz signal as a timebase so that the A and B scans are synchronised.

The controls for the raster scanner and the frame trigger originate from the LabView VI. It takes the kHz pixel enable signal from the RSOD which is synchronised with the A scans as an input. It then outputs 2 signals: a TTL signal which is high for 256 of the 7.9 kHz pulses as well as a ramp output beginning at 0V and increasing to 0.25V in 256 equal steps beginning as the
VI outputs the rising edge of the TTL pulse. The signals connected to the slow DAQ card are illustrated in Figure 4.3.2.

![Diagram of signal connections](image)

Figure 4.3.2: The signals connected to the connector box which is connected to the slower DAQ card. The kHz pixel enable signal from the galvanometer driver is used as a time base to generate a voltage ramp to move the mirrors on the raster scanner in the hand probe, and a frame clock so that the start of the frame is synchronised with the movement of the mirror.

### 4.3.3 Signal traces

Figures 4.3.3, 4.3.4 and 4.3.6 show the the single frame trigger and the hand probe voltage ramp, 7.9kHz pixel enable signal, and the MHz pixel clock respectively. While the kHz signal from the RSOD driver electronics is constant in frequency, the faster MHz signal varies slightly depending on the position within the A scan in order to keep the depth scan linear. Both of these signals are passed through differential filters to remove noise; the filter for the kHz was put together by experimental officer Mike Needham while the filter for the MHz signal was produced by laboratory technician Martin Coram. Despite being filtered the pixel enable signal still shows signs of interference from the MHz signal, which originates from the same driver board, and which appears as high frequency oscillations in Figure 4.3.4. The Fourier transform of this signal is shown in Figure 4.3.5, which shows a broad peak just after 7MHz.
Figure 4.3.3: The frame trigger to start the collection of a frame of data at the start of the corresponding voltage ramp which moves the mirror on the hand probe.

Figure 4.3.4: The pixel enable signal which, when high, indicates that the RSOD mirror is at a point during its scan where pixel data is valid. The oscillations visible on the signal are due to interference from the MHz signal which originates from the same electronic driver board.
Data is only collected during the middle 66% of the galvanometer mirrors rotation, while the change in the mirror's angle of rotation can be assumed to be linear in time. Data is also only collected during a one way scan; after taking an A scan the mirror must move back to its original position before the next A scan can commence, meaning pixel data is only valid 33% of the time. This is the point when the signal in Figure 4.3.4 is high, signalling that the pixel data can be taken.

![Figure 4.3.5: The Fourier transform of the pixel enable signal. Interference from the MHz signal can be seen in the broad peak beginning around 7 MHz.](image)

To correct for the change in the mirror’s velocity over the course of a single scan/mirror rotation (which is not linear) the frequency of the signal in Figure 4.3.6 varies. This signal is used to time the capture of individual pixels; the changing frequency ensures that data is taken at points corresponding to a linearly increasing depth. Without this variation parts of the image would be compressed in comparison to others.\[3\] The maximum scanning angle can be varied by a dial on the power supply for the galvanometer mirror; reducing the voltage supplied to the mirror reduces the amplitude of the scan and results in a shallower depth scan.

The data is collected in blocks of 131072 values, forming an image 512 pixels deep and 256 pixels wide. The maximum achievable frame rate of the system is limited by the frequency of the B scans; for an image 256 pixels wide the theoretical maximum is \( \frac{7910}{256} \approx 30 \text{ Hz} \). In reality this is limited by the speed
of the software which can not process the data as fast as the system could supply it.

![Pixel clock signal](image)

Figure 4.3.6: The pixel clock signal which triggers each individual data capture by the software. This signal is generated by the driver board for the RSOD. The change in frequency occurs on a time scale longer than shown here but has been included for completeness alongside the other analogue signals.

### 4.3.4 Flow of the software

On initialisation of the software the system is prepared for data acquisition; the fast DAQ card is initialised and default values of parameters including the voltage input range, impedance and coupling type are set. These can be changed by the user at any time via a menu. The LabView VI is also loaded via a DLL, which gives the software control of the raster scanner on the hand probe. At this point the VI is not running and no signals are generated. Once the initialisation is complete the system is ready to capture data. Capturing data commences when the user presses the capture button on the GUI, Figure 4.3.7, or selects capture from the drop-down menu. Before this, however, the hardware must be switched on. This is due to the reliance on the signals from the RSOD driver electronics; if the hardware is off the software returns an error alerting the user. Capturing is stopped with the stop button, when the software encounters an error or when 50000 images have been obtained.
Figure 4.3.7: A screenshot of the optical coherence tomography software graphical user interface while imaging a stationary mirror. The left-hand pane shows the structural image - in this case a stationary mirror which appears as a bright line due to its highly reflective surface. The right-hand pane shows - when the mode is selected using the "Doppler enabled" tick box - the Doppler, or flow, image. In this case there is no signal as the mirror is stationary. Both images are displayed as the post processed results and are scaled so that the full colour scale is used.
After the user has started the data capture, but before the first acquisition after the software is loaded, the software also checks for a FFTW wisdom configuration file which is loaded to create the plans for the Fourier transforms. If this file is not present or the software is unable to load it for any reason the user is informed and new wisdom is created and saved to a file for future use. The FFTW wisdom file contains the information required for the FFTW library to generate a plan to compute a Fourier transform of an array of data 256 values long - a single A scan. This plan is optimised for the particular computer on which the software is currently running on, choosing the algorithm which produces a correct output in the quickest time possible. The step of loading wisdom for the Fourier transform is only used for the phase resolved method of obtaining Doppler flow data.

The Alazar card is set up in traditional autoDMA mode which allows the AUX I/O input on the card to be used as an additional trigger to enable the essential synchronisation of the start of the data capture and the B scan voltage ramp. The data for a single frame is stored in the buffer of the card as it is collected, where a single channel can store 128 Msamples, until it is transferred to the application memory. The data is stored in records of 512 data points with 256 records - a single frame - per buffer. The overall flow of the software for a single frame is as follows:

1. The Alazar card is armed for a trigger event signalling the start of a frame.

2. The software then calls the LabView VI which controls the raster scanner on the hand probe via the slow DAQ card. It also outputs the frame trigger, with the start of the voltage ramp and the frame trigger synchronised. This is called in a separate thread to the main software thread so that the software can generate the B scan ramp and collect A scans simultaneously.

3. The frame trigger is connected to the fast DAQ card; when triggered the software waits for the next rising edge of the pixel enable signal from the galvanometer mirror in the RSOD. This prevents the software from beginning a capture at any point other than the start of a depth scan.

4. Once this is triggered, 131072 (256 by 512) samples of the detector voltage are taken. They are captured one A scan at a time in bursts of 512 after the card has received each pulse from the pixel enable signal from the
RSOD. The timing of the individual samples is governed by the pixel clock from the RSOD.

5. The program waits until all samples have been captured to the cards on board memory. If this does not occur within 5 seconds the software alerts the user and stops the capture.

6. This is repeated for a second frame if the correlation mapping method is being used.

7. If frame data has been captured it is then transferred to the application memory from the card buffer.

8. The data is converted into a usable voltage value from the 16-bit sample value recorded by the card.

9. If phase resolved OCT is used and the user has selected to generate a flow image, the data is Hilbert transformed and the Doppler signal calculated.

10. If the correlation mapping method is used then the flow signal is calculated from the two captured frames if flow imaging is selected.

11. The final structural image is rendered displayed to the screen as a greyscale image, as is the flow image if it has been generated.

The data capture function itself is placed inside a `for()` loop, which continues until 50000 frames have been captured and displayed or an error is encountered. If the software exits with an error message the user is informed with the cause. Once the loop exits the final frame is left displayed on screen. The GUI also includes buttons to allow the user to save the image or raw data to a file for later use, as well as copying the current image to the clipboard. These controls are available for both the structural image and the Doppler flow image.

### 4.3.5 Rendering the images

OpenGL is a graphics library capable of rendering 2D and 3D images in real-time. C++ does not include a standard graphics library hence OpenGL was chosen as it is supported by all recent graphics cards and there is an abundance of reference material available in both textbooks and via the internet. It also allows the GPU to take over some of the processing that would
otherwise be done by the CPU, freeing up the processor for other tasks. As mentioned above the graphics card used during software development only supports OpenGL 2.1, rather than the most recent version. However, version 2.1 is sufficient to be able to use concepts such as textures and pixel buffer images.

In order to render images a ‘canvas’ on the GUI has to be initialised and the images were drawn onto this. The software GUI included two 256 by 512 pixel panels, one for each image to be displayed, and a wxGLCanvas was set up on each one. Onto each of these canvases a 256 by 512 pixel 2D shape called a primitive quad was drawn and the image to be displayed was applied to the quad as a texture.

The canvas was first initialised when the first captured frame was ready to display. The initialisation routine is only used once each time the user presses the ‘Capture’ button to reduce overheads. For subsequent images within the same imaging session the texture is redrawn with the new data. As there were 2 images - structural and Doppler, there are 2 canvases, quads and textures when Doppler imaging is enabled.

The initialisation function initialise OpenGL, initialise the canvas on the appropriate wxPanel and sets the relevant OpenGL parameters so that the image is displayed as required. The two code extracts below are where the texture is prepared and where this texture is then applied to the primitive quad.

```c
glBindTexture (GL_TEXTURE_2D, texture [0] );
glTexImage2D (GL_TEXTURE_2D, 0, GL_LUMINANCE,
(GLint) width , (GLint) height , 0, GL_LUMINANCE,
GL_UNSIGNED_BYTE, textureData );
```

The above functions select the texture to be used and sets the height, width, colour scale, data format and data origin for that texture.

```c
glBegin (GL_QUADS);
    glColor3f (0.0, 0.7, 0.7);
    glTexCoord2f (0.0, 1.0);
    glVertex2f (256, 512);
    glTexCoord2f (1.0, 1.0);
    glVertex2f (0, 512);
```
The above code draws the 256 by 512 pixel primitive quad on the canvas and applies the selected texture to the shape. The resulting texture appears as a flat 2D image of the data assigned using the block of code above. Once the first frame has been displayed the next set of captured data can be displayed using a single command – `glTexImage2D()` – as the OpenGL canvas had been previously initialised. This command replaces the previous texture with new texture data, and everything else remains as it was with the previous image. Additional lines in this section of the code, such as `glFlush()` ensure that the image is rendered as soon as possible.

The same technique is used to render the Doppler images for both the phase resolved and correlation mapping methods. However, in the case of the correlation mapping, the scale of the images is first reversed before they are applied using a texture. As a result of the calculation highly correlated areas of the image as well as the background are set to 1, whereas areas which show low correlations are closer to 0. Due to the way colour mapping is applied (pixels with a value $\sim 0$ set to black, pixels $\sim 1$ set to white/a colour), this scale is reversed so that low correlations are closer to 1 and the highly correlated areas and background are set to 0 so the areas of flow appear white/coloured over a black background.

### 4.3.6 Phase resolved Doppler optical coherence tomography calculations

The first method used to try to obtain flow information from the raw data is the phase resolved method. This method has been used with success by many groups in a variety of systems for several years.[4, 5, 6] Although there are variations in how the data is processed, all methods involve generating the analytic continuation of the interferogram using a Hilbert transform.

This method described below, where the phase of a pixel on one A scan is calculated and then compared to the phase of the same pixel in adjacent A scans, was the main algorithm used for this work. Two alternatives - using a slightly different method to calculate the phase change between adjacent A
scans[6] and calculating the Doppler standard deviation[4] - were also tested, but gave similar results.

In early Doppler OCT publications the flow image was generated by looking for shifts in the power spectrum of the detected light using a short time Fourier transform (STFT). However, there were several disadvantages to this method, such as being computationally expensive and the velocity and spatial resolution being coupled via the size of the window used for the STFT. Using the phase resolved method removes the need to use a short-time Fourier transform to calculate the Doppler shift and results in an increase in imaging speed and velocity resolution.[6]

The following algorithm was followed:

1. Take the Hilbert transform of the original interferogram, \( \Gamma(t) \), a single A scan at a time.
2. Multiply the output by \( i \) and add to the original signal to give \( \Gamma(t) + iH[\Gamma(t)] \), the analytical signal.
3. Take use \( \phi = \arctan\left(\frac{\text{imag}(\Gamma(t) + iH[\Gamma(t)])}{\text{real}(\Gamma(t) + iH[\Gamma(t)])}\right) \) to obtain the phase of each pixel.
4. Starting from the second A scan compare each pixel to the equivalent pixel of the previous A scan. The change in the phase value, \( \Delta \phi \), indicates the magnitude of the frequency shift from one pixel to another.
5. The exact frequency shift can be calculated by \( \Delta f_D = \frac{\Delta \phi}{2\pi T} \), where \( T \) is the time between adjacent A scans.

The maximum phase shift which can be determined via this method is \( \pm \pi \), which corresponds to the Nyquist sampling frequency. Any phase shift exceeding this will be 'wrapped' and its exact phase shift will be unobtainable using this method. This method is also sensitive to the angle between the flow and the incident beam; an angle of 90° results in the flow being undetectable and prior knowledge of the sample structure is needed to determine the exact flow velocities.

As a test of the algorithm 2 interferograms representing A scans were simulated. One A scan was a pure sine wave, the other was an nearly identical wave with approximately 10% of the signal shifted by \( \pi \). The result of calculating the phase change is shown in Figure 4.3.8, where the change between the two A scans is clear.
In the case of real data a carrier frequency must be applied to the light to reduce noise. This is often achieved using a LiNbO$_3$ EOM placed in the reference arm.[7, 8, 9] This is driven by an external voltage and applies a varying phase shift to the light passing through it at a carrier frequency, $f_c$. Light reflecting from a moving scatterer adds a shift in this carrier frequency, $f_c \pm f_s$; the value of $f_s$ can be used to calculate the speed of the scatterer directly, provided that the geometry of the incident beam and flow are known. In the case of this phase resolved method, the change in the phase between two points in adjacent A scans indicate that there are varying values of $f_s$ at each point; light reflecting from one point has backscattered from a particle moving faster than the light backscattered from the adjacent point in the sample. In order for this phase change to be calculated the signal must be demodulated around the carrier frequency so that $f_s$ can be detected. With this method the RSOD must also be very carefully aligned, with the light centred on the axis of the galvanometer mirror, to avoid introducing an additional phase delay to the light in the reference arm.[10]

As areas of the image which do not contain structural data will not provide an accurate phase calculation, the phase difference for these areas is set to zero. The phase difference is only allowed to be non-zero for the locations where there is a data value above the background noise and their adjacent pixels.
Figure 4.3.8: Two simulated interferograms and the calculated phase change between them. (a) The two signals, one with a phase shift (blue). (b) The calculated phase shift between the two signals.
The phase stability of the system affects the minimum detectable Doppler shift; the greater the stability the lower the minimum detectable shift.\cite{11, 12, 13} The phase stability of this system is illustrated in Figure 4.3.9, which has been adjusted to account for phase wrapping, an effect which limits measurable phases to the range $-\pi < \phi < \pi$. The standard deviation of the phase difference is 0.136 radians compared to other reported phase stabilities in the region of 0.06 rad and lower.\cite{11, 14, 13, 15} Fourier domain systems, in particularly spectrometer based systems, have been reported to have a high phase stability.\cite{16, 17, 18, 19} This is in part due to the lack of moving parts; time domain systems with a rapid scanning delay line require very careful alignment. Using phase modulation to provide a carrier frequency also introduces another aspect of instability, although electro-optic based modulators are more stable than using mechanical methods.\cite{10} Jitter from the $x$-$y$ in the sample arm can also cause phase instabilities, leading to a noticeable change in the phase between adjacent A scans.\cite{13}

Figure 4.3.9: The phase difference of the interferogram as calculated between sequential A scans at the surface of a mirror. The phase has been corrected to negate the effects of phase wrapping. As the mirror is stationary there should be minimal signal as any signal corresponding to motion needs to be distinguishable from the noise. However, the background noise for this set-up is around 3 times higher than the values for phasestability reposted in the literature.
Issues with phase stability can be reduced by using a static surface as a reference point for each A scan. Since it is known that this point in the A scan is not generating an additional phase shift due to motion the phase values for each A scan can be adjusted according to this area. It is, however, difficult to do in practice as the stationary surface of a sample will move from one imaging session to another, and potentially between each frame. In addition it only removes phase instabilities which vary on a time scale greater than the time between A scans.

The phase modulator used in this set up - a Pockels cell based EOM - has a high insertion loss (3.9 dB) resulting in a 60% loss of the power in the reference arm. Combined with the high losses due to the free-space RSOD (measured to be greater than 95%), this reduced the power reaching the second beamsplitter from the reference arm to such an extent that there was a very large decrease in detector voltage and hence final image quality. To generate the clearest interferogram the intensity of the light from each arm at the point of recombination should be matched as equally as possible. With 10% of the light directed to the reference arm the light detected at the third port of the circulator in the reference arm (Figure 4.1.3, i.e. close to the point of recombination) was undetectable when measured with a power meter; The light exiting the third port of the circulator in the sample arm was measured as 7.5 µW.

If the first beamsplitter is changed to a 50:50 beamsplitter - providing 5 times as much light to the reference arm - then the powers of the light exiting the reference and sample arms are measured to be 4.2 µW and 3.5 µW respectively. This increases the highest voltage recorded by the software for a single frame from ~ 0.03 V to ~ 0.4 V if a mirror is used in the sample arm to provide a maximum signal. However this still does not provide a clear interferogram from which to make accurate phase calculations - Figure 4.3.10 shows a section of an interferogram for a mirror when a 50:50 beamsplitter is used. The interferogram does not contain a complete oscillation and is not highly sampled making it difficult to determine the exact phase at a particular point on the plot.
Figure 4.3.10: An A scan of a mirror with the phase modulator in the reference arm. With the interferometer present in the system there is a noticeable reduction in the signal-to-noise ratio compared to the same system without a modulator due to the additional losses in the optical system. The signal of interest - around data point 250 in this plot - is also less well defined, leading to lower image quality.

The reduction in the detector signal also has a noticeable effect on the quality of the signal and hence the final structural image quality - Figure 4.3.11. As the phase sensitivity of the system is also inversely proportional to the square-root of the signal to noise ratio[19] this has a direct effect on the ability of the system to measure the phase of the interferogram accurately and thus produce a Doppler signal.
Figure 4.3.11: A comparison of the structural images taken (a) with the phase modulator in place (and the light equally split between both arms of the interferometer) and (b) with the modulator replaced by a patch cable of equal length (with 90% of the source light directed to the sample arm). Both images have been normalised. It is clear that in the second image the signal to noise ratio is improved as fluctuations in the background noise are greatly reduced.

It is also believed that the use of single mode fibres - rather than polarisation maintaining fibres - causes additional power loss in the reference arm. The SLD source is unpolarised, however the polarisation of the light can be controlled by coiling of non-polarisation maintaining fibre. Any bending or twisting of the fibre creates birefringence and this effect can be exploited to create fibre based waveplates which change the polarisation of the light as it is transmitted.\[20\]

While the degree of polarisation does not change (i.e. the light remains unpolarised overall) the state of polarization is changed.\[21\] This effect, however, is wavelength dependent and so the polarization change varies for different wavelengths.

The phase modulator is highly polarisation dependent and contains a waveguide which does not transmit light not aligned to the correct axis.\[22\] It was observed that simply lightly bending or twisting the fibre pigtail on the SLD source (which has a single mode fibre pigtail, rather than polarisation maintaining fibre) can alter the amount of light detected at the exit of the phase modulator from
a maximum of 37% transmission to a minimum close to zero. Moving the fibre induces birefringence and as a result the output polarisation of the light is extremely sensitive to motion. This effect was tested with a 1310 nm laser diode (Thorlabs, New Jersey, USA) with a much narrower bandwidth than the SLD to investigate whether the extreme sensitivity to motion was linked to the spectral width of the source, with some wavelengths having their state of polarisation altered more than others caused by the wavelength-dependent nature of birefringence. This laser diode gave 37% transmission through the EOM (close to the expected 40% transmission), identical to the maximum transmission measured for the SLD. Motion of the fibre pigtail of the laser diode also had a very strong effect on the polarisation and hence modulator throughput.

The spectra of light originating from the SLD and after passing through the EOM are shown in Figure 4.3.12; spectra of the light at the output of the phase modulator when the intensity is near its maximum and minimum are shown. All spectra are normalised to aid comparison. There is no significant change in the spectrum after transmission through the EOM which suggests the effect is not wavelength dependent. A polariser and a fibre based polarisation controller were used to try to minimise the effect of the birefringence but had no improvement on final image quality.
Figure 4.3.12: Comparison of the spectra of broadband light before and after transmission through the phase modulator. Spectra for points near the minimum and maximum transmission through the phase modulator are given for comparison. There is no significant change in the spectra after the modulator when compared to the original spectrum of the source.

Due to the high losses produced by introducing the EOM to the system resulting in a degradation of the structural image quality and the fact that Doppler information could not be extracted, it was decided to investigate a second method of obtaining flow information which did not require additional equipment to be added to the system.

With just the structural images being captured and displayed the software can achieve a peak of 9 frames per second. With the phase resolved OCT method included this is reduced to around 7 fps. Not all of this increase in processing time is down to the additional calculations required for the Hilbert transform, as this additional time also includes the time required to rescale and display the image. The Hilbert transform itself is reasonably fast, with the entire image being processed in 0.015s.
4.3.7 Correlation mapping optical coherence tomography calculations

The second method of extracting flow information is achieved using a cross-correlation calculation of sequential frames. While the computation time for this method is much longer than the phased-resolved method, it removes the need for a phase modulator resulting in a higher quality interferogram to work with. Correlation techniques have only recently been used to extract flow information,[23, 24, 25, 26] with promising results. This technique looks at changes in the intensity of light backscattered from the sample. Moving particles produce a change in intensity which changes much faster than the change seen for stationary objects, provided that there is a suitable time gap between the images. Provided that the time between images is chosen such that stationary areas of the image appear highly correlated and moving areas show little correlation then a flow map can be produced. The technique can be so sensitive that Brownian motion in a fluid can be detected.[23, 24]

Equation 4.3.1 is the correlation calculation between 2 images, image A and image B.[24] A grid of size $M \times N$ is placed over the image where the correlation is to be calculated; the values of $I_A$ and $I_B$ are the values of the pixel intensity of the image at those points and $\overline{I}$ is the mean value of the grid. The difference between each of the $M \times N$ points and the average $\overline{I}$ is then calculated. This is repeated to produce values for the entire image. For an image of dimensions $x$ by $y$ pixels, the final result is a correlation map of $(x-M)$ by $(y-N)$ pixels which indicates the level of correlation in that area of the image using values between $-1$ and $1$. A value of 1 or $-1$ indicates the images are perfectly correlated whereas 0 means the images are completely uncorrelated.

$$c(x, y) = \sum_{p=0}^{M} \sum_{q=0}^{N} \frac{[I_A(x+p,y+q) - \overline{I_A}(x,y)] \times [I_B(x+p,y+q) - \overline{I_B}(x,y)]}{\sqrt{(I_A(x+p,y+q) - \overline{I_A}(x,y))^2 + (I_B(x+p,y+q) - \overline{I_B}(x,y))^2}}$$

(4.3.1)

To enable ease of programming for the movement of the grid over the images, the grid was aligned such that the current $(x, y)$ position is in the top left corner of the grid, as shown in Figure 4.3.13. The ability for the user to change the grid size was considered - if the grid is centred on the point of interest then computing the locations of the pixels to be used for the calculation requires
a different calculation for both odd and even lengths. However, due to how significant the changes in image noise and imaging time can be for even small changes in grid size the grid size was hard-coded into the software to provide standardisation between images.

Figure 4.3.13: Arrangement of a 5 by 5 grid around the current \((x,y)\) value (shaded).

The size of the grid used to generate the correlation map has a direct effect on the clarity of the resulting flow image[24] as well as the overall computation time of the correlation map. Small grid sizes can create a noisy correlation map sensitive to local changes in intensity; small changes in the structural information can result in lower correlations. Larger grid sizes can result in a blurry correlation map which is not sensitive to small areas of flow. These are also more time consuming to process due to the extra calculations involved.

Table 4.3.1 gives an example of the increase in computing time to calculate the end correlation result for increasing grid sizes. This increase in time for the correlation calculations directly affects the imaging speed of the software; for example when using a 10 by 10 grid the imaging speed is reduced from 9 frames a second (with no flow calculations being processed) to less than 1 frame a second.
Table 4.3.1: Time taken to calculate a single correlation map for two images.

<table>
<thead>
<tr>
<th>Grid size</th>
<th>Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 by 2</td>
<td>0.047</td>
</tr>
<tr>
<td>3 by 3</td>
<td>0.109</td>
</tr>
<tr>
<td>4 by 4</td>
<td>0.172</td>
</tr>
<tr>
<td>5 by 5</td>
<td>0.281</td>
</tr>
<tr>
<td>6 by 6</td>
<td>0.375</td>
</tr>
<tr>
<td>7 by 7</td>
<td>0.485</td>
</tr>
</tbody>
</table>

At any point in the image where structural information is not detected the correlation result is to be ignored; in the areas of the image where there is a signal above a background level the correlation is set to the value calculated using Equation 4.3.1. This is required because areas of background noise show up as areas of low correlation and might be confused for an area where there is a flow signal. This effect is achieved using a binary mask which is set to 1 in areas of the image where there is a signal above a specific level above the background noise and 0 elsewhere. If a pixel is set to 1 on the binary mask, and the corresponding point on the correlation map is within a range where the results show a low correlation (i.e. there is structural data which shows low correlation), then the value for that pixel in the final flow image is set to the value of the correlation map. Otherwise (either no structural data or the area shows high correlations) the value is set to 1. The range of $-0.6 \leq c \leq 0.6$ has been suggested as a boundary between the low/high correlations. [24]

As explained previously, in order for the flow information to show up as white/grey on a black background the values are reversed so that areas of no structural data or high correlations are set to black while the data of interest appears as grey/white. Figure 4.3.14 show examples of the correlation map, the binary map and the resulting flow image for a stationary mirror.
Figure 4.3.14: An example of a typical (a) correlation map (low correlation shown as white) (b) binary map (valid structural information shown as white) and (c) resulting correlation image (motion data shown as white) of a stationary mirror.

In order to capture images that are close enough in time for the stationary parts of the sample to be highly correlated, the data capture function of the software is altered to capture 2 sequential frames, increasing the number of buffers (frames) in a single function call to 2. As the speed of the RSOD is 7910 Hz, the minimum time difference between frames is $\frac{256}{7910} = 0.032$ s given that there are 256 frames in an image. However, the time between frames is longer due to the time required to rearm the trigger and for the raster scanner mirror in the sample arm to reset. These frames are low pass filtered and the resulting envelope of one of these is displayed as the structural image, while both of the images are used in the correlation calculation for the flow image using the above method.

While the computation time for the correlation method is longer than for the phase resolved method, this method is still able to image fast enough to display the flow image almost immediately, without having to save the data and post process separately.

4.4 Testing the software with the system

Initial testing of the software was carried out without the system triggers - a pulse generator was connected to the trigger port to provide the initial frame
trigger, and the timing of the sampling governed by an internal clock on the Alazar card set to sample at 5 MS/s. At this stage the source was connected directly to the detector, bypassing the whole interferometer. This was sufficient to be able to test the general data capturing and image display abilities of the Alazar card and the software.

Once the software was able to capture and display data at an adequate speed, it was connected to the triggers and the interferometer. The system was aligned such that the focal point of the hand probe corresponded to a point approximately \( \frac{1}{3} \) of the way through a depth scan as it is assumed that the areas of interest in a sample will be near the surface, rather than at a depth of several millimetres where light penetration is reduced. The depth of the scan is controlled by the voltage supplying the rotating mirror in the RSOD, so that the depth of the image can be increased or decreased as appropriate. Initial testing was conducted using highly reflective materials such as a silver-coated mirror, a microscope slide and an empty glass capillary which were placed in front of the hand probe at approximately the focal point of the lens. Figure 4.4.1 is a plot of the A scan of the surface of a mirror before processing.

![Figure 4.4.1: An unprocessed A scan of a mirror - large sinusoidal oscillations are visible in a small portion of the signal when the optical path length of both arms of the interferometer are nearly matched. This signal is squared and enveloped to minimise the light-dark fluctuations generated by the oscillations and to remove the negative components before being displayed by the software.](image)

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4.4.1 Structural images

Examples of images of a stationary empty glass capillary tube is shown in Figure 4.4.2. Samples such as this produced significant amounts of backscatter, thus generating a high detector voltage and a clear image.

![Images of empty capillary tubes](a) (b) (c)

Figure 4.4.2: Two empty capillary tubes with (a) 1.55mm outer diameter, 1.15mm inner diameter and (b) 0.46mm outer diameter, 0.36mm inner diameter, as imaged from the side of the tubes. (c) shows the 0.46mm tube as a cross section. The first two bright signals are from the two interfaces of the glass wall closest to the handprobe, while the second pair of signals - clearer in (b) than in (a) - are due to the back wall of the capillary.

The system is capable of imaging in-vivo although due to the lower reflectivity the images are not as clear - Figure 4.4.3.
Figure 4.4.3: OCT image of the palm side of the index finger (a) and the dorsum (back) of the hand (b).

4.4.2 Doppler images

Although the system was unable to image skin with enough detail so that flow information could be extracted using either the phase resolved or correlation mapping method, motion information was obtainable from highly reflective samples. Figure 4.4.4 shows the resulting 'flow' images for a stationary mirror and the same mirror being held by hand, as obtained using the correlation method.
Figure 4.4.4: Resulting 'flow' images from (a) a stationary mirror and (b) a mirror being held by hand which causes movement on a small scale.

The same technique was applied to a flow phantom consisting of a glass capillary tube containing diluted Intralipid, a fat emulsion with similar optical properties as tissue, which was pumped through the tube using a syringe. These images were compared to images obtained from a commercial system using a larger diameter capillary tube with Intralipid which showed a much clearer structural signal - Figure 4.4.5. The Intralipid in Figure 4.4.5a was not visible meaning that the correlations method was unable to extract flow information from the liquid.
Figure 4.4.5: A comparison of the structural images of capillary tubes containing Intralipid from our system (a) and with a commercial swept source system (b). The capillary in (a) has an external diameter of 0.46 mm and contains 2% Intralipid while (b) is a 1.55 mm capillary with 20% Intralipid.

4.5 Conclusions

Software was developed to collect and process data from a time domain Doppler optical coherence tomography imaging system and to display the resulting structural and flow images in real time; data capture was synchronised to the hardware to maintain appropriate timing for capturing frames. Modifications were also made to the optics of the system to improve the light returning from each arm of the interferometer and hence the quality of the data obtained.

The system is suitable for imaging highly reflective samples such as mirrors or objects made from glass. It is just able to image the surface of human skin but is unable to image to a significant depth to allow the measurement of the thickness of layers within the skin or the imaging of blood vessels or sweat glands.

Two methods to obtain flow information were tested - the Hilbert transform based phase resolved method, commonly seen in literature, and the recently developed correlation mapping method. Only the correlation mapping method
was able to image motion of highly reflective samples using the current optical system. The phase resolved method requires the use of a phase modulator in the reference arm of the interferometer to produce a carrier frequency, however this results in a significant loss of optical power. Combined with other losses in the system - particularly from the RSOD line - this made the signal to noise ratio too low to produce reliable data. However, even with the correlation mapping method, the image quality is too low to obtain flow data from Intralipid in glass capillary tubes.

The phase resolved method is an often used method of flow calculation and its ability to calculate accurate flow velocities is well documented. However due to its high sensitivity, dependent only on the time between frames used in the calculation, the correlation method is potentially better suited to determining qualitatively if an area of the sample is flowing or stationary than the phase resolved method.

4.6 Future work

There are several lines of work which could be followed to improve imaging speed and quality.

The software itself could also be improved, particularly in terms of computation speed. Very simple multithreading has been used so far, but only where it is absolutely necessary for the software to function. This could be expanded to allow concurrent processing of the structural information (rescaling and filtering) and flow information (either Hilbert transform or correlation calculations).

In terms of the structural image, the method of envelope detection could be improved; the current method of applying a low pass filter to each A scan occasionally does not envelope the entire interferogram, leaving bands of white and black patches at the surface of a highly reflective sample such as a mirror. Reducing the value of the upper edge of this pass band results in blurring of the image and potential loss of structural detail.

While the system and software are capable of imaging simple flow models and highly reflective samples, it is not capable of imaging in-vivo as was the original plan. One problem arises due to the loss of power in the RSOD. Due to its free space nature it requires careful alignment however it still looses a large proportion of the light entering the RSOD (> 95%). A CCD array camera and
Diffraction grating have been purchased to enable the system to be transformed into a Fourier domain system. This will completely remove the RSOD and associated losses and problems with alignment, hopefully producing a significant improvement in image quality.

In order to validate the final system and the images it produces it would be sensible to compare the images it produces to ones obtained with a commercial system. Chapter 5 describes a comparison of OCT images from such a system with other non-invasive imaging techniques in a study investigating skin thickness and microcirculation in patients with systemic sclerosis. Later in this thesis Chapter 6 discusses the development of a Monte Carlo model which could also be used as a comparison with the Doppler OCT system.
REFERENCES


Chapter 5

Development of software to measure skin thickness from optical coherence tomography and ultrasound images

5.1 Introduction

Systemic sclerosis (SSc) is a multisystem connective tissue disease characterised by thickened skin (fibrosis) and changes in the structure and function of small blood vessels. It is most commonly seen in women; the mean age at the time of diagnosis is 46 years and the median survival rate after diagnosis is 11 years.[1] There are two types of SSc: limited cutaneous and diffuse cutaneous. In limited cutaneous SSc only the extremities are affected: the face, forearm, hands, lower legs and feet. However patients with diffuse cutaneous SSc can also be affected on the trunk and are more likely to have problems with internal organs. The first symptom experienced by many patients is Raynaud’s phenomenon, where cold temperatures or emotional stress cause an episodic reduction blood flow in fingers and toes to such an extent that there is a significant change in skin colour, as seen in Figure 5.1.1. The thickening of the skin makes the skin feel tighter making it more difficult to accomplish everyday tasks. Sufferers can also develop extremely painful ulcers in the affected
areas which can take months to heal. The disease also affects internal organs including the heart, lungs and kidneys. [2] It is thought that the changes in skin reflect changes that are happening in internal organs and that the understanding of fibrosis and changes to blood vessels is fundamental to understanding the disease. [3] There are a variety of methods used to non-invasively assess disease severity and monitor changes in disease condition, with the majority focusing on investigating skin thickness or blood flow close to the surface of the skin.

![Figure 5.1.1: An example of the colour change in the fingers of someone suffering from Raynaud’s phenomenon.](image)

Ideally there would be a single non-invasive imaging method which could allow measurement of skin thickness with the same resolution as histology (or better) - since changes in the thickness of the skin are linked to the severity of the disease, with increasing thickness linked with an increase in mortality rates. [4] - at the same time as providing a way of studying microvasculature structure and function. Optical coherence tomography (OCT) is well suited to this as it is non-invasive, high speed and high resolution and it is capable of making measurements of flow velocity using the Doppler effect.

While the methods above have been widely used in studies of patients with SSc, OCT is a new technique in terms of monitoring the disease, although it has previously been used to measure skin thickness in healthy controls. [5, 6, 7] Only recently has it been applied as an imaging tool in SSc, where the results correlated highly with mRSS. [8] This chapter discusses the development of image analysis software which was used as part of a small scale study into non-invasive skin imaging techniques and
their ability to obtain information about the skin structure and microvasculature structure and function in patients with SSc and in healthy controls.

5.2 Measurement of skin thickness

Histology is the current gold standard in investigating the structure of the skin, with a resolution of $\sim 1\,\mu m$ however it is non-repeatable due to its invasive nature.[9, p539] The modified Rodnan skin score (mRSS) is also a clinically accepted method to estimate skin thickening[4, 10] and has been shown to be correlated with mortality.[11] To measure a patient’s skin score a trained clinician estimates skin thickness by palpitating the skin and rating it on a numerical scale where 0 is normal skin and 3 is defined as unable to move. The intra-rater reliability is higher than the inter-rater reliability; the skin score of a single person as measured by several scorers can vary to such an extent that it has been recommended that a patient is scored by the same person for the duration of a study.[12] High-frequency ultrasound (HFUS) is used to measure skin thickness non-invasively[13, 14, 15] and although it has a penetration depth in tissue of a few cms its resolution is a few tens of $\mu m$, much lower than histology.

5.3 The study of blood vessels in the skin

There are other techniques which are used to specifically investigate the structure and function of blood vessels. These include nailfold capillaroscopy, which uses high-magnification optical microscopy to image the capillaries in the nailfold of the finger. This technique is widely used in studies of SSc due to the significant change in size, shape and density of nailfold capillaries in people with the disease.[16, 17, 18] Laser Doppler imaging is also used in studies investigating SSc and can image blood flow over the whole hand.[19, 17] It utilises the Doppler effect by measuring frequency shifts of light which is scattered from moving particles, such as blood.
5.4 A comparison of non-invasive imaging methods

5.4.1 Study overview

The study was conducted at Salford Royal NHS Foundation Trust over several months in 2010. Imaging was conducted by Graham Dinsdale, a research associate, and Tonia Moore, a senior vascular technician, both based at the hospital. Imaging took place in a temperature-controlled room where participants were acclimatised for 20 minutes before imaging commenced; all images were taken during a single session.

Four non-invasive imaging techniques were used:

- OCT, a method of creating a tomographic depth image using infrared light.
- HFUS, a tomographic image produced using high frequency sound waves, but which has a lower resolution than OCT.
- Dual wavelength Laser Doppler imaging (LDI), an imaging method which uses the Doppler effect to quantify blood flow in tissue on a macroscopic scale.
- Nailfold capillary microscopy (NCM), a high magnification image of the capillaries in the nailfold of the finger.

HFUS, LDI (Figure 5.4.1) and NCM (Figure 5.4.2) are validated or partly validated imaging techniques to investigate skin structure or microvascular function. OCT has higher resolution than HFUS allowing more accurate measurements, giving it a potential advantage for determining epidermal thickness. However light penetration into skin at the wavelengths used in OCT imaging is much less than the penetration when using HFUS, limiting images to the first millimetre of tissue at most. Although histology is the current gold standard for investigating skin structure non-invasive imaging techniques like the ones used in this study may offer an alternative to skin biopsies.
Figure 5.4.1: Laser Doppler imaging in progress.[19]

Figure 5.4.2: Nailfold capillaroscopy equipment in use to image capillaries in the nailbed of the finger (circled).

Examples of laser Doppler images and nailfold capillaroscopy images are shown.

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in Figure 5.4.3.

![Figure 5.4.3: Examples of laser Doppler images from (a) a patient with SSc and (b) a healthy control. Both images are shown on the same colour scale (top centre); red indicates areas of higher blood perfusion. Perfusion is noticeably lower in (a), particularly in the fingers. The areas of the distal finger and dorsal regions used to measure a perfusion gradient in the hand are shown, indicated by black lines. Nailfold capillaroscopy images taken from the non-dominant ring finger of the hand from (a) a patient and (b) a healthy control are also shown. The capillaries in the nailbeds of the patient are generally more enlarged, less dense and more curly than in the healthy control.](image)

### 5.4.2 Patients and methods

Twenty patients with SSc and 20 healthy controls were recruited and assigned a unique number on entry into the study to identify them. The study was approved by Stockport Research Ethics Committee and all participants gave written consent. Before commencing imaging the skin thickness of patients at the locations to be imaged were assessed by mRSS by a trained clinician. LDI and NCM images were recorded from the non-dominant hand and OCT and HFUS images were taken from 5 sites over non-dominant side of the upper body; 3 on the hand and one each on the upper arm and forearm. The following section details the measurements which were made using each technique.
• OCT. A swept source OCT system (OCM 1300SS, Thorlabs, Ely, UK) was used to obtain 10 sequential images from each of the 5 imaging sites on the body. The system has a theoretical resolution of 7.7 microns in tissue, and is capable of imaging to a depth of 3 mm, although this is limited in tissue by attenuation of the light. 2 mm by 1 mm images were taken with an image resolution of 512 pixels (depth) by 1024 pixels (width). The thickness of the epidermis, the upper layer of the skin, was measured with the purpose built software detailed in this chapter.

• HFUS. The HFUS system, set to 35MHz, (Episcan I-200, Longport, Reading, UK) was used to obtain 16 sequential images from each imaging site and is capable of imaging to a depth of up to several centimetres in tissue. 21.57 mm (depth) by 14.9 mm (width) images were taken with an image resolution of 1024 pixels (depth) by 512 pixels (width). The thickness of the epidermis was also measured with the purpose built software described here.

• Dual wavelength Laser Doppler imaging (LDI). The distal-dorsal difference was measured; this gives a quantitative value representing the difference in arbitrary perfusion units between the tips of the fingers and the back of the palm of the hand. Blood perfusion at the distal phalanx is expected to be worse in patients with SSc than in healthy controls due to ongoing Raynaud’s phenomenon and vasculature damage.

• Nailfold capillary microscopy (NCM). In the nailbeds of the hands the capillaries lie parallel to the skin surface. These were imaged at high magnification and measurements of the architecture of the capillaries were made. Capillaries are expected to be less dense and have a larger width in patients.

Although the OCT and HFUS imaging software provided with the imaging systems provided a method to manually make measurements from the images, custom software was written to allow semi-automated detection of potential boundaries for both image types. This software could also export measurements to a spreadsheet file as well as saving a copy of the original image with the layer boundaries marked on top for future reference and comparison. The details of the development of this software and its application are discussed in this chapter and the source code is available on request.
OCT and HFUS images were analysed by myself using this software, with
other members of the team analysing the laser Doppler imaging and
nailfold capillary microscopy images. The nailfold capillaroscopy images
were measured by Dr Andrea Murray, a research fellow, using semi-automated
software. The quantities measured were the width of the top loop of
capillaries (Figure 5.4.4) and vessel density. The laser Doppler images were measured by Je Song Shin,
a medical student at the University of Manchester, using mooLDI Imaging
Processing software, version 5.0. The difference in blood perfusion between
two standardised areas on the back of the hand and near the finger tip (distal
digit) was measured to give a perfusion gradient.

Measurements were determined to be statistically significant if they had a
P-value of \( p < 0.05 \) i.e. there is less than a 5% chance to observe a difference
between the measurements of healthy controls and patients which is as extreme
as the one measured, even when the null hypothesis is true and no difference
actually exists between the measurements.

![Figure 5.4.4: The width of the apex and the density of the capillaries in the
nailfold capillaroscopy image were measured.](image)

### 5.4.3 Statistical analysis

The measured epidermal thickness for OCT and HFUS were compared with a
paired t-test between the patient and control groups. The thickness data from
all sites were then split into 2 groups based on the mRSS score of that site (0-1
and 2-3). These two groups were also compared using a paired t-test for both
OCT and HFUS.
In order to assess the reproducibility a Bland-Altman analysis on 3 pairs of measurements:

1. Single measurements from each of the two images selected
2. Two measurements from a single image
3. The mean of the 3 measurements from each image

5.5 Developing software to measure skin thickness from images

The Longport Episcan software provided with the ultrasound scanner allows the user to make measurements on screen directly from an image in the database in which it stores the images, however it has limitations which would have made it difficult to use for this study:

- There was no facility to record a measurement automatically to an external file
- The image zoom functionality was limited to just three levels of magnification
- The label placed on the image where the measurement was taken often obscures the part of the image which is being measured

The software for the OCT imaging system, Thorlabs OCM, also allows for measurements to be made from images but again it lacks a way to record them to a file. In addition, given the number of measurements to be made (6 per imaging location, for 5 imaging locations, for each of 40 subjects), manually recording measurements would be very time consuming. Instead of using the original software custom software was developed to measure epidermal thickness from .bmp files exported from the original database HFUS of images as well as the .img image files from the OCT system. A zoom function was added to the software so that the user can select any area of a image and it is immediately rescaled to fit the screen, allowing the user to chose an appropriate magnification for both OCT and HFUS images. In the case of the HFUS images, a comparison of the markers applied to the image in the original software and the custom written software is shown in Figure 5.5.1. Markers which obscure the image are avoided in the custom software by using 2 thin red
lines either side of the boundaries to be measured. For each imaging location 16 sequential images were saved. Two images with almost horizontal surfaces and regular cross-section were selected for measurement and were exported as a bitmap file.

![Figure 5.5.1](image)

Figure 5.5.1: Comparison of the same ultrasound image measured with (a) custom written software, with one epidermis measurement marked using small red lines and (b) original software with three measurements marked but slightly obscured by the measurement result. The red markings in (a) have been enhanced after the image has been saved so that they can be clearly identified.

The software is semi-automated as there is so much variation in some of the images that there is no guarantee that a fully automated system would mark the correct locations every time. By requiring user input this problem can be avoided.

### 5.5.1 Software overview

The measurement software was developed in Matlab (Ver. 7.8.0.347, R2009a) using the graphical user interface development environment (GUIDE). It comprises of 3 separate graphical user interfaces (GUIs), the first of which allows the user to extract vertical sections of the image (i.e. multiple A scans) and average the pixel intensity values to generate a single array of data, the results of which are saved to a .txt file. This GUI is used to extract A scans from both the OCT and HFUS images - see Figure 5.5.2.
Figure 5.5.2: A whole image is made by combining multiple A (depth) scans.

Figure 5.5.3 shows the first GUI in use with a HFUS image. The second and third GUIs have the same overall function - to use this extracted data to automatically plot potential layer boundaries over the original image and enable measurements to be recorded - but the method used is slightly different for each of the 2 imaging techniques leading to the development of separate GUIs, which are discussed in detail later in this chapter and are shown in Figure 5.9 (HFUS) and Figure 5.8.1 (OCT). After the potential boundaries are plotted on the image, the user determines by eye which of the boundaries were edges of skin layers and which were likely due to a local variation in the pixel intensity. The user can then save the measurements and accompanying information about imaging location, number of A scans and position on the image to a Microsoft Excel file using the `xlswrite()` command. It also saves a bmp image (in the case of HFUS) or a .jpg image (for OCT) with the boundaries marked on. The different file formats for this saved image were due to a slight blurring of the images of the greyscale OCT images when saved as a .bmp file using the `saveas()` command. Instead, a JPEG image with minimum compression was saved using the `print()` command for this image type.
Figure 5.5.3: The first GUI in use extracting A scans from a HFUS image. The image has been annotated to show, in increasing depth, the first 2 sets of peaks in the 'average of lines' plot which are due to the number at the top of the image, the the bottom of the ultrasound probe, the epidermis and the broad peak due to the dermis.

5.6 Extracting A scan data from images

5.6.1 OCT

The OCT system used has a central wavelength of 1325 $nm$ with a 100 $nm$ spectral bandwidth and average output power of 10 $mW$. It is capable of achieving a 16 $kHz$ axial scan rate. At 512 A scans per frame it can generate 2D images at 25 fps over a maximum area of $10 \times 3 \ mm$ (width by depth). The OCT image data saved by the system software in the .img file is the post-processed image, rather than raw fringe data. All 10 images saved from a single imaging location are stored in a single file and each frame has to be read into memory by the GUI for the image to be displayed. The data contained in each .img file is stored as follows in Table 5.6.1 and Table 5.6.2. The section of code to
extract the header and image data was adapted from code previously written
by Dr Graham Dinsdale, a research associate at the University of Manchester.
Clicking 'Read header' shows a standard dialogue box to open a file. The
selected file is then opened using 
\texttt{fopen()}, and bytes 1 to 40 read into memory
using \texttt{fread()} with each variable in the header data saved as a separate string.
This data can be used to determine the correct dimensions at which to display
the image as well as the total number of images stored in the file.

<table>
<thead>
<tr>
<th>Start byte</th>
<th>Length in bytes</th>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>String</td>
<td>File identification string</td>
</tr>
<tr>
<td>17</td>
<td>4</td>
<td>Long integer</td>
<td>Number of images saved in the file</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>Long integer</td>
<td>Image width</td>
</tr>
<tr>
<td>25</td>
<td>4</td>
<td>Long integer</td>
<td>Image depth</td>
</tr>
<tr>
<td>29</td>
<td>4</td>
<td>Long integer</td>
<td>Number of frames in each 3D volume</td>
</tr>
<tr>
<td>33</td>
<td>4</td>
<td>Long integer</td>
<td>Number of 3D volumes in the file</td>
</tr>
<tr>
<td>37</td>
<td>4</td>
<td>Long integer</td>
<td>FFT length</td>
</tr>
<tr>
<td>41</td>
<td>472</td>
<td></td>
<td>Reserved</td>
</tr>
<tr>
<td>513</td>
<td>Frame data</td>
<td>Frame data (processed images)</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.6.1: The contents of the header of the .img files as saved by the OCT system. From byte 513 the frame data is saved one frame at a time. [20]

<table>
<thead>
<tr>
<th>Start byte</th>
<th>Length in bytes</th>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Long integer</td>
<td>Time elapsed between previous and current frame</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td></td>
<td>System time of current frame</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td></td>
<td>Reserved</td>
</tr>
<tr>
<td>41</td>
<td>Frame size</td>
<td>2 Dimensional array of the image data</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.6.2: The contents of the frame data section of the .img OCT image file as saved by the software. [20]

Since each byte of the image data represents one pixel the required number
of bytes per image can be calculated using data from the header by using the
The equation \( \text{frame size} = \text{image width} \times \text{image depth} \). The function \( \text{fseek}() \) is used to find the point in the file containing the start of the data for the selected frame number and \( \text{fread}() \) is again used to read the appropriate number of bytes into memory. The user is required to read the file header file before selecting the frame to be displayed using a drop-down menu to ensure that the correct number of bytes for each image are read, as the software is able to display images of varying size. A screenshot of this GUI being used to extract A scans from an OCT image is shown in Figure 5.6.1.

![Screenshot of program to extract A scans from HFUS and OCT images. An OCT image is used as an example.](image)

After selecting the number of A scans to be averaged using the drop down menu on the bottom right, clicking 'Select lines' allows the user to click on the OCT image to select an area to extract the A scans from using the \( \text{ginput}() \) command. This command selects the \((x, y)\) values of the current mouse location when the user clicks on the image. Only the \(x\) value is of interest which is rounded to the nearest integer and taken to be the central A scan of the area of the image to be extracted. The A scans around this point are then averaged to give an array representing the average pixel intensity over that area; the length of the array is the same as the pixel depth of the image. This is then displayed.
for the user in the top right frame of the GUI. Averaging, rather than using a single line, helps to reduce the impact of local variations when identifying boundaries which are signified by a change in intensity. At the other extreme averaging over the entire image would only give an accurate representation of the boundaries if they are all parallel to the \( x \) axis across the entire image, which is rarely the case for \textit{in-vivo} images. The appropriate number of A scans to extract is discussed in Section 5.6.3 and an example of the plot from an OCT image is shown in Figure 5.6.2. After extracting the lines the user can save the average line data to a text file ('Save extracted scans' button), or can make measurements from the line data using the 'Calculate thickness' button. The 'Calculate thickness' prompts the user to select 2 points on the right hand image, and the difference between the \( x \) values is calculated and displayed. However, at this point any distances calculated are given in terms of pixels and must be converted to microns.

The file name of each of the sets of extracted A scans is unique and contains all of the relevant information which will later exported to the spreadsheet along with the thickness measurement. This includes ID number, imaging location, position of the central line of the extracted A scans, and the number of A scans extracted.

![Figure 5.6.2: A typical plot of the extracted A scans from an OCT image. The peak at \( \sim 50 \) pixels deep corresponds to the air-tissue interface. The next broad peak is the point where the epidermis and dermis meet.](image)

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5.6.2 HFUS

HFUS images are stored by the original software in a database, with the individual images themselves saved in .ima format. While the image data can be extracted from this file type in a similar method to the .img files, the boundaries in the images did not appear clear when a standard Matlab colour scale was used. This was not a problem for OCT images as they were greyscale, however HFUS images are colour. Instead the individual images need to be exported from the original database and saved as a bitmap file to be read into the Matlab software using the imread() command; a standard Matlab colour map is used to display the image. The image is loaded through the ‘Load image’ button on the GUI (Figure 5.5.3), which presents the user with a standard dialogue to open the file. The image is then displayed on the appropriate frame on the GUI. Once the image is displayed the user can select where on the image they would like to make a measurement and save it to a file, as with the OCT images.

5.6.3 Number of A scans to extract

To test what affect the number of A scans used to generate an average would have on the clarity of the plot, varying thicknesses were extracted and the extracted array of values plotted. An example of a single A scan is shown in Figure 5.6.3, while some of the plotted averages of multiple A scans from a HFUS image are shown in Figure 5.6.3.
Figure 5.6.3: A single A scan from a HFUS image. The point of interest signifying the epidermis is the peak at around 85 pixels deep. The peaks at approx 60 pixels deep are due to the HFUS handprobe. The probe itself is covered with water over which a membrane is placed; the membrane is generally the upper most object viable on a HFUS image.
(a) Average of 30 A scans

(b) Average of 50 A scans
Figure 5.6.3. Plots of the average pixel intensity of a HFUS image for 3 different numbers of A scans, all plotted on the same scale for comparison. The extracted plots were saved from the same region of the same image as the single A scan in Figure 5.6.3. Averaging over 30 sequential A scans shows an improvement in the clarity of the peak compared to a single A scan making it easier to determine. However, by increasing the number of A scans to be averaged over the average intensity decreases.

The peaks in the plots correspond to higher pixel intensity at that point, where there is more light or sound reflected back from the tissue. The greater the number of A scans used the less defined the plot is, which can reduce the accuracy of the program to detect the boundary between layers. Blurring of the plot would have been particularly noticeable in the case of highly skewed images such as those taken from patients with problems with hand mobility, because the depths of the peaks of interest vary from one A scan to another. In order to minimise the affect of skewed images on measurements the orientation of the image was taken into account when choosing which image frame to use out of the 10 OCT and 16 HFUS images for each location on each subject. When measuring the image any skewed sections of the images were avoided as well as any other area which could potentially be problematic such as when imaging near a hair follicle using OCT – Figure 5.6.4.
Figure 5.6.4: An example of an OCT image where a hair prevents imaging as light reflecting from it reduces the depth of penetration. Both the reflection and the shadow it creates are marked.

Once strips of 30 pixels were determined to be an optimal thickness data was extracted for both the OCT and HFUS images. Two images which were deemed to be the most suitable from each imaging site for each subject were selected and measured at 3 points on the image, giving 6 measurements overall. Once the 6 sets of A scans were extracted and saved to text files they were used with the second GUI. As the GUI dealt with the OCT and US images differently due to one being greyscale and the other being 32 bit bitmap this GUI will be discussed separately in Section 5.8 and Section 5.9 respectively.

### 5.7 Resolution test of OCT and HFUS measurement software

Before the extracted A scans could be measured in microns the conversion factor to convert from a distance in pixels to a distance in microns was needed for both imaging techniques. A resolution test was conducted using both the OCT and HFUS imaging systems by measuring the width of a microscope side. A screenshot of the measurement of the OCT image is shown in Figure 5.7.1.
Figure 5.7.1: The microscope slide as imaged with OCT.

The resolution test allowed accurate determination the micron-to-pixel ratio of each of the systems. It also acted as a test of the of the program to extract measurements from the .img OCT or .bmp HFUS images. As with the skin images 3 measurements were taken from 2 separate images. An average of these 6 values is shown in Table 5.7.1 along side the measurement obtained by measuring the slide with a digital micrometer, accurate to 0.01 mm.

<table>
<thead>
<tr>
<th>Micrometer</th>
<th>HFUS</th>
<th>OCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.97 mm</td>
<td>267.67 ± 0.52 pixels</td>
<td>48.33 ± 0.82 pixels</td>
</tr>
</tbody>
</table>

Table 5.7.1: Resolution test using a microscope slide of OCT and HFUS, with a comparison of the micrometer measurement.

The measurements in Table 5.7.1 give values of 3.62 microns per pixel for the OCT and 20.07 microns per pixel for HFUS.
5.8 OCT measurements of skin thickness from extracted A scans

The second GUI dealt exclusively with OCT images, making measurements of epidermal thickness from the previously extracted A scans. Figure 5.8.1 shows an image which has been marked with pink lines representing the boundaries of the epidermis. In most in-vivo OCT images there is some local variation in pixel intensity meaning that some of the boundaries plotted by the software need to be removed before the measurements are taken. The software might also pick up boundaries deeper in the tissue than the epidermis. To aid in selecting the correct markers on the image the software has the option to zoom in using the 'Rescale' button. When clicked the user is able to select 2 points on the image representing 2 opposite corners of the area that they want to focus in on. This can be repeated to zoom in further if required, and the the original zoom reset using the 'Reset scale' button. There are also 'Shift up' and 'Shift down' buttons to allow the user to move the boundaries up and down if they are blocking the image below and making determination of the correct measurement difficult. When using these buttons all of the boundaries will be moved up/down by the same amount so the relative distances between points, used for calculating layer thicknesses, remain unchanged.
Figure 5.8.1: Screenshot of the analysis program for OCT images with boundaries marked in pink, after removal of any boundaries deemed to be due to local variation in pixel intensity or noise. The stratum corneum (SC), epidermis (E), dermal-epidermal junction (DEJ) and dermis (D) are shown. However the boundary with the dermis was not visible in most images measured.

The following method was used to obtain thickness measurements from the extracted A scans using this GUI.

1. The file containing the average line/pixel intensity data from the first GUI is loaded and stored in a 1-D array in Matlab using ‘Load line data’ button.

2. This average is then differentiated using the Matlab \texttt{diff()} command and stored in a new array. The modulus is taken so that any negative values become positive. It is the magnitude rather than the sign of the differential which is of interest.

3. The GUI has a sliding scale - on the left hand side of the GUI - where the user can select a gradient value as a lower limit. Any rows of pixels where the change in average pixel intensity leads to a gradient value above this limit are marked by the software when the user clicks the ‘Find boundaries’ button. The lines placed over the image to mark potential boundaries cover only the section of the image which has been analysed.

4. The user can then determine by eye if the appropriate boundaries have been marked, changing the gradient limit as necessary, and deleting any
boundaries which they believe to be due to local variations within the image until they are satisfied that the appropriate boundaries have been marked.

5. The user can then select the numbers of the marked boundaries (1 being the upper-most boundary) between which the measurement will be made and select a name tag for the layer from a drop-down list. These can be written to a .xls spreadsheet and a copy of the marked up image can be saved as a .jpg file using the ‘Save stored data + image’ button.

The \texttt{diff()} command in Matlab, Equation 5.8.1, is used to achieve an approximated differential of the array of average A scans which can be used to look for large changes in the average grey values of pixels. In an OCT image a pixel lighter than the surrounding area indicates that more light was reflected from that point in the tissue than nearby tissue. The higher the value of the differential, the greater the difference in pixel intensity between 2 adjacent pixels. This may signal a potential a boundary between layers in the skin where there is a sudden change in the amount of light backscattered.

\begin{equation}
\text{diff}(x) = [x(2) - x(1) \quad x(3) - x(2) \quad ... \quad x(n) - x(n-1)] \quad (5.8.1)
\end{equation}

There appears to be little consensus in the literature over the exact measurement from an OCT image which corresponds to the epidermal thickness. Suggestions have included measuring the distance between the first entrance peak at the air-tissue interface and the next significant peak[21, 6] and from the first peak to the first significant decrease in signal.[5, 7] For this study the distance measured was the distance between the initial peak of the differential - the start of the air-tissue interface - and a point of the rising edge of the next significant peak - the end of the first decrease in signal intensity/beginning of the second increase. This is the measurement which is exported to a spreadsheet along with information about the original image and where on the image the measurement was taken from; an example of a part of this spreadsheet is illustrated in Figure 5.8.2.
5.9 HFUS measurements of skin thickness from extracted A scans

The extracted A scans from HFUS images are analysed with a similar GUI to the one discussed in the previous section, with a slight difference in how the boundaries are determined. Rather than analysing the gradient of the average A scans the magnitude of the pixel intensity was used to mark boundaries. The higher the intensity, the more sound that was detected from that point in the tissue. The colour scale used to display the image ranges from black for no signal through blue, green, pink and finally white, which signals the highest signal. The pink/white pixels in the image are few and far between and are usually down to local variation and/or noise rather than a boundary across the entire image. Screenshots of the software in use are given in Figure 5.9.1.
Figure 5.9.1: A screenshot of the analysis program for HFUS images with the boundaries of the top and bottom of the epidermis marked in red. (a) Shows the image zoomed in before the extra marked boundaries are deleted. (b) Shows the final marked image.
In this GUI the user sets the minimum value of the pixel colour intensity to signify a boundary, rather than a minimum gradient value. Unlike in the OCT images where the epidermis appears as a different colour on the greyscale than tissue underneath (and so looking for a peak in the gradient is appropriate), in the HFUS images the upper and lower boundaries of the epidermis were taken to be the top and bottom of a set of bright ‘tramlines’ as shown in Figure 5.9.2. This method of measuring epidermal thickness is consistent with previously published measurements.[13]

![Figure 5.9.2: The epidermis was taken to be the ‘tramlines’, which are clearer at some points on the body, such as (a) the finger than (b) the forearm.](image)

As with the OCT images 6 measurements were taken and averaged from 2 images (3 from each image) for each imaging location. Measurements were taken from the parts of the image where the tramlines were clear and near-horizontal. These measurements were exported to an .xls spreadsheet and an image with the boundaries marked on was saved for reference.

### 5.10 Results

#### 5.10.1 OCT and HFUS results

The results of the OCT and HFUS measurements are given in Table 5.10.1. The differences in skin thickness between the healthy controls and patients were significantly different for 2 imaging locations for OCT and 4 for HFUS; the proximal digit and back of the hand were significantly different for both techniques. The lack of significance for upper arm measurements using either technique can be explained by the fact that most of the patients who were imaged suffered from limited cutaneous SSc where the upper arm is not usually affected, as indicated by the majority of patients having a zero skin score in this
location. Hence skin thickening would not always be expected here for those patients.

Overall the OCT and HFUS results are comparable - they were both able to detect statistically significant differences between patients and healthy controls at several imaging sites. However, HFUS was able to detect differences in the distal digit while OCT was not. This is believed to be because of the thicker skin at this point of the finger - the quality of OCT images decreases with increasing depth, with some images of the finger tips being particularly difficult to measure as the boundaries were less obvious. HFUS has a greater penetration depth and there were no noticeable reduction in image intensity due to increased losses.

When the measurements of all imaging locations for all patients were grouped according to the mRSS score recorded at the location imaged (0 – 1 and 2 – 3) statistically significant differences between the two groups were seen with both OCT (mRSS 0 – 1: 231(208 – 251) µm, mRSS 2 – 3: 249 (218 – 307) µm, p < 0.001) and HFUS (mRSS 0 – 1: 214(181 – 249) µm, mRSS 2 – 3: 237 (211 – 271) µm, p < 0.001). Skin thickening with both techniques also correlated with mRSS score when all of the measurement sites were considered together (OCT: \( R = 0.345, p < 0.001 \). HFUS: \( R = 0.296, p = 0.003 \)).

5.10.2 Reproducibility of OCT and HFUS measurements

In order for OCT and HFUS to become validated techniques they need to be reproducible i.e. if the same image is measured twice (or more), the results should be the comparable. The results of several reproducibility measurements are given in Table 5.10.2. The intra-class correlation coefficient (ICC) is a measure of how well correlated measurements within the same group are. The closer the ICC is to 1, the higher the correlation between the values and the more reliable the measurement is.
<table>
<thead>
<tr>
<th>Site of epidermal thickness measurement (μm)</th>
<th>Controls (N=20)</th>
<th>Patients (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OCT</td>
<td>HFUS</td>
</tr>
<tr>
<td></td>
<td>p = 0.442</td>
<td></td>
</tr>
<tr>
<td>Proximal digit</td>
<td>251 (233 – 280)</td>
<td>244 (217 – 268)</td>
</tr>
<tr>
<td></td>
<td>p = 0.007*</td>
<td></td>
</tr>
<tr>
<td>Hand</td>
<td>212 (194 – 234)</td>
<td>176 (159 – 201)</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.001*</td>
<td></td>
</tr>
<tr>
<td>Forearm</td>
<td>210 (196 – 219)</td>
<td>190 (166 – 222)</td>
</tr>
<tr>
<td></td>
<td>p = 0.174</td>
<td></td>
</tr>
<tr>
<td>Upper arm</td>
<td>214 (203 – 230)</td>
<td>181 (171 – 217)</td>
</tr>
<tr>
<td></td>
<td>p = 0.078</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.10.1: Results of the epidermis measurements of the OCT and HFUS images, along side the mRSS values for each location. Values given are median (IQR). * indicates a statistically significant result i.e. $p < 0.05$. 
Intra-class correlation coefficients (95% CI)

<table>
<thead>
<tr>
<th></th>
<th>OCT</th>
<th>HFUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparing a single</td>
<td>0.82 (-66.8 to 60.6)</td>
<td>0.71 (-91.2 to 86.7)</td>
</tr>
<tr>
<td>measurement site from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>each of the two images</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparing 2 measurement</td>
<td>0.89 (-55.4 to 42.2)</td>
<td>0.70 (-86.1 to 86.6)</td>
</tr>
<tr>
<td>sites from the same</td>
<td></td>
<td></td>
</tr>
<tr>
<td>image</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparing the average</td>
<td>0.92 (-39.6 to 39.0)</td>
<td>0.83 (-61.1 to 57.2)</td>
</tr>
<tr>
<td>thickness calculated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>from 3 measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>from one image with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>the same measurement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>from the second image</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.10.2: Measurement of the reproducibility of OCT and HFUS measurements.

For both imaging methods comparing a single measurement from each of 2 images gave the highest correlation coefficients, although all 6 calculated ICC values indicate a strong agreement between measurements.

5.10.3 NCM and LDI results

The results of the NCM and LDI measurements are given in Table 5.10.3. There was no correlation between these results and the measurements obtained using either OCT or HFUS.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Controls (N=20)</th>
<th>Patients (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical width</td>
<td>11.2(10 − 13)</td>
<td>14.7(13 − 19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( p = 0.001^{*} )</td>
</tr>
<tr>
<td>Density</td>
<td>31.5(27 − 34)</td>
<td>23.0(14 − 34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( p = 0.031^{*} )</td>
</tr>
<tr>
<td>LDI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal-dorsal difference</td>
<td>191.5(142 − 262)</td>
<td>123.1(31 − 179)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( p = 0.003^{*} )</td>
</tr>
</tbody>
</table>

Table 5.10.3: Results of the measurement of the width and density of capillaries as measured by NCM, and the LDI distal dorsal perfusion difference. Values given are median (IQR). * indicates a statistically significant result i.e. \( p < 0.05 \).
5.11 Conclusion and discussion

This chapter discussed the development of semi-automated image analysis software to measure the thickness of the epidermis from both HFUS and OCT images. It also discussed the application of this software in a study to measure epidermal thickness in patients with systemic sclerosis as well as healthy controls. The software is able to open and display various image types and allows the user to select a section of the image to be analysed. It then determines potential boundaries within that section using either the absolute value of the pixels in the image, in the case of ultrasound images, or a gradient method, as for OCT images. The user chooses the most appropriate of these boundaries; the software then measures the distance between these two points and exports the data to a .xls file for later analysis.

The results of the study show that both OCT and HFUS are able to measure a statistically significant difference in epidermal thickness between patients and healthy controls over some of the body areas imaged. Both methods also show high reproducibility. Two imaging methods used to investigate the function of microvessels in the skin - nailfold capillaroscopy and laser Doppler imaging - were also able to measure statistically significant differences between patients and healthy controls.
REFERENCES


Chapter 6

Monte Carlo modelling of a Doppler optical coherence tomography signal and its comparison with a flow phantom

Monte Carlo simulations are a versatile method of simulating a physical system, particularly where the direct calculations might otherwise be difficult or impossible. They rely on the generation of random numbers to calculate the expected value of each quantity to be studied. After many iterations the expected value can be assumed to be equal to the actual value under investigation. Monte Carlo computer simulations using a computer use a pseudo-random number generator as they are unable to generate truly random numbers. In this case the computer uses a seed number to which it applies set mathematical operations to generate sets of numbers which are unrelated. The seed is generally taken from a changing value, such as the system clock, as using the same seed twice will result in the same set of numbers. This chapter discusses the adaptation of Monte Carlo simulation of multi-layered media (MCML),[1] a simulation of light propagation in a multi layered sample which is freely available online, in order to simulate Doppler optical coherence
tomography imaging of a flow phantom. Both the modifications made to the original source code and a comparison of the simulation to experimental data are given.

6.1 Monte Carlo simulations

One of the simplest examples of a Monte Carlo simulation is the calculation of \( \pi \) using geometry like the one in Figure 6.1.1. Points representing \((x, y)\) values within a square with sides unit length are sampled. After many selections the ratio of the points within the shaded area to the total number of points sampled becomes \( \pi/4 \). In order for this to be true it is clear that it must be equally likely for every point within the square to be chosen i.e. the numbers must be chosen at random and should be sampled from a uniform distribution.

![Figure 6.1.1: A unit square with all points satisfying \( x^2 + y^2 \leq 1 \) shaded.](image)

A similar algorithm can be applied to, for example, computing the mean distance travelled by a photon between scattering events in a tissue by sampling the probability distribution \( P\{s \geq s'\} \) for \( s \), the photon’s mean free path.\([2]\) If the photon travels a distance \( s' \) then the probability of scattering or being absorbed per unit path length is given by Equation 6.1.1, where the total attenuation coefficient is \( \mu_t = \mu_a + \mu_s \) and \( \mu_s \) and \( \mu_a \) are the scattering and absorption coefficients respectively. This can be rearranged and integrated over \( s' \) over the range \((s, s_1)\) to give Equation 6.1.2, the cumulative distribution function of \( s \).\([2]\)

\[
\mu_t = \frac{-dP\{s \geq s'\}}{P\{s \geq s'\} ds'} \tag{6.1.1}
\]
\[ P \{ s < s_1 \} = 1 - \exp (-\mu t s_1) \quad (6.1.2) \]

As a cumulative distribution function \( P \{ s < s_1 \} \) only takes on values between 0 and 1. If this is set equal to a uniform random number, \( \zeta \), then rearranging gives Equation 6.1.3. As the distribution for \( \zeta \) and \( 1 - \zeta \) are equal they can be used interchangeably, resulting in Equation 6.1.4.

\[ 1 - P \{ s < s_1 \} = 1 - \zeta = \exp (-\mu t s_1) \quad (6.1.3) \]

\[ s_1 = \frac{-\ln \zeta}{\mu t} \quad (6.1.4) \]

If \( \zeta \) is sampled repeatedly then the estimated value of \( s_1 \) can be calculated provided that the value of \( \mu t \) is known. In the case of modelling propagation of photons in tissue the expected value of the mean free path is not necessarily needed, but the same method can be used to calculate a set of path lengths travelled between interactions for that tissue. When combined with a similar algorithm to calculate scattering angles after the interactions and a method to account for photon absorption this method becomes a good base to begin simulating light propagation within tissue.

### 6.2 Monte Carlo simulation of multi-layered media (MCML)

The source code for the Monte Carlo simulation discussed in this chapter was adapted from version 1.2 of Monte Carlo simulation of multi-layered media (MCML),[1] the source code of which is freely available online. The original simulation models the propagation of an infinitely narrow beam of photons, orthogonally incident to the sample surface, through a multi-layered turbid sample where the layer boundaries are perpendicular to the initial direction of propagation of the photon. It traces packets of photons through layers defined by the user using a random number generator to determine path lengths, scattering angles and reflections through the media based on the user-set properties of the sample. These properties are set at runtime and include the optical properties of the tissue as well as the number and thickness of each of the layers. The optical properties required are \( n, \mu_o, \mu_s \) and \( g \) which
are the refractive index, absorption and scattering coefficients and anisotropy respectively. The value of $g$ and the ratio of $\mu_s/\mu_a$ have a strong influence on the running time of the simulation; the higher the values, the longer the simulation will take to complete as there will on average be more scattering events before the photon leaves the tissue or is absorbed.\cite{2} The user must also set the number of photons to be traced, which will also have a direct effect on simulation time; more photons means a longer computation time. The original MCML simulation generates information about the absorption of light within the tissue, specular reflection and transmittance through the sample; this data is saved to a file at the end of the simulation. The simulation is based on Cartesian geometry, with the positive $z$ axis pointing down into the tissue - Figure 6.2.1.

![3D geometry of the simulation](image)

Figure 6.2.1: 3 dimensional geometry of the simulation. The surface of the simulated sample is in the $x$-$y$ plane, as are all boundaries between layers within the sample. The positive $z$ axis is in the direction of increasing depth into the sample.

The original MCML model simulates radiant energy transport, and does not track properties such as phase or polarization.\cite{2} However, information about the field or phase are not necessarily needed for OCT modelling, only information about the intensity of the light.\cite{3, p93} Hence MCML can be used as a base to adapt for simulating optical coherence tomography images, both structural and Doppler.

### 6.2.1 Modifications to MCML

The original source code was heavily modified so that structural and Doppler flow optical coherence tomography images could be simulated. This included the inclusion of vessels containing a flow within the sample, changing the
ininitely narrow beam to one with a finite diameter and introducing a detection scheme to better model a physical optical coherence tomography system. However the overall structure of how the photon packets are propagated remains largely the same. As the simulation was modified to include a Doppler OCT simulation the angle of incidence was changed to be user set in the region $0 < \theta < \pi/2$ radians with respect to the tissue surface normal. $\theta = 0$ radians was not included as phase resolved Doppler OCT is insensitive to flow perpendicular to the direction of photon propagation. The following is a summary of the major changes made:

- Addition of velocity as an input parameter which changes the shape of that layer if it is non-zero. The layer changes from an infinitely wide layer of a fixed depth to a vessel centred on $(x, z) = (0, (d/2 + z_{min}))$ where $z_{min}$ is the bottom of the layer above and $d$ is the diameter of the vessel. This geometry change is depicted in Figure 6.2.2. The vessel runs parallel to the $y$ axis.
- The ability to record frequency shifts applied to photons after scattering from a moving particle.
- Incident beam changed from an infinitely narrow beam to a finite diameter Gaussian beam.
- The beam was focused onto the tissue by modelling the effect of a lens with focal length $f = 30$ mm.
- A detection scheme was implemented where the photon information is only recorded if particular angular and location conditions are met, meaning stray photons do not contribute to the signal.
- Added the ability to generate a B scan by moving the location of the incident beam on the tissue surface linearly along the $x$ axis.
- Added the recording of values required to produce an OCT image from the simulation output, such as total path length travelled and maximum depth reached.
- If a single photon is traced, every $(x, y, z)$ location is stored and written to the output file so that the movement of individual photons can be traced.
Figure 6.2.2: Examples of the geometry of a 3 layer sample where the middle layer has a zero (a) and non-zero (b) velocity. In the case of flow the input file still contains details of 3 layers but the layer above and below the vessel must have the same optical properties. This set of properties is used in the area of the sample surrounding the vessel. The software ignores the horizontal boundary that the layers above and below the vessel make with the flow layer itself, and instead only recognises the boundary with the vessel.

The MCML source files provided by the original authors were accompanied by a set of Matlab .m files to read the output files created by the simulation and display the information graphically. These files were also modified to generate OCT and Doppler images from the additional data that was saved to the output files, and to read in multiple A scans in a loop. In order to generate the OCT images from the recorded data a coherence gate was applied to each A scan and any photons with a non-zero Doppler shift extracted. Both the OCT and DOCT images are then plotted automatically.

6.3 Simulating photon propagation

The simulation is conducted a single photon packet at a time, which is moved independently of the path taken by past photons. A large number of photons are used for each simulation which can be assumed to be simulating a single A scan - using as many as $10^7$ photons is not unusual in a simulation of this type.[4] An OCT image simulation is made up of many of these individual A scan simulations, with the initial $x$ location of sequential scans changed between each set to simulate the beam focus moving across the surface of the sample. When a photon packet is initialised 3 of the values which are set are the directional
cosines, \((\mu_x, \mu_y, \mu_z)\). These are calculated from the angle between the direction of propagation and the \(x\), \(y\), and \(z\) axes respectively, as in Equation 6.3.1.[2] The \(x\) and \(y\) axes are in the plane of the top layer of the sample and \(z\) axis is positive in the direction of increasing depth.

\[
\begin{align*}
\mu_x &= \cos \theta_x \\
\mu_y &= \cos \theta_y \\
\mu_z &= \cos \theta_z
\end{align*}
\] (6.3.1)

The incident light is assumed to have left a collimator as a perfectly collimated Gaussian beam, and is focused towards the sample with a lens; the initial \((x, y, z)\) location of the photon is taken to be at the lens, after having the directional cosines set such that it is heading towards the focal point. The incident angle, \(\theta_i\), is set at runtime by the user. Values of \((x, y, z)\), the location of the photon, and \(\mu_x\), \(\mu_y\) and \(\mu_z\), its direction, are calculated using this angle as well as a Gaussian random number generator. The 2D geometry of the launching scheme is shown in Figure 6.3.1.

The first layer in the sample is the material that the lens and collimator is in; this is assumed to be air in all simulations, however the optical properties can be changed in the input file. The line \(z = 0\) is in line with the point of the lens furthest from the surface of the tissue leading to only positive values of \(z\) in the simulation. \(x = 0\) and \(y = 0\) correspond to the centre of the collimator at the centre A scan. While \(x\) and \(y\) can take on negative values, \(z\) is always \(\geq 0\) as a negative value would take it above the point of the lens which is an end point of the simulation.
6.3.1 Beginning the simulation

The simulation begins at the lens. The photon packets are assigned $x$ and $y$ values based on a randomly sampled number from a Gaussian distribution with mean 0 and standard deviation equal to $1/e^2$ value of the diameter for the collimator being simulated, $sd$. $z$ values are calculated according to the value of $x$ using Equation 6.3.2. The actual $x$ location of the initialised photon is then modified by an offset value to take into account the changing starting location for each B scan.

\[
  z = \begin{cases} 
    0 & \text{for } x \leq -sd \\
    (sd + x) \tan \left( \frac{\pi}{2} - \theta_i \right) & \text{for } -sd < x < sd \\
    2sd \tan \left( \frac{\pi}{2} - \theta_i \right) & \text{for } x \geq sd
  \end{cases} \tag{6.3.2}
\]

The directional cosines are then calculated so as to aim the light towards the surface of the tissue in a focused manner. This is determined by the spot size that the OCT system beam makes at the focal point of the lens. The angle between each of the $x$, $y$ and $z$ axes and the line made between the photon’s starting point and the equivalent point in the smaller, focused beam profile at the surface of the sample is used to calculate $\mu_x$, $\mu_y$ and $\mu_z$. The photon is launched towards this point.
6.3.2 Calculating the step size

The size for each step between interactions in the tissue, \( s \), is determined by Equation 6.3.3, where \( \zeta \) is a randomly generated number from a uniform distribution. After this calculation, but before moving the photon, the software checks to see if it would cross an interface within the tissue during this step. If it remains in the same layer then it is moved to the point given by Equation 6.3.4.[2]

\[
s = \frac{-\log(\zeta)}{\mu_a + \mu_s}
\]  
(6.3.3)

\[
x' = x + \mu_x s \\
y' = y + \mu_y s \\
z' = z + \mu_z s
\]  
(6.3.4)

6.3.3 Interaction with a flat interface

If the photon would cross a flat interface (i.e. the photon will not move into/out of a vessel) by moving to this point the movement is split into 2 separate steps. First the photon is moved to the boundary and the remaining step size stored in a new variable. The software then determines whether the photon will be transmitted or reflected, by comparing another uniform random number to the reflectance \( R \), which is determined by Fresnel’s formula - Equation 6.3.5. \( \alpha_i \) is the angle of incidence to the interface, and \( \alpha_t \) is the angle at which the photon would be transmitted if it were not reflected. If \( \zeta > R \), then the photon is transmitted otherwise it is reflected from the interface.[2]

\[
R(\alpha_i) = \frac{1}{2} \left[ \frac{\sin^2(\alpha_i - \alpha_t)}{\sin^2(\alpha_i + \alpha_t)} + \frac{\tan^2(\alpha_i - \alpha_t)}{\tan^2(\alpha_i + \alpha_t)} \right]
\]  
(6.3.5)

New values for the directional cosines are then calculated depending on whether the photon was reflected or transmitted. Reflection within the tissue was dealt with using \( \sin(\theta_{\text{incident}}) = \sin(\theta_{\text{reflected}}) \). In the case of the photon meeting a boundary parallel to the tissue surface orthogonally the directional cosine \( \mu_z \) is multiplied by \(-1\) while \( \mu_x \) and \( \mu_y \) remain the same.
6.3.4 Interaction with a vessel

In the case of a vessel the point of reflection was treated as an infinitely small point on a line. If that line makes an angle $\alpha$ with the $x$ axis, then Equations 6.3.6 and 6.3.7 for reflection of a point, $(x_0, z_0)$, across a straight line through the origin $(0,0)$ can be used. From the resulting reflected point, $(x', z')$, the angle that the line between this point and the original point of reflection on the vessel wall makes with each of the two axes can be used to calculate new values of the directional cosines. The assumption of the line crossing $(0,0)$ can be neglected as it is only the angle between the outgoing photon packet and the $x$ and $z$ axes that are required, rather than a specific co-ordinate location. The $y$ axis runs parallel to the vessel walls, through the centre of the vessel. Due to this the $\mu_y$ value does not change after reflection from the vessel, and the problem is reduced to 2 dimensions. The vessel wall is assumed to have zero thickness. The geometry used for calculation is shown in Figure 6.3.2.

\[
x' = x_0 \cos(2\alpha) + z_0 \sin(2\alpha) \quad (6.3.6)
\]

\[
z' = x_0 \sin(2\alpha) - z_0 \cos(2\alpha) \quad (6.3.7)
\]

![Figure 6.3.2: Geometry of the reflection from the point $(x_{ves}, z_{ves})$ on the surface of the vessel.](image)

In order to calculate $(x', z')$ the location of $(x_0, z_0)$, a point on the photons...
current line of propagation, must be calculated. Any location on this line can be used and can be calculated using the point on the vessel wall that the photon is considered to be reflecting from, \((x_{ves}, z_{ves})\), and the values of \(\mu_x\) and \(\mu_z\) at the point of reflection using Equation 6.3.8. The constant 0.1 can be replaced with any value, provided the constant used for both the \(x_0\) and \(z_0\) calculations is the same.

\[
(x_0, z_0) = (x_{ves} - 0.1\mu_x, z_{ves} - 0.1\mu_z)
\]  

(6.3.8)

Once \((x', z')\) are known the new values of the directional cosines can be calculated via Equation 6.3.9. Whether the values are positive or negative is determined by the difference between \((x', z')\) and \((x_{ves}, z_{ves})\). If \(x' < x_{ves}\) then \(\mu_x\) is negative, otherwise it is positive (or zero). The same relationship holds for \(\mu_z\).

\[
\mu_x = \cos\left[\tan\left(\frac{z' - z_{ves}}{x' - x_{ves}}\right)\right], \quad \mu_z = \cos\left[\tan\left(\frac{x' - x_{ves}}{z' - z_{ves}}\right)\right]
\]  

(6.3.9)

### 6.3.5 Post-interaction scattering

Scattering within the sample is governed by the Henyey-Greenstein phase function, the generating function for which is given in Equation 6.3.10, where \(\theta\) is the deflection angle and \(g\) is the anisotropy of the sample.[2]

\[
\cos \theta = \begin{cases} 
\frac{1}{2g} \left(1 + g^2 - \left[\frac{1 - g^2}{1 - g^2 + 2g^2}\right]^2\right) & g \neq 0 \\
2\zeta - 1 & g = 0
\end{cases}
\]  

(6.3.10)

The azimuthal angle \(\psi\) is considered to be evenly distributed between 0 and \(2\pi\) via Equation 6.3.11.

\[
\psi = 2\pi \zeta
\]  

(6.3.11)

### 6.3.6 Accounting for absorption within tissue

To take into account the absorption of light within the sample and to prevent photons from propagating in the sample indefinitely each photon is initially assigned a weight value, \(w = 1\). This is decreased after each scattering event by an amount, \(\Delta w\), as determined by Equation 6.3.12.[2] If this value drops below \(w = 0.0001\) then the photon packet is considered to have been completely
absorbed by the tissue and is marked as 'dead'. The simulation of this photon is then terminated and it is not recorded as having reached the lens and collimator in order to contribute to the final signal.

\[
\Delta w = w \frac{\mu_a}{(\mu_a + \mu_s)} 
\]

6.3.7 Simulating flow

Flow in a vessel is modelled as laminar flow following Poiseuille’s Laws. The velocity at each location in the vessel is given by Equation 6.3.13, where \( v_{\text{max}} \) is the peak flow velocity of the vessel, \( R_{\text{ves}} \) is the vessel radius and \( r \) is the distance of the current photon location from the centre of the vessel.

\[
v(r) = v_{\text{max}} \left(1 - \frac{r^2}{R_{\text{ves}}^2}\right) 
\]

With each scattering event in a flowing medium a Doppler shift was applied to the photon according to Equation 6.3.14, where \( k_r \) and \( k_i \) are the wavevectors of the outgoing and incoming light respectively. These Doppler shifts are summed over all interactions to give a single total frequency shift for each propagated photon.

\[
\Delta f = (k_r - k_i) \cdot v(r) 
\]

As Equation 6.3.14 uses the scalar product of \( \Delta k \) and the velocity its value is depending on the direction of the flow relative to the wavevectors of the light, not just its magnitude.

6.3.8 Detection scheme

If the photon exits the tissue before being absorbed, terminating the simulation of that packet, the software must determine if it has exited at a point where it will be detected and will contribute to the signal or if it is lost. This is achieved using a confocal detection scheme. The light is assumed to be collected from the same collimator from which it is incident on the sample. This places requirements on the \((x, y, z)\) position of the photon packet, as well as \( \mu_x, \mu_y \) and \( \mu_z \) due to the numerical aperture, NA, of the lens in the system. Any photon packets not meeting the required criteria are considered lost and are not recorded. The detection scheme is illustrated in Figure 6.3.3. Any photons
exiting the bottom of the sample (positive $\mu_z$ value and $z \sim z_{total}$) are considered lost.

6.3.9 Simulating an imaging system

As the simulation is to be compared to experimental data the simulation was designed to model an actual imaging system. The NA is determined from the focal length, $f$, and diameter, $d$, of the lens in the handprobe of the OCT system detailed in Chapter 4 using Equation 6.3.15. With a diameter of 2.54 cm and focal length of 3.00 cm, the NA of the lens is 0.423. The angle to the normal of the lens within which the light must be propagating towards can then be calculated to be $\beta = 14.684^\circ$ using Equation 6.3.16, assuming the refractive index, $n$, of the lens material - Barium flint N-BAF10 - to be 1.670.[6] In order to be detected the photon packet must have a negative $\mu_z$ value signifying that it has left the tissue at the top surface, and the $\mu_x$ and $\mu_y$ values must be within the bounds determined by $\beta$ and $\theta_i$.

$$F \text{ number} = \frac{f}{d} = \frac{1}{2NA} \quad (6.3.15)$$

$$NA = n \sin \beta \quad (6.3.16)$$

Figure 6.3.3: The detection scheme of the simulation. The angle of acceptance is determined by the numerical aperture of the system which is chosen by the user at run time.

The $(x, y)$ constraints were determined from the diameter of collimator used in
the same handprobe rather than the focusing lens, as it has a smaller diameter; not all of the light that meets the angular and spatial requirements of the lens enters the collimator. It is assumed that the collimator and lens do not move between launching the light and collecting it, therefore the \((x, y, z)\) condition to be met is the same plane as where the photon packets are launched from i.e. at the focusing lens.

6.3.10 Overview of the propagation simulation

The overall flow of the Monte Carlo simulation from start to finish is as follows:

1. Ask the user for the filename of the input file containing information to set up the layers at runtime.

2. Check if the file exists. If yes, open it and read in data. Otherwise ask user for filename until correct or user inputs ‘.’ to exit.

3. Ask the user for the number of A scans required.

4. Begin the first A scan by setting up a photon packet for propagation; assign properties such as \((x, y, z)\) locations, \((\mu_x, \mu_y, \mu_z)\) directional cosine values.

5. Begin photon packet propagation by generating the first value for the photon distance \(s\).

6. Check whether this would cause the photon packet to cross into another layer. If no, the photon is moved according to this value. Otherwise the step is split into two parts. The photon is moved to the boundary, and the remaining step size stored in the variable \(s_{left}\) and used in place of generating a new value for \(s\) in step 5.

7. Scatter the photon based on Equation 6.3.10 or, if at a boundary, decide whether the photon is transmitted or reflected. If the photon is in a layer with a velocity then a Doppler shift is applied based on the velocity at that point.

8. If \(w < 0.0001\) then the photon is terminated. Otherwise repeat steps 5 to 7.

9. Repeat steps 4 to 8 for all other photons, and output the complete A scan data to a file.
10. Repeat for all other A scans, changing the initial $x$ value between each A scan to generate a full OCT image.

If a single A scan is selected then the photon propagation begins at $x = 0$, otherwise the photon start locations are evenly spaced between $x = x_{\text{min}}$ and $x = x_{\text{max}}$, centred on $x = 0$. No other beam or sample properties change between A scans.

6.4 Simulating an optical coherence tomography image

The Monte Carlo simulation does not simulate the OCT image itself; it is solely a method of determining the optical path length and Doppler shift of each photon packet entering the sample and if it will be coupled back into the optical fibre by the lens and collimator. If a photon is detected the path length that the photon packet travelled during the simulation and its Doppler shift is written to an output file at the end of the simulation of each A scan. Additional information, such as the number of scattering events each detected photon packet undergoes, is also provided in this file for reference but is not used to generate the OCT image. A coherence gate to generate the structural and Doppler flow images is applied separately in MATLAB after the simulation. The result is a set of A scans which resemble post-processed/enveloped data rather than the interferogram. Figure 6.4.1 shows an intensity profile of a simulated A scan of a sample with low scattering and absorption coefficients, such as a microscope slide.
Figure 6.4.1: The intensity profile of a simulated A scan from 100000 photon packets incident on an infinitely wide 1mm deep sample with $n = 1.45$ and low scattering and absorption coefficients (1.00 and 0.02cm$^{-1}$ respectively).

An example of the path taken by a photon in a sample is shown in Figure 6.4.2, where the only boundary within the sample is marked with a horizontal line at depth = 1, and Figure 6.4.3 shows the distribution of absorbed energy within the same sample.

Figure 6.4.2: An example $x - z$ projection of the path of a single photon in a 2 layered non-flowing sample, the point of entry of the photon is approximately (0, 0), at an angle 40$^\circ$ to the normal. Both layers $n = 1.35$, $\mu_a = 0.01$cm$^{-1}$, $g = 0.9$. Layer 1 depth 1cm, $\mu_s = 3$cm$^{-1}$. Layer 2 depth 4cm, $\mu_s = 25$cm$^{-1}$.
6.4.1 Applying a coherence gate in MATLAB

The output files from the simulation are read into Matlab where coherence gating is applied to the data and the resultant images displayed. Code supplied with the original MCML simulation, originally designed to plot data such as the energy distribution plot above, was modified for this purpose. Photon packets which are deemed to have been detected by the simulation can be split into two categories: class 1 photons which have been backscattered from the point of interest and class 2 which have only been scattered at shallower depths but have a path length long enough for them to contribute to the signal from the point of interest when a coherence gate is applied - see Figure 6.4.4. In order to simulate a true OCT image class 2 photons were included in the calculation of the coherence gate, as in practice there is no way to distinguish them from class 1 photons when they are collected from the sample.
Figure 6.4.4: An example of how class 2 photons can contribute to the OCT signal. (a) is a class 1 photon and has backscattered directly from the point of interest, whereas (b) has undergone many more scatters but has a very similar time of flight. In OCT imaging there is no way to distinguish the two types.

The flow of the MATLAB .m file to apply the coherence gate is as follows:

1. Ask the user for the format of the file names and how many A scans are to be read in.

2. Generate an equally spaced linear array from 0 to the maximum imaging depth as set in the .m source file. The array is the same length as the required pixel depth of the resultant image, $z_{\text{pixels}}$.

3. Compare half of the total path length travelled by each photon, which the depth the photon can be assumed to have reached if it had undergone a single backscatter in the sample, to this array one by one. If they are equal to within the coherence length, which is also set in the .m file, then the value of the corresponding pixel in the A scan array is increased by 1.

4. For each A scan the Doppler shift and total path length of all photons which meet this criteria are stored in a separate array, $\text{DetectedDoppler}$.

5. The structural image is generated from several A scans made up of arrays containing of a histogram of half of the total path length. The more photons which are in each bin then the more photons which were detected from that point.
6. To produce the Doppler image the DetectedDoppler array is arranged in order of the path lengths. The values are then binned based on their depth; there are $z_{\text{pixels}}$ bins in total. Another array records the total number of photons detected for that bin plus the average Doppler shift over all detected photons for each depth.

7. The RMS Doppler shift for each pixel is calculated. These arrays are then used to create the full Doppler image.

The resulting structural image, an example of which is in Figure 6.4.5, can be displayed in greyscale, as is typical for OCT images. As the plot is produced in Matlab a number of other colormaps can also be applied.

Figure 6.4.5: An example of a structural image of a multi-layered non-flowing sample as generated by a Monte Carlo simulation. The anisotropy and absorption coefficient are equal for all layers (0.9 and 0.2 cm$^{-1}$ respectively). The scattering coefficient and refractive index of the middle layer is greater than the layers above and below ($\mu_s = 50$ cm$^{-1}$ and $n = 1.40$ whilst other layers are $\mu_s = 0.1$ cm$^{-1}$ and $n = 1.45$). 1 million photons were used for each of the 256 A scans which are 1024 pixels deep. The image is 2 cm by 1 cm (width by depth).

In reality the clarity of the interference fringe, and hence the final image,
is dependent on the relative intensity of the optical signal in both arms of
the interferometer, not just the light backscattered from the sample. For the
purpose of the simulation it is assumed that the intensities from the arms of the
interferometer are matched as close as is possible, so that the photons from the
sample arm achieve their maximum contribution towards the signal.

6.5 Comparison with a flow phantom

6.5.1 Simulation model

The aim of the Monte Carlo simulation is to provide a model which could be
compared to a flow phantom to be imaged with a commercial swept-source
DOCT system (OCM 1300SS, Thorlabs, Ely, UK) which has a theoretical
resolution of 7.7 microns in tissue. The optical properties of the fluid within
the vessel are matched as close as possible to those of Intralipid at 1300 nm
using values found in literature. Intralipid is a fat emulsion which is often used
in flow phantoms as it has similar optical properties to tissue [7, 8] The material
outside the vessel was taken to have low scattering and absorption coefficients as
the capillary in the flow model is surrounded by air. The geometry simulated
is shown in Figure 6.5.1.

![Figure 6.5.1: The geometry of the simulated model.](image)

6.5.2 Flow phantom

A flow phantom - Figure 6.5.2 - was imaged using a Doppler OCT system.
The phantom consists of glass capillaries of varying internal diameter all of
which can be connected individually to a syringe containing Intralipid which is
pumped through the capillaries. The phantom was not connected to a syringe
pump making it difficult to give an exact speed for the flow, however visually
it was estimated around the order of 1 cms$^{-1}$. The second largest capillary has an external diameter of 1.55mm and internal diameter of 1.15mm. This was imaged for comparison with the results of Monte Carlo simulation for vessels of matching diameter containing a liquid of similar optical properties as Intralipid.

![Figure 6.5.2: The flow phantom containing 5 glass capillaries of varying widths through which fluid can be injected using a syringe.](image)

6.5.3 Comparison of simulation and experimental results

In order to mimic these images as accurately as possible values of the scattering and absorption coefficients used for the simulation must be as close as possible to actual values. The values used for the flowing medium are given in Table 6.5.1, which are based on optical properties of Intralipid and blood found in literature. The surrounding air is considered to have negligible values of $\mu_a$ and $\mu_s$, $n = 1$ and $g = 0.999$. Due to the negligible scattering coefficient of air the vast majority of the scattering events occur in the capillary.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>1.36$[9]$</td>
</tr>
<tr>
<td>$\mu_s$</td>
<td>4mm$^{-1}[10]$</td>
</tr>
<tr>
<td>$\mu_a$</td>
<td>9.95mm$^{-1}[11]$</td>
</tr>
<tr>
<td>$g$</td>
<td>0.35$[12]$</td>
</tr>
</tbody>
</table>

Table 6.5.1: Optical properties of the Intralipid solution modelled with the simulation. The value of $\mu_a$ is calculated from the value of $\mu_t$ given in the referenced paper, assuming that $\mu_s = 4$mm$^{-1}$ and $g = 0.35$. 

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The results of the simulation are shown in Figure 6.5.3, along with the corresponding images from the flow phantom. The raw data of the simulation is shown, whereas the images from the phantom have been post processed by the OCT system software. The central wavelength of the simulated source was set to 1300 nm, the numerical aperture to 0.42 and incident angle to 5.0°. 40 million photons were used for each A scan. A tube of internal diameter 1.15 mm was simulated, corresponding to the second largest tube in the flow phantom. Peak flow was set to 1 mms$^{-1}$ although as Laminar flow was simulated - Equation 6.3.13 - most of the photons will have scattered from flow with a lower speed than this. To produce the OCT image the total number of photons detected at that optical path length was used. For the DOCT image the RMS value of the detected frequency shifts at each pixel was recorded. The simulated images are 0.3 cm by 0.4 cm or 2048 by 1024 pixels in size (width by depth). The experimental Doppler image for the capillary tube is subject to 'banding', where the image contains rings in the area of flow. This is due to phase wrapping, where the change in phase is greater than π but is measured to be in the range $-π < \text{phase change} < π$, and only occurs in areas of high flow. As the simulated results do not depend on the phase change but the frequency shift this effect is not visible.
Using an equation from Chapter 1, Equation 6.5.1, allows the velocity component parallel to the vessel axis of the scatterer, $v_s$, to be calculated from the frequency shift recorded, $f_D$, when given the central wavelength of the source, $\lambda_0$, the refractive index of the sample, $n_t$, and the angle between the flow and the incoming beam, $\theta$. Assuming $n_t = 1.36$, $\lambda_0 = 1300\text{nm}$ and an
angle between flow and beam of $\theta = 85^\circ$ (based on an angle of incidence of $5^\circ$ to the normal), typical calculated velocities are around 0.01 to 0.03 cm$^{-1}$ through the centre of the vessel parallel to the beam direction.

$$f_D = \frac{2v_sn_t \cos \theta}{\lambda_0} \quad (6.5.1)$$

The simulation does not appear to completely account for absorption as the penetration depth is higher than in the flow phantom. The reduction in signal intensity as a function of depth is higher in the images from the commercial system compared to the simulated results. However there is still a noticeable reduction in the number detected photons as depth increases in a single A scan, for example the A scan from the centre of the above image which is shown in Figure 6.5.4a, as less photons are scattering from the deeper locations.

The resulting flow information from the same A scan is shown in Figure 6.5.4b. No Doppler noise, an effect which has been noted in other simulations in the literature,[13] is visible in this A scan. This is likely due to the low scattering coefficient of the medium surrounding the vessel and the majority of photons in the vessel scattering once before being detected meaning that optical path lengths longer than the bottom of the vessel were very unlikely.
Figure 6.5.4: Plots of simulated structural (a) and Doppler (b) data from a simulation of 10 million photons through a 0.115 cm internal diameter vessel surrounded by air, including the 0.05 cm gap above the vessel which can be seen in the plot. The flow was set to 1 mm s$^{-1}$. Despite the number of simulated photons there were only 622 photons detected for this A scan.

Applying a low-pass filter to the structural A scan smooths out some of the high frequency noise - Figure 6.5.5. The full image after filtering is shown in Figure 6.5.6.
Figure 6.5.5: The OCT A scan before (black line) and after (blue line) low-pass filtering.

Figure 6.5.6: The structural image after low-pass filtering has been applied to each A scan. The filtering allows the gradation of the flow to be seen. As expected the highest flow is in the centre with the speed generally reducing with increasing distance from this point.

Although the flow profile is noisy its overall structure is close to the flow that was modelled, with the velocity increasing towards the centre of the vessel; applying a low-pass filter in Matlab results in Figure 6.5.7. The peak flow values calculated are lower than expected for the simulated velocity of 1.0 mms$^{-1}$, even at the vessel centre.
If each Doppler A scan is filtered individually then the flow profile is much clearer - Figure 6.5.8 - although the measured peak flow value is reduced as a result of filtering. It is not known what sort of post-processing the commercial system uses for either the structural or flow data, so a comparison of different filtering methods with the phantom images can not be made.

Both the simulated structural and Doppler images suffer from horizontal 'blurring' where photons are detected outside the vessel in areas where the scattering coefficient is orders of magnitude lower. The vast majority of photons scattered once before being detected, ruling out multiple scattering events being the cause. Given that this does not occur vertically it is believed that this is due to an error in the focus mechanism of the simulation, where the beam is
not focused correctly towards the vessel. This leads to photons having a larger component of horizontal motion than would be expected; some of these photons may then reach and scatter within the vessel despite being expected to follow an almost-vertical path and not scatter at all. As a result A scans either side of the flow show detections of photons which have reached the scattering medium.

6.6 Conclusions and discussion

While the simulation can give an idea of the distribution of flow data expected from a Doppler OCT image, it does not completely simulate the interaction of light with the tissue, in particular to generate a structural image as evidenced by the increased penetration depth of the simulated photons. It is possible that the value of $\mu_a$ is incorrect as it is calculated from the values of $\mu_t$ and $\mu_s$ from separate sources. While both were measured using a 1300 nm source, the value of $\mu_s$ was calculated for a 5% Intralipid solution whereas $\mu_t$ was calculated for porcine blood.

From the flow phantom it can be seen that Doppler information is not always obtained even in locations where there is structural data present - see Figure 6.5.3c and 6.5.3d. Despite the difference in penetration depth between the two sets of images a similar effect is seen in the simulated images. However it is reversed; the data in the structural image when all A scans from the image are considered together extends to approximately 440 pixels deep while the Doppler reaches 460 pixels. This implies that the simulation is more sensitive to Doppler shifts than a DOCT system. It is not known what method the commercial system uses to obtain flow information from the raw data, however given the appearance of phase wrapping in the flow images it is likely a method such as phase-resolved DOCT was used, where the phase shift between sequential A scans is calculated. As the simulation uses the raw frequency shifts to calculate flow the difference in how the data is calculated is a possible cause of this. Future work in this area could potentially look at a comparison of different measurement techniques.

Different equations were also used to calculate the frequency shift during the simulation - Equation 6.3.14 - and to calculate the equivalent flow speed from the results - Equation 6.5.1. The former requires the precise direction of the incident light to be known, as it is dependent on the wavevectors of the incoming and outgoing light. This varies from photon to photon as the incident beam
is not collimated. However, to compute the results as plotted above the same value \( \theta = 85^\circ \) was used for all photons. Although the angles would have been close to this value, especially as it was rare for a photon to scatter more than once thus changing its angle, this could be a source of the disagreement between the measured and simulated flow magnitudes. As actual OCT imaging systems also use a focused beam this problem would also be present when calculating DOCT data using a method which relies on a measurement of the frequency shift.

The simulation could be further adapted to allow simulation of multiple vessels at varying depths and orientations to allow a comparison with a more complicated flow phantom which models microvasculature structure more closely. If this was to be studied further, the computational speed of the simulation could be an issue as was it was based on code originally written in C and hence is not optimised for modern computers; the simulation to generate the results above ran for almost 21 hours. The simulation could be adapted to run in multiple threads, simulating several photons at a time. While this would reduce the computation time it would not have any effect on the accuracy of the simulation.
REFERENCES


Chapter 7

Summary, discussion and future work

7.1 Thesis overview

Several chapters in this thesis centre on a custom built OCT system at the Photon Science Institute, University of Manchester. The optics of this system were already in place as a result of the work by Dr Graham Dinsdale during his PhD. However, there was no software to capture data or control the galvanometer mirror in the delay line and the raster scanner in the hand probe. A phase modulator had been purchased with a view to add this to the system to enable Doppler measurements to be taken, but it had not been tested or characterised. The system is designed to eventually be used in imaging studies with patients with diseases such as systemic sclerosis where other imaging methods have shown there is a detectable difference in skin thickness and blood flow, particularly in the hands. As Doppler OCT is able to image into skin while providing information about the structure and function of small vessels in real time it is potentially a very useful tool in this area of research.

During the course of this PhD the modulator was incorporated into the system and tested. Software was also developed to enable real-time structural and flow images to be taken, both using the phase modulator and using an alternative cross-correlation method which had more success in obtaining flow data. A freely available simulation of light-tissue interaction, Monte Carlo simulation of multi-layered media (MCML), was also adapted to allow the comparison of
simulated structural and flow images to those obtained using a commercial OCT system by imaging Intralipid in a glass capillary tube. As the final application of the purpose built system is clinical studies a study was also undertaken at Salford Royal NHS Foundation Trust in order to compare the capabilities of the commercial OCT system with previously validated techniques.

Below is a list of significant achievements from this thesis and a summary of each of the chapters. They are followed by ideas for future work at the end of this chapter.

- Characterisation of an electro-optic phase modulator for use in Doppler OCT imaging
- Production of software to control a Doppler OCT system, including synchronisation of data capture and hardware in addition to post-processing
- Application of a correlation technique for measuring flow with a DOCT system
- Development of software to measure skin thickness from OCT and HFUS images
- Modification of a Monte Carlo model to simulate OCT and DOCT images
- Measurements on a flow model for comparison with simulated results

### 7.1.1 Chapter 3: Characterisation of an electro-optic modulator for use in a Doppler optical coherence tomography system

This chapter discussed the characterisation of a LiNbO$_3$ electro-optic phase modulator for use in a phase-resolved Doppler OCT system. The phase modulator modulates the light at the same frequency as the applied voltage with a phase shift determined by the magnitude of the voltage. When the modulator was purchased, however, the relationship between the applied voltage and resulting phase modulation was unknown for all but one frequency and voltage combination.

The effect which changing the frequency and voltage applied to the modulator had on the resulting interferogram recorded at the output of an interferometer was studied and a general relationship between the frequency and voltage was
obtained. From this the voltage required to apply a π phase shift to a source with a wavelength \(\sim 1300\,\text{nm}\) can be calculated for the frequencies likely to be used in DOCT imaging - values of several hundred kHz are normally mentioned in the literature. The modulator was then integrated into the previously-built OCT system which did not have Doppler capabilities.

7.1.2 Chapter 4: Software for a Doppler optical coherence tomography system

Chapter 4 discussed the development of software designed to control an OCT imaging system as well as post-process and display structural and flow data in real-time. Two methods were tested; a Hilbert-transform based phase-resolved method and a correlation mapping method. Only the correlation mapping method was successful in detecting the motion of highly reflective samples using the optical system as it is currently set up. The phase-resolved method relied on a phase modulator with was a significant source of optical power loss. Due to issues with loss of power, particularly when a phase modulator was used to try to obtain flow information from the system and from the free space RSOD line, Fourier Domain OCT may be a better solution for the system. A CCD array camera and diffraction grating have been purchased to enable the system to be transformed into a Fourier domain system. This will completely remove the RSOD hopefully providing a noticeable improvement in image quality. However, this will require sections of the software to be re-written as raw FD-OCT data from a camera is obtained in a different manner to TD-OCT which uses a single detector.

7.1.3 Chapter 5: Development of software to measure skin thickness from optical coherence tomography and ultrasound images

This chapter discussed the development of semi-automated image analysis software to measure the thickness of the epidermis using both high frequency ultrasound and OCT. This software was then used to measure epidermal thickness in patients with systemic sclerosis as well as healthy controls as part of a clinical study into non-invasive imaging methods at Salford Royal NHS Foundation Trust.

The software was designed to allow the user to select a location on the image to
be analysed. The software then displays potential boundaries on that section of the image using either the absolute value of the pixels in the image, in the case of ultrasound images, or a gradient method, as for OCT images. The user chooses the most appropriate of these boundaries to eliminate incorrect markings due to local variations. From the remaining boundaries a measurement is taken and recorded to a .xls file. Images from 5 locations on the body were measured, including locations which are typically affected by the two forms of systemic sclerosis: limited cutaneous and diffuse cutaneous.

Both imaging methods were able to measure a statistically significant difference in epidermal thickness between patients and healthy controls over some of the body areas imaged. Both methods also show high reproducibility. Two additional imaging methods were used to investigate the function of microvessels in the skin. Both of these - nailfold capillaroscopy and laser Doppler imaging - were able to measure statistically significant differences between patients and healthy controls. However, there was no correlation between either blood perfusion or capillary density and thickness. A future aim is to obtain Doppler OCT images in order to determine its effectiveness at measuring blood perfusion in the skin in patients with this disease.

This chapter has resulted in a manuscript which is being prepared for publication.

7.1.4 Chapter 6: Monte Carlo modelling of a Doppler optical coherence tomography signal and its comparison with a flow phantom

A modified version of Monte Carlo simulation of multi-layered media (MCML) was designed to simulate structural and Doppler images from an OCT system. Simulated images of Intralipid flowing through a capillary tube were compared to images of the same set-up obtained using a commercial OCT system, however this was not as successful as was hoped. Absorption in the Intralipid did not appear to be fully accounted, as the amount of light penetrating deeper parts of the sample reduced at different rates between the two methods. There also appeared to be an issue with the method of focusing the beam towards the sample which resulted in horizontal blurring in both the structural and Doppler images.

The simulation was able to extract the magnitude of the simulated flow accurately to within an order of magnitude, and after a simple filter was applied
to eliminate fluctuations in the data the structure of the Doppler image closely matched what was modelled, with the highest flow measured in the centre of the vessel.

7.2 Discussion and future work

The work discussed in this thesis covers several aspects of OCT, from the development of software and continued development of the optics for a custom built system to a clinical study investigating the suitability of OCT in measuring skin thickness compared to an established technique. The ultimate aim is to develop OCT as a potential imaging tool for investigating skin thickness and microvasculature structure and function in patients with systemic sclerosis; numerous researchers across several disciplines are involved in this long-term project. The work presented in this thesis work forms a significant contribution towards the final objective. While there have been challenges it is hoped the work presented in this thesis can provide a solid base from where this goal can be pursued.

The resulting images from the custom built system, both structural and Doppler, were not as successful as had been hoped. There were many sources of losses in the system, notably in the reference arm of the interferometer where there was a 60% insertion loss caused by the electro-optic modulator when it was included in the system and a 95% loss in the rapid scanning delay line. These significantly reduced the intensity of the light reaching the detector and thus the image quality. The low phase stability of the system made it difficult to extract Doppler information from the interferogram using a phased resolved method. Although the phase modulator was able to be successfully characterised, integrating it into the system in order to use this method resulted in a significant reduction in image quality.

Without the phase modulator in place in-vivo images could be obtained, although the penetration depth was not sufficient enough to allow the full epidermis to be imaged as had been hoped. However by using a correlation mapping method the phase modulator was no longer required and motion data from highly reflective samples could be obtained.

The software developed for this system was successful in achieving its aims; the analogue signals from the delay line are used to control both the data capture using the data acquisition card and the movement of the handprobe so that
the A and B scans are synchronised. As a result a full structural image can be displayed. Using the correlation mapping method motion data can be obtained from the same raw data without additional equipment, although additional processing time is required which results in a slower rate of imaging. Currently the imaging speed of the system is software limited; a maximum speed of 9 frames per second for the structural image only was achieved, with the system theoretically capable of supplying data at 30 frames per second.

The second set of software which was developed - to enable semi-automated measurements from OCT and high frequency ultrasound images - was also able to perform as designed. Making measurements from the images proved to be time-intensive due to the number of measurements to be made. While a fully-automated method would have been preferred in terms of speed, local variations within the image made this impossible due to the unreasonably high rate of falsely marked boundaries. This made user interaction necessary. Using a threshold method, compared to measurements solely by human eye, also removed some of the uncertainty that would have existed for measurements of boundaries which spanned several pixels. However measurements were still limited by the resolution of the images for both techniques. The final results of the study for which this software was developed were promising; OCT was able to measure statistically significant differences between patients with systemic sclerosis and healthy controls at several locations on the upper body.

The Monte Carlo simulation discussed in Chapter 6 does not completely account for the interactions between light and tissue, in particular the level of absorption is believed to be too low. As mentioned in the chapter, this could have been due to an incorrect value for the absorption coefficient which was derived from values from two sources for two liquids; 5% Intralipid and porcine blood. However as dilute Intralipid is often used as a blood substitute in flow phantoms due to its similar optical properties to tissue this is unlikely to be the sole cause of the discrepancy. There was also a difference between the relative locations in the images where structural and Doppler information data is obtained. In particular it was difficult to properly simulate the Doppler images for comparison with a commercial system without knowing how the experimental data is post processed and how the Doppler shifts are calculated. Ideally the simulation results would have been compared to images from the custom built system but the quality of the images from this system were insufficient for this. Overall the results of the simulation were disappointing, and investigations into the problems that arose were hampered due to time limitations.
7.2.1 Future work

The OCT system is intended to be able to image in-vivo at a resolution and depth suitable for measuring skin structure as well as the structure and function of blood vessels in the dermis. It is recommended that the focus should be on improving the optical system of the custom built OCT system in order to obtain clear images at sufficient resolution so that measurements of skin thickness can be made. Although the bandwidth of the source is sufficient enough to provide an appropriate level resolution, currently the system can not achieve penetration depths to enable imaging of the full epidermis at any locations which were tested on the hands and forearms. In order to achieve sufficient image quality so that the dermis is visible changes will need to be made to the optical system and to the software controlling the data acquisition.

A line scan camera has been purchased in order to convert the system from time-domain to Fourier Domain. This will hopefully increase the SNR enabling clearer structural images to be obtained and a penetration depth sufficient enough for in-vivo imaging for clinical studies. As a result of the switch to Fourier domain imaging the rapid scanning optical delay line will no longer be required. However, as this currently provides the signals used by the software to trigger the data acquisition card as well as the motion of the hand probe mirror, a new method of synchronisation will need to be developed. This improvement will also remove the need for additional equipment, such as the phase modulator, in order to extract Doppler information, as it should hopefully be obtainable from the raw data from the line camera. Work has also begun to investigate multi- and hyper-spectral imaging as a method of measuring blood perfusion. This could potentially be combined with DOCT imaging to provide additional information about flow via a different method to Doppler imaging.

It is hoped that the work undertaken for this thesis will provide a foundation from which the OCT system can be further developed and improved so that it can fulfil its ultimate purpose of being used in clinical studies to investigate skin structure as well as microvasculature structure and function. Given the limited research in this area previously - OCT as a technique has been in development for around 20 years however its first application to systemic sclerosis research was only recently published - it is important to continue this line of research to see what potential OCT and Doppler OCT may have to offer.