Development of a High Resolution Microvascular Imaging Toolkit for Optical Coherence Tomography

By

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Department of Medical Biophysics

University of Toronto

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Abstract

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This thesis presents the development of new optical coherence tomography imaging systems and techniques to improve in vivo 3D microvascular imaging. Specifically these systems and techniques were proposed to address three main problems with 3D Doppler optical coherence tomography imaging: (a) Motion artefacts, (b) angle dependence of the signal, and (c) relatively high minimum detectable velocity of conventional color Doppler algorithms (~500 µm/s). In order to overcome these limitations a multi-pronged strategy was employed:

(1) Construction of a retrospectively gated OCT system for the mitigation of periodic motion artefacts. Proof of principle in vivo B-mode imaging of Xenopus Laevis (embryo of African clawed frog) cardiovascular function up to 1000 frames per second (fps) from data acquired at 12 fps. Additionally, 4D imaging of the Xenopus Laevis heart at 45 volumes per second was demonstrated.

(2) Construction of a Fourier domain mode locked laser for high speed swept source optical coherence tomography imaging. This laser was capable of reaching sweep rates of 67 kHz and was optimized to function in the SNR limited phase noise regimes upto approximately 55 dB structural SNR.

(3) Development of a novel speckle variance image processing algorithm for velocity and angle independent 3D microvascular imaging. The velocity and angle independence of the technique was validated through phantom studies.
In vivo demonstration of the speckle variance algorithm was performed by imaging the capillary network in the dorsal skin-fold window chamber model, with the results being validated using fluorescence confocal microscopy.

In the final part of this thesis, these newly developed technologies were applied to the assessment of anti-vascular and anti-angiogenic therapies in preclinical models, specifically, photodynamic therapy and targeted degradation of HIF-α.
Acknowledgements

There are a number of people who helped to shape the course of my PhD research and without them I would not have been able to complete this work. First and foremost I would like to sincerely thank my supervisor Prof. Alex Vitkin for accepting me into his research group and giving me the freedom to explore and develop my own research directions. His mentorship, encouragement and enthusiasm have been extremely motivational to me.

During my first few weeks in the lab I met Dr. Victor Yang, at the time a recent graduate of the MD/PhD program at the University of Toronto, who built the first optical coherence tomography system at Princess Margaret Hospital and in Canada. He is probably best described as a fountain of enthusiasm and ideas. It was during my many discussions with Victor that I finally decided upon a research project that focused on improving the microvascular imaging capability of OCT and for this and for I am truly grateful. Without his continued mentorship over the last 5 years this project would not have been possible.

I would also like to thank the members of my thesis supervisory committee, Profs. Brian Wilson, Michael Kolios and Peter Burns. They have continuously challenged me with their insightful questions and have always found the time to discuss my project with me.

Special thanks also goes to the members and students of the biophotonics group at UHN that I have worked closely with over the past 5 years. Especially, Beau Standish, Nigel Munce, Michael Leung, Mark Jarvi and Eduardo Moriyama; they made the lab an enjoyable place to be and have taught me more than I could have imagined.

Lastly, I would like to thank my family and friends for their continuing support and encouragement.
To my mother and father, thank you for all your support
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Abbreviations and Symbols used in thesis

Abbreviations

2D: Two Dimensional

3D: Three Dimensional

4D: Four Dimensional

ANSI: American National Safety Institute

ASE: Amplified Spontaneous Emission

BTM: Bulk Tissue Motion

CCD: Charge Coupled Device

CD31: Cluster of Differentiation 31

CIR: Circulator

CT: Computed Tomography

DAQ: Data Acquisition

DBP: Dual Balanced Photodetector

DOCT: Doppler Optical Coherence Tomography

ED: Early Diastole

LD: Late Diastole

FBG: Fiber Bragg Grating

FD: Fourier Domain

FDML: Fourier Domain Mode Locked

FPS: Frames Per Second

FSR: Free Spectral Range
FWHM: Full Width Half Max
HFUS: High Frequency Ultrasound
HIF-α: Hypoxia Inducible Factor Alpha
ITBF: Inter-Trabecular Blood Flow
I.V.: Intravenously
LVA: Luminal Vascular Area
MPCM: Multi-Photon Confocal Microscopy
MRI: Magnetic Resonance Imaging
MVD: Mean Vascular Density
MZI-Clock: Mach-Zender Interferometry Clock
OCT: Optical Coherence Tomography
OAG: Optical Angiography
PC: Polarization Controller
PDT: Photodynamic Therapy
PET: Positron Emission Tomography
PMH: Princess Margaret Hospital
PS: Peak Systole
PSF: Point Spread Function
RA: Right Atrium
RAo: Right Aortic Arch
LA: Left Atrium
LAo: Left Aortic Arch
LD: Late Diastole
LS: Late Systole
RSOD: Rapid Scanning Optical Delay Line
SCL: Short Cavity Laser
SD-OCT: Spectral Domain Optical Coherence Tomography
SEM: Scanning Electron Microscope
SM: Single Mode
SNR: Signal to Noise Ratio
SOA: Semiconductor Optical Amplifier
SS: Swept Source
SV: Speckle Variance Intensity
TA: Truncus Arteriosis
TD: Time Domain
TWF: Tunable Wavelength Filter
US: Ultrasound
VEGF: Vascular Endothelial Growth Factor
VHL: Von Hippel-Lindau
VWF: Von Willebrand Factor
WCM: Window Chamber Model
Symbols

\( a(z) \): Axial Reflectivity Profile

\( C \): Speed of Light

\( \vec{E} \): Electric Field Vector

\( f_a \): Axial Scan Frequency

\( f_D \): Doppler Frequency Shift

\( \text{FT} \): Fourier Transform

\( g \): Coefficient of Anisotropy

\( I \): In-Phase Signal

\( l_c \): Coherence Length

\( I(k) \): Intensity as a Function of Wavevector

\( I(v) \): Intensity as Function of Frequency

\( k \): Wavevector

\( k_B \): Boltzmann Factor

\( L \): Laser Cavity Length

\( \vec{n} \): Noise Vector

\( n \): Index of Refraction

\( Q \): Quadrature Signal

\( S^2 \): Structural Signal

\( S(k) \): Source Spectrum

\( \text{SV}_{\text{fluid}} \): Speckle Variance Intensity for Fluid

\( \text{SV}_{\text{solid}} \): Speckle Variance for Solid
$T_0$: Period of Laser Sweep

$\nu$: Velocity

$w_0$: Beam Waist

$2z_0$: Confocal Parameter

$\lambda_0$: Central Wavelength

$\Delta\lambda$: Spectral Bandwidth

$\Delta\phi$: Change in Phase

$\Omega$: Mean Phase Shift

$\mu$: Mean

$\nu$: Frequency

$\nu_1$: Rate of Change of Frequency

$\sigma^2$: Variance

$\sigma_{\text{fluid}}$: Standard Deviation of Fluid Intensity Pixels

$\sigma_{\text{solid}}$: Standard Deviation of Solid Intensity Pixels

$\sigma_{\Delta\phi}$: Standard deviation of phase difference
Format of thesis
The thesis begins with an introductory chapter which describes the microcirculatory system, tumor angiogenesis, vascular targeted therapies, current microvascular imaging techniques and finally Doppler Optical Coherence Tomography (DOCT) as a potential technology to monitor and quantify 3D vascular structure. Chapter 2 deals with the development of a retrospectively gated DOCT system that is able to mitigate periodic bulk motion. Chapter 3 describes the development of a high speed Fourier domain mode lock laser with low phase noise. Chapter 4 details the development of an angle and velocity insensitive 3D microvascular imaging technique called speckle variance OCT. Chapter 5 describes the preclinical studies which I have participated in that utilized the techniques and systems developed in Chapters 2, 3 and 4. Lastly, the thesis ends with a summary and future research chapter.
Contributions to manuscripts generated

**SYSTEMS DEVELOPMENT**


**Contribution:** Designed and set up the majority of experiments and wrote the majority of the manuscript


**Contribution:** Independently built and characterized the laser source used in this study.


**Contribution:** Designed and set up the majority of experiments and wrote the majority of the manuscript


**Contribution:** Designed and set up the majority of experiments and wrote the majority of the manuscript
Pre-Clinical Applications


**Contribution:** Performed all optical coherence tomography imaging and subsequently processed data and wrote the corresponding section of the manuscript.


**Contribution:** Performed all optical coherence tomography imaging and subsequently processed data and wrote the corresponding section of the manuscript.


**Contribution:** Performed fluorescence and optical coherence tomography imaging and subsequently processed data and wrote the corresponding section of the manuscript.


**Contribution:** Designed, setup and performed wide field photodynamic therapy treatments and optical coherence tomography imaging. Subsequently performed related image processing and wrote corresponding section of the manuscript.
Chapter 1: Introduction

Microcirculatory changes play important roles in the pathophysiology of diseases such as cancer [1], age-related macular degeneration [2], atherosclerosis [3], and spinal cord injury [4]. These changes are often subtle, yet can be critical for both the early detection and monitoring/optimization of treatment efficacy if appropriate detection techniques are available.

Nowhere are these changes more evident than in the pathophysiology of solid tumors which, in order to grow beyond 1-2 mm, must recruit and develop their microvascular networks in a process known as tumor angiogenesis. In normal tissues the development of microvasculature by angiogenesis is governed by a delicate balance between pro-angiogenic and anti-angiogenic molecules. However, in tumors the balance between pro and anti-angiogenic signaling is lopsided resulting in the formation of structurally and functionally abnormal vasculature [5].

This observation has opened up a new set of treatment options whose target is this abnormal microvasculature. These treatments can be divided into two categories, anti-vascular and anti-angiogenic therapies. Anti-vascular therapies aim to rapidly coagulate and cutoff the vascular supply to the tumor, causing tumor cell death from lack of oxygen and nutrients. Examples of these include ablative techniques such as laser photocoagulation and radio-frequency ablation as well as photochemical techniques such as photodynamic therapy. Anti-angiogenic therapies may antagonize vascular growth receptors that are most active on newly branching vessels and dividing endothelial cells associated with angiogenesis in an attempt to force the tumor back into a dormant state thereby controlling tumor growth [5].

High resolution three-dimensional imaging of microvascular networks pre-clinically and clinically has the potential to play an important role in the future development of strategies to (a) improve the efficacy of antiangiogenic and anti-vascular therapies (dosing, scheduling, metronomic and combination therapies) and (b) overcome a tumor’s resistance mechanisms to these therapies. Unfortunately, the ability to visualize
microvasculature is limited by the resolution and sensitivity of imaging modalities used in medical imaging.

The development of new high resolution 3-Dimensional (3D) optical technologies to image the microcirculatory network may overcome these limitations. Doppler optical coherence tomography (DOCT), which is usually referred to as the optical analogue to ultrasound, is one such potential imaging technique. DOCT typically provides ~10 µm isotropic resolution and exhibits microvascular imaging capabilities that are sensitive to blood flow velocities greater than ~500 µm/s. However, this velocity limit as well as other limitations, severely impacts the sensitivity of the technique and the ability to generate 3D microvascular maps in normal tissue and tumors.

Several key challenges/topics of interest in the field of microvascular imaging were identified that this thesis aims to investigate:

1. Microvasculature plays a key role in the pathophysiology of solid tumors, thus it may be possible to use a high resolution 3D microvasculature imaging technique to help develop, optimize and monitor the efficacy of anti-vascular and anti-angiogenic therapies.
2. Current clinical imaging techniques lack the spatial resolution and blood-flow sensitivity necessary to image microcirculation on a scale smaller than 50µm.
3. Doppler optical coherence tomography, in its current form, has sufficient resolution to image microcirculation. However, it suffers from a number of problems that limits its sensitivity to low blood flow (slowly moving red blood cells). Overcoming these limitations may increase the 3D microvascular imaging capability of the technique.

The following 3 sections expand upon and explore each of these motivations in detail.

**1.1 The microcirculation and tumor angiogenesis**

The adult human body contains over 160 000 km of blood vessels. Without this extensive network tissues would not receive the oxygen and other vital nutrients necessary to carry out their normal functions. The vascular network consists of an ordered network of arteries, capillaries and veins with each component having a specific
A scanning electron microscope image of a polymer cast of normal tissue microvasculature shown in Figure 1.1. An orderly branching of arteriole and venous vessels is observed, with the capillaries neatly forming the interconnect between the two. The arteries move oxygen rich blood to all parts of the body where it is forced into smaller and smaller arteries and arterioles. Due to the high peak pressure (90-140 mmHg) required to move blood from the heart to the rest of the body, arteries and arterioles incorporate significant layers of smooth muscle and elastic tissue into their architectures to provide strength and flexibility. When imaging the microcirculation, we are primarily interested in the arterioles, venules and capillaries systems (for a summary of physical specifications see Table 1.1). The arterioles range in size from 20-200 µm and have typical peak flow velocities of 2-15 mm/s depending on the local capillary recruitment. The arterioles, due to their small size and large number, are the primary source of vascular resistance within the circulatory system and are able to regulate the flow of blood to the entire body through vasoconstriction and vasodilation [6].

Figure 1.1: Scanning electron microscope polymer corrosion cast of normal vasculature from the vasa vasorum of rat carotid sinus (adapted from [7]).

Capillaries branch from the arterioles as shown in Figure 1.1 and due to their enormous numbers provide a large total surface area through which oxygen and other nutrients diffuse. For cells to receive the necessary oxygen to survive, the capillary bed must bring oxygen-rich blood to within the diffusion length of oxygen in tissues to every cell (~100 µm) [8]. At the junction between arterioles and capillaries there is a small band of
smooth muscle known as a precapillary sphincter which acts to control flow into each capillary and thus control local tissue perfusion. Capillaries are comprised of only a monolayer of endothelial cells and basement membrane. This simple structure facilitates diffusion of oxygen to the surrounding tissue. Most capillaries also have pores between cells to allow for diffusion of dissolved nutrients and waste products in and out of the capillary. Capillary size ranges from 7-15 µm and typical flow velocities within capillaries are 0.5-1.5 mm/s.

The venous network is responsible for returning oxygen-depleted blood to the heart and starts with the small venules attached to the capillary network. Since the venous network is a low-pressure system, the elastic membranes that are found in arteries and arterioles are not found in venules and larger veins. Veins also contain valves made up of flaps of endothelial cells which prevent the backflow of blood, thus keeping the deoxygenated blood always moving towards the heart. Typical venules range in size from 20-200 µm and have flow velocities which range from 2-3 mm/s. The ordered structure and normal function of the microvascular network described above is different from the microvascular network formed during tumor development.

<table>
<thead>
<tr>
<th>Microvessel Type</th>
<th>Diameter (µm)</th>
<th>Velocity Range (mm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteriole</td>
<td>20-200</td>
<td>2-15</td>
</tr>
<tr>
<td>Venule</td>
<td>20-200</td>
<td>2-3</td>
</tr>
<tr>
<td>Capillary</td>
<td>7-15</td>
<td>0.5-1.5</td>
</tr>
</tbody>
</table>

**Table 1.1:** Approximate physical specifications for microvasculature (arterioles, venules and capillaries).

As a cancer develops from a single neoplastic cell to a deadly tumor it undergoes a number of genetic mutations which increases its reproductive and survival capability, including self sufficient growth signaling, insensitivity to antigrowth signaling, unresponsiveness to apoptotic signaling and finally the ability to replicate indefinitely [1]. However, during this progression there is another critical transition which a tumor must undergo in order to evolve into a highly lethal phenotype: it must recruit and maintain its own blood supply. This need is driven by the simple fact that oxygen is only able to diffuse about 100 µm into tissue (at sea level) [8]. Thus as an unvascularized tumor
grows beyond this limiting size there is a fraction of cells which receive an insufficient supply of oxygen; a state referred to as hypoxia. If a tumor is unable to recruit a blood supply, then, as the tumor continues to grow, cellular apoptosis will balance proliferation and tumor growth stops. At some point these tumors may activate their “angiogenic switch” in response to their hypoxic conditions and recruit nearby blood vessels through the release of pro-angiogenic factors. These factors include hypoxia inducible factor (HIF) and vascular endothelial growth factor (VEGF) [9].

During normal angiogenesis VEGF binds to the endothelial cells of preexisting blood vessels which initially causes them to dilate and become leaky [10]. VEGF also activates a signaling cascade that results in numerous other factors being released. This cascade subsequently leads to breakdown of the basement membrane, proliferation and migration of endothelial cells, and finally vessel maturation through formation of a new basement membrane.

The vascular network formed during tumor angiogenesis differs from the network formed in normal tissues due to the large over-expression of VEGF and other pro-angiogenic compounds. This over-expression leads to a vascular network that is both structurally and functionally abnormal in many ways (Table 1.2). Structurally, the vascular network loses its defined hierarchy of arteries, capillaries and venules and is replaced with a chaotic structure and a haphazard pattern of interconnection, an example of which is shown in Figure 1.2. The vessels also become abnormally shaped, with regions of dilation and constriction appearing randomly. Endothelial cells divide excessively during tumor angiogenesis, leading to growth of irregular shaped and tortuous vessels [11]. Regions of tumor vessels may also have excessive coverage of endothelial cells, while other regions may show insufficient coverage and only a partially developed basement membrane [12]. These structural abnormalities also give rise to functional differences, most notably the hyper-permeability of tumor vessels and uneven tumor perfusion. Additionally, in normal tissue there is a gradient of pressure from inside the blood vessel to outside which is maintained by proper endothelial cell coverage and an intact basement membrane.
Figure 1.2: Scanning electron microscope polymer corrosion cast of microvasculature from a human tumor implanted in nude mouse (adapted from [7]).

Within tumors the hyper-permeability of vessels may cause interstitial fluid pressure to build up and become comparable to the vascular pressure. As the delivery of drugs to regions of the tumor with high interstitial fluid pressure and/or low perfusion may be inhibited [13] by this abnormal vasculature, the resulting functional abnormalities have important implications for the effectiveness of treatments that are delivered through the vasculature or are based on oxygen dependent mechanisms.

<table>
<thead>
<tr>
<th>Normal Vasculature</th>
<th>Tumor Vasculature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organized</td>
<td>Disorganized</td>
</tr>
<tr>
<td>Evenly distributed</td>
<td>Unevenly Distributed</td>
</tr>
<tr>
<td>Uniformly shaped</td>
<td>Twisted</td>
</tr>
<tr>
<td>Non-permeable</td>
<td>Leaky</td>
</tr>
<tr>
<td>Vascular pressure &gt; Interstitial pressure</td>
<td>Vascular pressure ~ Interstitial pressure</td>
</tr>
<tr>
<td>Properly matured</td>
<td>Immature</td>
</tr>
<tr>
<td>Supporting cells present (pericytes)</td>
<td>Supporting cells absent</td>
</tr>
<tr>
<td>Appropriate membrane expression</td>
<td>Inappropriate membrane expression</td>
</tr>
<tr>
<td>Independent of cell survival factors</td>
<td>Dependent on cell survival factors (VEGF)</td>
</tr>
</tbody>
</table>

Table 1.2: Structural and functional differences between normal and tumor vasculature. (Adapted from [10])
The abnormal vasculature and its formation represent potential therapeutic targets for anti-vascular and anti-angiogenic therapies. Anti-vascular therapies rapidly coagulate the vasculature in and surrounding the tumor to effectively cut off oxygen and nutrient supply, causing cell death. Anti-angiogenic therapies on the other hand target the molecular pathways that stimulate angiogenesis in attempt to inhibit endothelial cell proliferation and force the tumor into a state of dormancy where cell death balances cell proliferation. Of particular interest is the anti-vascular treatment photodynamic therapy (PDT).

The development of PDT as a clinical tool began in earnest in the 1960’s with studies by Lipson and Baldes at the Mayo clinic [14]. PDT induces cell death primarily through the generation of singlet oxygen by photoexcitation of a photosensitizer. Thus, an effective PDT treatment requires the presence of oxygen, photosensitizer and light in sufficient quantity within the treatment region to achieve cell kill [15]. This constraint and the ability to accurately control the deposition of light makes it possible to treat highly localized regions of interest and avoid collateral damage to adjacent structures. PDT has been shown to be effective in the treatment of solid tumors, dysplasia and age related macular degeneration [16, 17]. An important aspect of PDT is the uptake of the drug, specifically whether the drug is predominantly found in the vasculature (vascular targeted) or has extravasated into the tissue bed (cellular targeted) at the time of light exposure. Vascular targeted PDT acts by causing endothelial cell damage, increased vessel permeability, thrombus formation and vasoconstriction [18]. These microvascular changes induce an ischemic state within the tumor, resulting in cell death through apoptosis or necrosis in the days following therapy. Standish et al. also demonstrated using 2 Dimensional Doppler OCT imaging techniques, that microvascular changes occur on the time scale of minutes during and immediately after PDT and that these changes are dependent on the fluence rate of light being delivered [19]. Further investigations by our group have also revealed that in some cases these rapid microvascular changes were predictive of local tumor necrosis [20]. However, these initial experiments only imaged a 15 µm × 1mm × 2 mm region of the treatment volume and thus only provided a very localized view of the PDT-induced vascular response. Additionally, the DOCT system used in these studies was not able to image blood flow.
below 500 µm/s, greatly limiting the observation of vascular changes in areas of slow flow such as arterioles, venules and the capillary network. The interesting and clinically relevant results from these initial experiments, although limited, indicate that vascular targeted PDT could indeed benefit from an improved 3D microvascular OCT imaging technique. Chapter 5 details this application, pursued through collaborations within our and other research groups.

While PDT is an interesting therapy it represents only one of a number of therapies that are in development which target the vasculature of tumors, the majority of which are anti-angiogenic therapies. In this context we have also applied our newly developed imaging techniques to longitudinal imaging of vascular response to an anti-angiogenic therapy that targets hypoxia inducible factor (HIF) in collaboration with Sufan et al. [21]. This study is also described in chapter 5 and helps to demonstrate the wider scope of applicability of the imaging techniques we are developing.

1.2 Overview of microvascular imaging techniques

The discussion in the previous section illustrates the need for high resolution imaging of tumor microvascular networks, primarily as a tool for imaging the response to anti-vascular and anti-angiogenic therapies. In order to help illustrate why currently available techniques are insufficient for the task we present a brief review of vascular imaging technologies.

Methods for imaging microvascular structure and/or function can be divided into two groups, microscopic methods and clinical methods. Microscopic methods provide high resolution but have a limited penetration depth or can only be performed ex vivo. Table 1.3 summarizes these methods and their applicability to microvascular imaging.
<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Approximate Resolution</th>
<th>Penetration Depth</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning Electron Microscopy</td>
<td>10 nm</td>
<td>Surface Only</td>
<td>- Vascular casting in excised tissues</td>
</tr>
<tr>
<td>Histochemical Staining</td>
<td>1µm</td>
<td>5µm</td>
<td>- Stains for CD31 positive cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Assess functionality through injections of fluorescent markers prior to tissue fixation</td>
</tr>
<tr>
<td>Confocal Microscopy</td>
<td>1µm</td>
<td>500µm</td>
<td>- Intravital imaging in limited set of preclinical models using fluorescently labeled dextran's</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Retinal imaging clinically using fluorescein</td>
</tr>
<tr>
<td>High Frequency Ultrasound</td>
<td>50µm</td>
<td>5mm</td>
<td>- Intravital imaging in preclinical models</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Clinical imaging of retina and intravascular applications</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Doppler, Power Doppler and microbubble contrast to assess blood flow, perfusion</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>250µm</td>
<td>20cm</td>
<td>- Doppler, Power Doppler and microbubble contrast to assess blood flow, perfusion</td>
</tr>
<tr>
<td>Computed Tomography</td>
<td>500µm</td>
<td>Whole Body</td>
<td>- Iodinated contrast agents can be used to measure blood volume in the brain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- µCT in preclinical models provides resolution down to ~10µm</td>
</tr>
<tr>
<td>Magnetic Resonance Imaging</td>
<td>1mm</td>
<td>Whole Body</td>
<td>- Gadolinium and Super-paramagnetic based contrast agents to measure blood volume</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- µMRI in preclinical models provides resolution down to ~10µm</td>
</tr>
<tr>
<td>Positron Emission Tomography</td>
<td>5mm</td>
<td>Whole Body</td>
<td>- Blood volume measurements made using radioactive isotopes of carbon monoxide</td>
</tr>
</tbody>
</table>

**Table 1.3**: Imaging modalities used in vascular imaging, approximate specifications and capabilities.

Microscopic methods include scanning electron microscopy (SEM), confocal and multi-photon confocal microscopy. SEM is performed by first casting the vascular network with a polymer which hardens inside blood vessels. The tissue is subsequently eroded away and imaged with a scanning electron microscope, which yields 3D information on vessel dimension, branching and tortuosity (defined as the ratio of the distance between
two points in the vascular network measured along the blood vessel and the corresponding geometric distance between the two points). In one recent study this technique was used to assess the efficacy of the anti-angiogenic agent ZD6474 for the treatment of an orthotopically implanted renal cell carcinoma model [22]. The study demonstrated a decrease in vascular density and tumor volume in treated tumors relative to untreated controls. Interestingly this study also showed that tumors which were administered treatments of ZD6474 immediately after implantation had a vascular phenotype (characterized using metrics for intravascular distance, vessel diameter and branching distance) resembling that of normal kidney, whereas tumors which received treatments starting 10 days after implantation had a vascular phenotype more closely resembling the untreated control group. Representative images are shown in Figure 1.3.

Studies like this help demonstrate the power of SEM as a tool to assess microvascular morphology. However, it also comes with some practical limitations, specifically: (1) SEM cannot be performed longitudinally (2) Scanning electron microscopes are costly and not widely available.

![Figure 1.3: Scanning electron microscope images of corrosion casts of tumor microvasculature, adapted from [22]. (a) untreated tumor (b) treated tumor with anti-angiogenic therapy ZD6474 beginning 1 day after implantation (c) treated tumor with anti-angiogenic therapy ZD6474 beginning 10 days after implantation.](image)

Much more practical and more widely available than SEM are light based microscopes. These can be used in a limited set of in vivo preclinical models (mouse ear, retinal imaging, dorsal and cranial window chamber models) to perform longitudinal microvascular imaging. Specifically, intravital multi-photon confocal microscopy (MPCM) can be used in conjunction with fluorescently labeled dextrans to obtain 3D mages of the microvascular network. This technique has been used in a number of preclinical studies involving anti-VEGF and other anti-angiogenic therapies to evaluate vascular
response and effectiveness [23-25]. A typical example of an intravital fluorescence confocal image from mouse skin is shown in Figure 1.4. Standard metrics used to quantify vascular networks obtained with MPCM are vascular density, vessel diameter, branching points and vessel tortuosity. MPCM, like SEM, suffers from a number of drawbacks that makes it impractical for large preclinical studies (1) it is time consuming; scanning a 5mm x 5mm region takes over one hour (2) fluorescent dye leaking out of tumor vasculature making repeated longitudinal vascular imaging difficult and (3) limited depth of penetration (<400um).

Figure 1.4: FITC dextran fluorescence confocal image of microvasculature from normal mouse skin.

Due to the limitations of intravital multi-photon confocal microscopy and the limited number of suitable animal models, the vast majority of preclinical studies involving measurements of angiogenic response still rely on animal sacrifice followed by histochemical staining with CD31 or von Willebrand factor (VWF), and quantitative analysis using metrics such as Mean Vascular Density (MVD, number of vessel per unit area) and Luminal Vascular Area (LVA, which is the cross sectional area of vasculature in the stained histological section) . Functionality of blood vessels can also be assessed using intra-vital tracers (fluorescent lectins) that are injected into the circulatory system prior to tissue fixation. Figure 1.5 shows an example of xenograft tumor that has been sectioned and stained for CD31: image (a) represents an untreated tumor, whereas (b) is from a tumor that has been treated with an anti-VEGF therapy [26]. The obvious limitation of histochemical staining techniques is that only a single time point or snapshot of the vascular network is obtained and the longitudinal effects of disease progression and response to therapy cannot be followed in a single animal.
Furthermore, 3D metrics to quantify the vascular structure are difficult to obtain from a stack of histological sections, primarily due to physical processing artefacts.

Figure 1.5: CD31 labeled histological section of untreated and anti-VEGF treated tumor, adapted from [26]. Brown regions represent CD31 positive endothelial cells, a few vessels are pointed to with arrows. Treated tumor shows lower CD31 staining.

Clinical imaging methods such magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET) and ultrasound (US) and high frequency ultrasound (HFUS) all have the ability to longitudinally image deeply seated tissue, but lack the spatial resolution to actually resolve microvasculature in the 10-50 μm diameter range and, therefore, are unable to assess the full 3D structure. However, each of these methods does have the capability to make either perfusion (moving blood volume) or static blood volume measurements through the use of various contrast agents. MRI macromolecular contrast agents such as superparamagnetic iron oxide particles [27] and gadolinium-labeled albumin, dendrimers or liposomes can be used to estimate fractional plasma volume [28]. CT can be used with iodinated contrast agents to measure cerebral blood volume [29]. However, due to the low molecular weight of the iodinated contrast agents, there is rapid diffusion into the surrounding tissue, making measurements inaccurate in the absence of a blood brain barrier. PET can be used to measure blood volume by inhaling small amounts of carbon monoxide composed of radioactive isotopes $^{11}$C or $^{15}$O [30]. The carbon monoxide permanently binds to red blood cells, which distribute according to the vascular volume. US and HFUS can be used in conjunction with microbubbles composed of a hydrophobic shell that encapsulates various types of perfluoro hydrocarbon gas [31]. The microbubbles can be burst using high intensity ultrasound, yielding a strong transient signal that reflects the
microvascular perfusion. Additionally a number of different nonlinear imaging techniques for these microbubble contrast agents, of which an example is shown in Figure 1.7, are being developed to non-destructively ascertain perfusion [32].

Both microscopic and clinical imaging techniques have limitations when it comes to imaging subsurface microvasculature \textit{in vivo} with high resolution. In the following section I present an introduction to the Doppler optical coherence tomography imaging technology that aims to bridge the gap between microscopic and clinical methods by providing high resolution (∼10μm) imaging to depths of several millimeters and which has the capability of detecting microvasculature \textit{in vivo}. Figure 1.8 shows how the various imaging modalities fit together in terms of resolution and depth of penetration and how OCT fills a gap between the microscopic and clinical imaging methods, specifically confocal microscopy and high frequency ultrasound.

Figure 1.7: High frequency ultrasound image demonstrating microbubble contrast enhancement (green) of microvasculature in a hind limb xenograft tumor model, adapted from [33].
Figure 1.8: Schematic diagram depicting the tradeoff between imaging depth and resolution encountered in medical imaging (Resolution elements not to scale).

1.3 Doppler optical coherence tomography

Time domain Doppler optical coherence tomography (TD-DOCT) is an optical imaging method based on low coherence interferometry that is capable of achieving ~10μm isotropic resolution and flow sensitivity of ~500 μm/s. This technique was first applied to biological systems by Fercher et al. in 1990 to image the retinal pigment epithelium [34]. Fujimoto et al. introduced the first fiberoptic-enabled TD-OCT system for imaging in non-ocular applications in 1991[35]. The price for this high resolution and flow sensitivity is a limited penetration depth of 2-3 mm (but still approximately one order of magnitude better than fluorescence confocal microscopy, which typically only penetrates ~400 μm). However, a significant advantage of TD-DOCT, when compared to other microscopic imaging techniques, is that it is a fiberoptic based imaging method. Therefore, in vivo imaging, such as the luminal surface of the upper and lower
gastrointestinal tract and lung are accessible with endoscopes [36, 37], along with solid organ imaging through minimally invasive interstitial probes [38-40].

There are several similarities between TD-DOCT and Doppler ultrasound, as many image processing techniques used in TD-DOCT were originally developed for ultrasound. Instead of measuring backscattered pressure waves, TD-DOCT measures backscattered photons by interferometry, avoiding the difficult task of directly measuring the time of flight of the backscattered light. A Michelson interferometer is the most commonly configuration used in TD-DOCT and is displayed in Figure 1.9. A beam splitter divides the light equally with 50% incident on the sample arm mirror and 50% incident on a reference arm mirror. The light is reflected from the two mirrors, producing constructive or destructive interference depending on the optical path length differences in the reference and sample arms. If the sample mirror is replaced by a biological specimen, the intensity of light waves that interfere at the detector will be proportional to the local reflectivity in the sample. By scanning the reference mirror along the optical beam, the reflected intensity will then be proportional to the local reflectivity of the sample, as a function of depth, producing an axial or A-scan. A 2D image, known as a B-scan, can be created if the sample arm is then translated laterally, providing a subsurface structural image of the sample. In order to achieve spatial localization, a broadband source is used to produce interference patterns only detectable within the coherence length of the light, defined as:

$$l_c = \frac{2 \ln 2 \lambda_0^2}{n m \Delta \lambda}$$  \hspace{1cm} (1.1)

where $\lambda_0$ is the center wavelength of the source, n is sample index of refraction, and $\Delta \lambda$ is the spectral bandwidth of the source, assuming a Gaussian shaped spectrum [41].
**Figure 1.9**: Simplified schematic of a TD-OCT system. Light from the low coherence light sources is split between the two arms of the Michelson interferometer. Half directed to the translating reference arm and half to the tissue being probed. The coherence properties of the light source and the position of the reference arm determine the depth of tissue probed.

A B-mode structural image from African clawed tadpole heart is shown in Figure 1.10 and the displayed signal, $S$, was calculated using

$$S = \sqrt{\frac{1}{NM} \sum_{n=1}^{N} \sum_{m=1}^{M} I_{n.m}^2 + Q_{n.m}^2}$$  \hspace{0.5cm} (1.2)$$

Here $I$ and $Q$ are the In-phase and Quadrature components of the coherently demodulated detector signal and the indices $m$ and $n$ represent the lateral averaging index and axial averaging index respectively. The image is that of the ventricle and the outflow tract of the heart called the *Truncus Arteriosis*.
Figure 1.10: In vivo structural OCT image of the heart of the Xenopus Laevis tadpole. The ventricle (V) pushes blood from the heart out through the Truncus Arteriosus (TA) to the rest of the embryo.

By acquiring two A-scans taken at the same position at slightly different times, phase shifts induced by scatterer particles moving with a component in the direction of the optical beam may be detected using autocorrelation analysis. To perform the autocorrelation analysis $N$ consecutive A-scans ($N$ is referred to as the packet length in the ultrasound literature) are acquired from approximately the same location. A simplified situation of a single scatterer moving in the direction of the optical axis is shown in Figure 1.11a. A schematic of a subset of the $N$ a-scans acquired as the scatterer moves within the beam, at depth indicated by the dashed line, is shown in Figure 1.11b. The sampled signal traced out along the dashed line is called the slow time signal. The phase shift between any two samples can be used estimate the frequency of the slow time signal, which is referred to as the Doppler frequency, $f_d$, in ultrasound. Typically, using a larger number of samples ($N=16$ or 32) improves the estimate of the phase shift. The frequency of the slow time signal is related straightforwardly to the phase shift through the equation,

$$f_d = \Omega f_s$$  \hspace{1cm} (1.3)

where $\Omega$ is the measured phase shift and $f_s$ is the A-scan rate (assuming a linear change in time over the time interval $\Delta t=1/f_s$).
Figure 1.11: Schematic representation of how Doppler detection is performed in OCT, adapted from [42]. (a) single scatterer moving in the optical beam with a velocity $V$. (b) Schematic representation of the acquired OCT A-scans from the single scatterer in (a) as it moves. (c) Slow time axis signal generated by sampling the set of A-scans in (b).

In the more general situation, where the particles are moving at an angle $\theta$ relative to the optical axis, depicted in the Figure 1.12, only the component of the velocity along the optical axis is detected. In this situation the measured phase shift can be related to the flow velocity,

$$\langle V \rangle = \frac{\lambda_0 \Omega f_s}{4n \cos(\theta)}$$

(1.4)

where $V$ is velocity of the particle, $n$ is the index of refraction of the sample and $\theta$ is the Doppler angle (the angle between the direction of flow and the optical axis). This method of analysis gives rise to the color Doppler images commonly seen in clinical ultrasound and has been extended to OCT flow measurement techniques [42].
Figure 1.12: Coordinate system for Doppler OCT measurements. The red structure represents a blood vessel making an angle $\theta$ relative to the optical beam (blue).

This strong angle dependence creates serious problems when trying to perform 3D reconstructions of tumor vascular networks from color Doppler data, as the angle between the optical axis and direction of flow is highly variable.

In practice the Doppler frequency is most commonly estimated using the Kasai velocity estimator,

$$f_D = \frac{f_a}{2\pi} \operatorname{arctan} \left( \frac{1}{M(N-1)} \sum_{m=1}^{M} \sum_{n=1}^{N-1} (Q_{m,n+1} - Q_{m,n+1}) \left( I_{m,n+1} + I_{m,n} \right) \right)$$

Here $I$ and $Q$ are the In-phase and Quadrature components of the coherently demodulated detector signal and the index $m$ is the axial averaging index. The index $n$ represents the direction (lateral), in which the autocorrelation analysis takes place, which is why there are cross terms in both the numerator and denominator of the form $X_n Y_{n+1}$. This estimator provides a computationally efficient way to estimate the Doppler shift over a packet length of $N$ A-scans [42]. The corresponding Doppler image to the structural image shown in Figure 1.10 is shown in Figure 1.13.
Figure 1.13: Corresponding Doppler flow image from Figure 1.10 showing blood being ejected out of the Truncus Arteriosis (TA) in the Xenopus Laevis tadpole heart. To the right of the Truncus Arteriosis is the ventricle (V).

To estimate the SNR limited Doppler noise floor, consider the simple theoretical model, which defines the SNR as

\[ SNR = \left( \frac{|E|}{|n|} \right)^2 \]  \hspace{1cm} (1.6)

Here \( \vec{n} \) is a random noise vector and \( \vec{E} \) is the reflected electric field. In the regime where we have good SNR (\(|E| >> |n|\)), the standard deviation of the phase shift between two A-scans is given by [41],

\[ \sigma_{\Delta \phi} = \frac{1}{\sqrt{SNR}} \]  \hspace{1cm} (1.7)

The SNR limited phase noise is the limiting phase shift that can be measured between two A-scans, and for optimal Doppler detection, the system should always be operating in this regime. However, even when operating in the SNR limited regime and using large packet lengths (\(N=16\) or \(32\) A-scans), the typical in vivo minimum detectable velocity for TD-OCT is only on the order of \(500 \mu m/s\) for B-mode imaging [43-45]. This is clearly not adequate when attempting to detect the slow microcirculation of tumors. In chapter 3 we discuss the theory and the construction of a new type of OCT system.
known as swept source OCT. SS-OCT provides higher SNR than TD-OCT systems making it a better choice for phase sensitive imaging in general.

Another important factor that contributes, in part, to the DOCT noise floor is the fact that the majority of OCT systems use only a single linearly-swept optical scanning element to generate B-mode images. This introduces a lateral displacement between each A-scan during B-mode imaging, which increases the Doppler noise floor [43]. Standard protocols to minimize these effects require very large amounts of oversampling, which reduces frame rate of the system [46, 47]. Contrast this to ultrasound systems which use multi-element transducers to acquire multiple A-scans from exactly the same positions while maintain imaging frame rates of 30fps.

Another practical issue is that of motion artefacts. These can make it difficult to extract regions of blood flow from tissue which is moving. Methods to reject phase shifts from moving tissue are known as clutter rejection techniques and have previously been discussed in the OCT literature [47, 48]. A larger problem is that, since 3D microvascular maps are generated from a stack of 2D B-mode images, motion artefacts can cause misalignment errors between frames which end up distorting the true 3D structure of the microvascular network.

1.5 Thesis Motivation: Development of a high resolution OCT toolkit capable of visualizing normal and tumor 3D vascular structure

3D microvasculature maps provide additional biological, and potentially important, information when compared to their 2D counterparts, since metrics such as tortuosity, vessel diameter and branch points can be quantified. However, 3 major limitations, alluded to in the previous section, inhibit our ability to generate these 3D maps in vivo when using OCT. Specifically, they are,

1. Motion artefacts that cause difficulty in 3D reconstruction, such as discontinuities in vessel structure and high background phase noise.
2. The angle dependence of Doppler detection makes it difficult to image highly tortuous vessel structures.

3. Finite velocity noise floor of conventional Doppler OCT techniques makes it difficult to image below ~500 µm/s.

The following 4 chapters present the work conducted to overcome these obstacles and the application of these techniques to biological problems for which knowledge of the microvascular network is important.

Chapter 2 details our implementation of a retrospectively-gated OCT imaging systems for the removal of periodic motion artefacts in studies of embryonic cardiovascular development.

Chapter 3 outlines the construction and characterization of a high speed swept source OCT system that has SNR limited phase noise up to 50 dB. The high imaging speed of the system also leads to a direct decrease of non-periodic motion artefacts in 3D reconstructions.

Chapter 4 details the implementation and optimization of speckle variance imaging, which is both an angle and velocity independent technique for high resolution 3D microvascular reconstruction in vivo.

Chapter 5 details our in vivo biological experiments using the systems and algorithms developed. Specifically, monitoring vascular response to one and two photon photodynamic therapy and targeted degradation of HIF-α.

Chapter 6 concludes this work with a summary and the future directions for further improvement and increasing the utility of techniques developed.
Chapter 2: Retrospectively Gated OCT

3D microvasculature maps provide extensive clinical and biological information when compared to their 2D counterparts, as 3D aspects such as the tortuosity of the vascular network can be assessed. Motion during the acquisition of 3D volumes causes artefacts in the reconstructed vascular networks, such as discontinuities and artificial changes in vessel morphology. We can subdivide motion artefacts into two categories: (1) periodic motion artefacts, such as those due to breathing and the heart beat (2) non-periodic motion artefacts such as muscular spasm. In this chapter we discuss the implementation of a retrospectively gated DOCT system for small animal imaging that is able to mitigate periodic motion artefacts due to heart beat [49]. This was the first such system to be built in the OCT field. As a proof of principle experiment the technique was used to generate 4D reconstructions at 45 volumes per second, of a *xenopus laevis* heart, a commonly used model in developmental biology. This technique was also used to generate high temporal resolution blood flow maps in the *xenopus laevis* heart at up to 1000 fps.

2.1 Design concept

The retrospectively gated DOCT system consisted of two main components, the DOCT imaging system and the gating system. The core component of the DOCT imaging system was a commercially available 16 kHz swept source laser (Thorlabs, SL1325-P16). The theory behind swept source (SS) OCT is discussed in detail in chapter 3. The SS-DOCT system acquired the structural and color Doppler B-mode images from the *xenopus laevis* tadpole, these images could then be retrospectively gated using a simultaneously acquired cardiogram signal. However, obtaining an electrocardiogram from a tadpole is extremely difficult primarily due to the requirements for electrode placement. Therefore, a Doppler optical cardiogram (DOC) was designed and implemented. The DOC provided a novel method of gating the highly periodic flow and tissue motion in the cardiovascular system and was generated by using a second time-domain DOCT system that had a 13 kHz A-scan rate. A small fiber optic probe, coupled
to the sample arm of the DOC system’s interferometer, was placed directly over one of the major arteries near the tadpole heart so that a high temporal resolution M-mode signal of the blood flow dynamics could be acquired. The M-mode signal was subsequently processed to generate a cardiogram and used to gate the images acquired by the 16 kHz SS-DOCT imaging system. The details of the system, implementation of Doppler algorithms and the results of in vivo experiments were published as,


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Doppler optical cardiology gated 2D color flow imaging at 1000 fps and 4D in vivo visualization of embryonic heart at 45 fps on a swept source OCT system

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Abstract: We report a Doppler optical cardiology gating technique for increasing the effective frame rate of Doppler optical coherence tomography (DOCT) when imaging periodic motion as found in the cardiovascular system of embryos. This was accomplished with a Thorlabs swept-source DOCT system that simultaneously acquired and displayed structural and Doppler images at 12 frames per second (fps). The gating technique allowed for ultra-high speed visualization of the blood flow pattern in the developing hearts of African clawed frog embryos (Xenopus laevis) at up to 1000 fps. In addition, four-dimensional (three spatial dimensions + temporal) Doppler imaging at 45 fps was demonstrated using this gating technique, producing detailed visualization of the complex cardiac motion and hemodynamics in a beating heart.

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References and links

1. Introduction

Optical coherence tomography (OCT) is a high resolution ($1 - 10 \mu m$) imaging technique capable of visualizing structures to a depth of 2-3 mm in soft tissues. The high spatial resolution provided by OCT has been used to study the developing cardiovascular system of various models in developmental biology including Drosophila (fruit fly) [1], Xenopus laevis (African clawed frog tadpole) [2-5], chick [6, 7] and mouse embryos [7, 8]. Doppler OCT (DOCT) is a functional extension of OCT, analogous to Doppler ultrasound, that can provide a flow sensitivity down to 10-100 $\mu m/s$ [9-12]. DOCT imaging of Xenopus embryos has been demonstrated at up to 16 frames per second (fps) [4]. In rodent models the heart rate can range from 200 to 500 beats per minute, which makes high temporal resolution a necessity for accurate characterization of cardiovascular function and development. To date, the highest in vivo structural OCT frame rate has been 360 fps [13]. High frame rate color Doppler flow imaging in OCT is very challenging due to the increased Doppler noise floor that accompanies the higher scanning optics velocities [14]. In Doppler ultrasound, this effect is negated through the use of arrayed transducers and electronic scanning such that no physical scanning motion is required. The concept of fiber array OCT systems has previously been described [15], but its use in DOCT has yet to be demonstrated.

The first demonstration of artificially increasing the imaging frame rate for OCT was by Yanzizafar et al., who used a retrospective gating technique on the structural signal [3] to generate two-dimensional structural and color Doppler images from a time domain OCT system with a slow delay line frequency of 8 Hz. Jenkins et al. have used a prospective gating technique based on electrical pacing of excised hearts of chick and mouse embryo models to allow ex vivo 4D structural imaging at 16 fps [7]. Most recently, in the related field of high

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frequency ultrasound (~50 µm resolution), Cherin et al. have performed *in vivo* structural and colour Doppler flow imaging of the mouse heart at 1000 fps using a retrospective gating technique based on a simultaneously acquired electrocardiogram (ECG) [16].

The retrospective gating techniques demonstrated by both Yanzafar and Cherin have the potential to increase the frame rate for color Doppler flow imaging while maintaining an acceptable noise floor. In this paper, we demonstrate a retrospective gating technique based on a Doppler optical cardiology as it proved difficult to obtain an ECG signal from the *Xenopus*. In addition for *in utero* mouse embryos, obtaining ECG signal would be problematic due to the difficulty in electrode placement and interference from the maternal heart or surrounding sibling’s embryonic hearts. We demonstrate 1 kHz frame rates for both structural and Doppler images of the *Xenopus* cardiac dynamics as well as 4D (3D spatial + temporal) imaging at 45 fps. This was accomplished on a swept source (SS) OCT system, while using a separate Time Domain (TD) DOCT to provide the Doppler optical cardiology.

2. Methods and materials

2.1 SS-DOCT system hardware

The Swept Source Doppler OCT (SS-DOCT) system is based on a commercially available frequency swept source (Thorlabs, SL1325-P16) that is centered at 1325 nm, with a FWHM of the optical spectrum of >100 nm, and an average output power of 12 mW. Further details about the swept source can be found in Ref. [17]. The OCT interferometer is based on a 90/10 Mach-Zehnder interferometer (MZI) where 90% of the power goes to the sample arm and 10% to the reference arm. The interference fringe signal is measured by a 15 MHz balanced photodetector (Thorlabs, PDB140C). Both the MZI clock signal and the interference fringe signal are digitized at 50 MS/s with 14-bit A/D resolution. The interference fringe data is recalibrated by using the MZI clock signal as described in Ref. [17] yielding ~1024 points per laser sweep. The SS-OCT system also outputs two waveforms, through a 16-bit digital to analog card (National Instruments, PCI-6731), to drive the XY galvanometer and scanning mirror assembly (Cambridge technology, 6210). The TD-OCT system is triggered by the start of the scanning driver waveform from the SS-DOCT system and streams the optical cardiology data continuously to the hard drive. The SS-DOCT system acquires time-stamped B-mode structural images at 25 fps and streams the raw data to disk at the same time. Real time Doppler OCT imaging is performed at 12 fps using unidirectional wavelength sweeps from the laser. This imaging rate allowed for real-time feedback, which was required for tadpole positioning and visualization of breathing motion artifacts. Each B-mode image consisted of 512 A-scans per image. Prior to *in vivo* *Xenopus* cardiac imaging, the phase stability of the SS-OCT system was measured.

2.2 Doppler optical cardiology hardware

A schematic of the imaging setup is shown in Fig. 1. The TD-OCT system has been previously described with a Michelson interferometer and a rapid scanning optical delay (RSOD) line at a 12.95 kHz resonant frequency [4]. The light source (Inphirex, IPSDM-1325) is centered at 1310 nm with ~55 nm full width half maximum (FWHM) bandwidth and an output power of 22 mW. The interference signal was measured with a 10 MHz dual balanced photodetector (New Focus, 2117-FC). A 4.3 MHz electro-optic phase modulator is placed in the delay line providing stability for phase measurements while also providing the carrier frequency for in-phase (I) and quadrature (Q) demodulation. The II and Q signals were digitized with a 10 MHz data acquisition card (DAQ) (National Instruments, PCI-6115). The optical cardiology was obtained via a previously described GRIN fiber imaging probe [18, 19].
2.3 Structural and Doppler signal processing

The results presented in this paper used only the forward wavelength (short to long) sweeps of the laser. Similar results can be produced from analyzing the backward wavelength sweeps of the laser. The recalibrated interference fringe pattern \( \hat{F}(k) \) can be processed to obtain both the structural and Doppler information encoded in the raw data. The Fourier transform of \( \hat{F}(k) \) gives the complex signal \( I(y) + iQ(y) \). The structural image can be obtained by calculating the magnitude of the complex signal. A sliding window spatial averaging mask for the structural images defined in Eq. (1), is used to improve signal-to-noise (SNR) as has been described previously [4]. Briefly, \( M \) defines the size of the mask in the axial direction while \( N \) defines the size of the spatial averaging mask in the transverse direction. The B-mode structural images shown in this paper were generated using \( N=4 \) and \( M=2 \).

\[
\left\langle S^2 \right\rangle = \frac{1}{MN} \sum_{m=1}^{M} \sum_{n=1}^{N} \left[ I_{m,n}^2 + Q_{m,n}^2 \right]
\]  

(1)

The frequency shift induced by moving scatterers in the direction of the incident laser beam can be estimated using the Kasai autocorrelation function [20]. The Kasai autocorrelation function measures phase shifts between two adjacent A-scans and is shown in Eq. (2).

\[
\Delta \phi = \arctan \left( \frac{\frac{1}{M(N-1)} \sum_{m=1}^{M} \sum_{n=1}^{N} \left( I_{m,n} Q_{n,m} - Q_{m,n} I_{m,n} \right)}{\frac{1}{M(N-1)} \sum_{m=1}^{M} \sum_{n=1}^{N} \left( Q_{m,n} Q_{n,m} + I_{m,n} I_{m,n} \right)} \right)
\]  

(2)

The spatial averaging mask values used for all B-mode Doppler images acquired by the SS-D OCT system were \( N=8 \) and \( M=2 \).

The phase shift induced by moving scatterers is displayed using a standard Doppler colormap with red and yellow indicating flow in the direction of the optical beam while blue and turquoise coloring indicate flow in the opposite direction in unaliased images. The maximum detectable phase shift without aliasing is \( \pm \pi \), due to the complicated blood flow profiles and the presence of noise no attempt is made to phase unwrap images. Furthermore, due to the difficulty in determining the Doppler angle in the complicated structures of the heart we do not relate the phase shift to the velocity of moving scatterers.

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2.4 Doppler optical cardiogram and retrospective gating

The RSOD frequency was measured at the beginning of each imaging session, allowing for accurate calculation of the elapsed time during data acquisition by counting the number of A-scans acquired. An example of the M-mode structural image through one of the two great vessels leaving the tadpole heart is shown in Fig. 2(a). The corresponding M-mode Doppler image is shown in Fig 2(b), the size of the averaging mask used to generate this image was N=13 and M=1. The pulsatile nature of the blood flow is clearly discernible in this image. Averaging the phase shift across the vessel shown in Fig 2(b) generates the cardiogram signal shown in Fig. 2(c). A low pass finite impulse response filter was used to remove the high frequency noise with the result demonstrated in Fig. 2(d). One cardiac cycle was defined by the region between the vertical dotted lines. This allowed for the calculation of the duration in each cardiac cycle. The mean and standard deviation of the *Xenopus* cardiac cycle duration during the entire acquisition were calculated, and cardiac cycles with variations greater than 1.75 standard deviations were rejected.

![Images of Doppler optical cardiogram M-Mode data collected from one of the two great vessels leaving the heart. Data was used to generate the optical cardiogram signal needed to retrospectively gate B-mode images.](image)

**Fig. 2.** Doppler optical cardiogram M-Mode data was collected from one of the two great vessels leaving the heart. Data was used to generate the optical cardiogram signal needed to retrospectively gate B-mode images. (a) Structural M-mode of great vessel. (b) Doppler M-mode of great vessel. (c) Raw optical cardiogram generated by averaging axially across the Doppler M-mode of the vessel. (d) Filtered cardiogram signal generated by filtering the raw signal with a 128th order finite impulse response low pass filter with 3dB cutoff at ~200 Hz and 80 dB stop band attenuation.

The overall system timing accuracy was 1 ms, and thus the highest frame rate achievable was 1000 fps for 2D movies. To achieve this maximum frame rate each cardiac cycle within the 1.75 standard deviation was divided into a number of equally spaced temporal bins determined by the mean cardiac cycle length over the image acquisition time of 2-3 minutes. For 1000 fps movies shown in section 3.2 the mean cardiac cycle length was approximately 800 ms and thus each cardiac cycle was divided into 800 temporal bins. B-Mode frames acquired by the SS-DOCT system were placed into the correct temporal bin using the time stamp attached to each frame. If multiple frames were found to originate from the same bin...
only the first frame was kept. The frames were then re-ordered according to their temporal bin location to achieve the higher effective frame rate.

To reconstruct the 4D structural and Doppler models, the tadpole heart was imaged at approximately 150 different spatial locations. Each position was imaged for 15 seconds after which the 2D scanner stepped 10 μm and the process repeated. During each 15 second acquisition the cardigram signal was also simultaneously collected with the TD-OCT system. An identical process to that described above was also followed for increasing the frame rate from 12 to 45 fps, using 36 temporal bins. The use of 36 temporal bin here was chosen as a trade off between good temporal resolution and performing manageable 4D reconstructions. The heart was then segmented in each of 36 different bins using the structural images. Masks were created to generate the surface reconstruction at each of the 36 bins as well as to segment the corresponding Doppler images.

2.5 Animal protocols

*Xenopus laevis* embryos were provided by Dr. Winklbauer in the Department of Zoology at the University of Toronto and were housed in standard conditions under an animal protocol approved by the institutional animal care committee at Princess Margaret Hospital, Toronto, Canada. Stage 51 tadpoles were anesthetized with 21°C (room temperature) Lidocaine solution diluted to 0.001% with tap water treated with Aqua Plus water conditioner. *Xenopus laevis* were allowed to sit in this solution for approximately 10 minutes before being placed in a shallow V-groove with a small amount of the solution. Images where then taken for approximately 1-2 hours and finally the animals were euthanized by anesthetic overdose.

In previous studies we have used 0.01% Tricaine as our anesthetic of choice. We chose Lidocaine in this study as a lower cost alternative. We compared its performance to Tricaine and found no significant degree of arrythmia under our experimental conditions since over an imaging time of 180 secs, the cardiac cycle variation was only 2%. This may be attributed to the species difference between mammalian and amphibian cardiac systems.

The stage 51 *Xenopus* heart is a three-chambered heart consisting of two atria and one ventricle. Unlike the four-chambered mammalian heart the amphibian heart mixes oxygenated (left atrium) and deoxygenated (right atrium) blood inside the single ventricle. This mixture is pumped out of the ventricle to the systemic capillaries and the lungs through the common outflow tract known as the truncus arteriosus. The oxygenated blood from the lungs then returns to the left atrium while the deoxygenated blood from the systemic capillaries returns to the right atrium.

3. Results

3.1 Phase stability results

Prior to *Xenopus* imaging it was necessary to characterize the phase noise properties of the SS-DOCT system. The results are summarized in Fig. 3 and indicate a factor of five improvement (for N=2) in the intrinsic phase noise as compared to our TD-OCT system [14], as shown in Fig. 3(a). However, the overall Doppler noise in the system was still dominated by speckle modulation and sample arm scanning noise, which increased as a function of frame rate as shown in Figs. 3(b) and 3(c). The TD-OCT system used in this study, exhibits phase stabilities as a function of frame rate, that are similar to those found in Ref. [14].
Fig. 3. (a). Normalized phase noise measured from a diffuse stationary reflector for various spatial averaging masks \( (M=1, N=2, 4, 8, 16, 32, 64) \). Each data set consists of measurements from 100,000 pixels and was fit with a Gaussian distribution. (b). Doppler noise floor measured in RMS when imaging stationary 0.5% Intralipid solution at 3, 6, 12, 24 fps, or equivalently at 0, 18, 36, and 72 mm/s lateral scanning speed. The resultant images were split into 20 regions, each with different SNR for the structural OCT signal. The Doppler noise was measured in each region and plotted against the SNR. Spatial averaging mask values were \( (M=1, N=16) \) for the 3 and 6 fps images and \( (M=1, N=8) \) for the 12 and 24 fps images. (c). At optimal SNR, the minimum Doppler noise floor plotted against lateral scanning speed. For the remainder of the paper, the imaging was performed at 12 fps or 36 mm/s scanning speed.

3.2 Color Doppler visualization at 1000 fps

An example of the original real-time 12 fps movie acquired by the SS-DOCT system is shown in Fig. 4. Structural intensity based thresholding of the Doppler images were performed in real-time to minimize Doppler noise, resulting in the movie in Fig. 4(b). This provided necessary feedback for proper positioning of the sample, identification of blood flow, and monitoring the tadpole for breathing motion during data acquisition to generate higher effective frame rate as described in Section 2.2.

Fig. 4. SS-DOCT imaging of *Xenopus laevis* heart with real-time acquisition and display at 12 fps. (a) Cross-sectional structural movie at the level of the aortic arches (Movie figure_4a.mov, 220 kB). Vessel walls and blood pumped through the vessels are clearly seen. (b) Doppler movie at the same position, demonstrating flow through the vessels and the wall motion (Movie figure_4b.mov, 448 kB). RAO: right aortic arch, LAO: left aortic arch and V: pulmonary/gill vessels.

The resulting 1000 fps movies are shown in Fig. 5. Gated imaging at effectively 1000 fps gave us the option to implement a sliding window temporal averaging scheme over several
frames to improve the SNR in both the structural and Doppler images. Such a high effective frame rate allowed detailed capture of the complex cardiac motion and hemodynamics during the heart cycle. Even more importantly, this technique will allow for proper imaging of the mouse cardiovascular system, where heartbeat can exceed 6 beats per second.

![Optical cardiogram gated movies at the same position as shown in Fig. 4 with an effective frame rate of 1000 fps, but played back at 30 fps. Sliding window temporal averaging was used to improve SNR. (a) Structural movie with a sliding window over 3 frames (Movie figure_5a.mov, 1783 KB). (b) Doppler movie with a sliding window over 5 frames (Movie figure_5b.mov, 1892 KB). Small artefacts (see discussion) contribute to the jittering motion, more prominently seen in the structural movie. Note the improved Doppler movie with a decreased noise level and more defined aliasing rings through the RAo and LAo. Notice the difference in Doppler shift between the blood flow and the vessel wall motion, both of which are present through out the cardiac cycle and visualized with high temporal resolution. Since the frame acquisition time is 83 ms, and the effective frame rate is 1000 fps, spatial-temporal artefacts are also present. These are demonstrated in (b), such as the right to left “flash” of red shifted Doppler frequencies in the vessel wall during the transition from late diastole to early systole, when the aortic arches move rapidly in the dorsal (downward) direction. The spatial-temporal artefacts are also depicted in Fig. 6, with data reconstruction in the x-t plane.

Furthermore, the left and right arches traverse through different angles (which changes during the cardiac cycle); these angles represent different incident Doppler angles to the optical axis. In addition, since the branching point moves into and out of the 2D imaging plane, with the downstream branches (especially of the right aortic arch) pointing in different directions, positive and negative Doppler frequencies are observed due to this diverging flow.

![Reconstructed 1000 fps data in the x-t plane, played back as movies through the y axis. (a) Structure movie (Movie figure_6a.mov, 4070 KB). (b) Doppler movie (Movie figure_6b.mov, 5309 KB). LD: late diastole, ES: early systole, PS: peak systole, LS: late systole, ED: early diastole. Equal-temporal lines (dash) every 100 ms are drawn. Note the spatial-temporal effects occur in the orientation parallel to the equal-temporal lines, as expected. Black arrow heads indicate the original 12 fps data acquisition time points, which are much more sparse when compared to the gated data. The fast hemodynamic changes during the cardiac cycle, especially during ES and PS phases, are better visualized with the gated data, demonstrating the rapid acceleration of flow from approximately 0 to 22 kHz (0 to 17.3 radians) Doppler shift within 60 ms.

The difference in flow pattern between the right and left aortic arches further exemplifies the need of 4D imaging to fully visualize and comprehend the complex nature of cardiac hemodynamics, even in a simple amphibian model.
The 1000 fps B-mode structural and Doppler images can be reconstructed to provide details in different planes. An example is shown in Fig. 6, where the data is presented in the x-t plane for better visualization of the temporal flow pattern, and appreciation of the different phases of the cardiac cycle.

3.3 Three dimensional + temporal (4D) imaging of the tadpole heart

Starting from the head end of the tadpole heart, we acquired SS-DOCT and Doppler optical cardiogram data as described in section 2.2 at different slice positions towards the tail. Three examples of the 45 fps structural and Doppler movies generated are shown in Figs. 7-9.

Fig. 7. Optical cardiogram gated structural movie (Movie figure_7a.mov, 1343 kB) at the level of truncus arteriosus (TA) branching into the left and right aortic arches (L Ao, R Ao) during early systolic (a) and peak systolic (b) phases of the cardiac cycle at 45 ms and 160 ms, respectively. The image acquisition is at 12 fps, the effective frame rate is 45 fps, and the movies are played back at 30 fps. The corresponding Doppler movies (Movie figure_7b.mov, 1789 kB) are shown in (a*) and (b*). These are shown without any threshold or spatial filtering to demonstrate the original system performance. Most of the noise in the Doppler background occurs at low structure intensity regions, which can be removed through simple thresholding performed in real-time.

Fig. 8. Optical cardiogram gated structural movie (Movie figure_8a.mov, 1362 kB) at the level of spiral valve (SV) and atrio-ventricular valve (AVv) during peak systolic (a) and diastolic (b) phases of the cardiac cycle at 160 ms and 775 ms, respectively. Imaging conditions are identical to Fig. 7 and the corresponding Doppler movie (Movie figure_8b.mov, 1809 kB) is shown in (a*) and (b*). While the truncus arteriosus (TA) is within the imaging plane for the entire duration, the central ridge of the SV is only visible during the systolic phase due to the complex motion of the TA (as shown in Fig. 10). The blood flow (F) around the central ridge of SV is clearly visible during systole. In diastole, the atrium (A) provides blood flow (open arrow) to the ventricle (V) through the AVv, which is more prominent during atrial contraction (AC).
Fig. 9. Optical cardiogram gated structural movie (Movie figure_9a.mov, 1370 kB) at the level of ventricle (V) and ventricular outflow tract (VOT) during systolic (a) and diastolic (b) phases of the cardiac cycle at 364 ms and 820 ms, respectively. Imaging condition is identical to Fig. 7 and the corresponding Doppler movies (Movie figure_9b.mov, 1802 kB) are shown in (a*) and (b*). During systole, the blood flow in the VOT can be visualized, which is continuous with that in the TA. Ventricle trabeculae (VT) are also visible during systole. In diastole, blood flows into the ventricle and fills the space in between the trabeculae. This is demonstrated by the inter-trabecular blood flow (ITBF) in the Doppler image.

Fig. 10. 4D surface reconstruction (Movie figure_10.mov, 14908 kB) of the tadpole heart demonstrating the complex cardiac motion, and the relative position of the various components (V: ventricle, TA: truncus arteriosus, RA: right atrium, RAo: right aortic arch, LAo: left aortic arch, LA: left atrium) of the heart.

The entire 4D data set can then be used for other visualization reconstructions to better illustrate the complex cardiac motion during a heart beat cycle. Figure 10 shows a surface reconstruction of the tadpole heart, rotating while beating, to demonstrate the dynamic anatomical relationships. For example, the truncus arteriosus (TA) oscillates over a significant distance relative to the right and left atria (RA and LA), which are situated more posterior. During each cardiac cycle, the TA not only changes position, but its shape and diameter also vary periodically to accommodate the output blood flow from the ventricle (V).
Internal to the heart, the blood flow pattern and the heart wall motion velocity can also be visualized at different positions of the same cardiac phase (Fig. 11) or at the same position during different phases (Fig. 12).

Fig. 11. Doppler shift within the tadpole heart during mid-systolic phase of the cardiac cycle, presented as a movie slice (Movie figure. 11.mov, 12811 kb) moving through the heart in the ventral to dorsal direction. The surface of the heart is rendered semi-transparent to demonstrate the complex blood flow pattern in 3D.

Fig. 12. Arbitrary oblique slice through the heart (Movie figure. 12.mov, 3839 kb) to demonstrate the advantage of 4D Doppler imaging data set. Here the data visualization plane is chosen to be perpendicular to the TA for the majority of the cardiac cycle. Blood flow through the SV and ITBF in the ventricle are shown to illustrate the complex blood flow pattern.

4. Discussion
The demonstration above reports the highest effective frame rate in OCT and DOCT imaging of embryonic heart samples in which the motion is periodic over 2 to 3 minutes of time. It is also the first demonstration of combined 4D structural and blood flow OCT imaging, where the full complexity of the embryonic heart motion and blood flow can be visualized in detail. The Doppler optical cardiogram provides a novel method of tracking the highly periodic flow and motion in the cardiovascular system. In situations where conventional ECG signals are difficult to obtain, such as mouse embryos in utero, it provides a relatively easy way to accomplish gated 2D, 3D and 4D reconstructions. The techniques presented are suitable for in utero high speed 4D imaging and can be extended to visualize mouse embryo hearts at frame rates over 300 fps with low Doppler noise background.
Using a second OCT probe to provide the Doppler optical cardiogram also offers the possibility of imaging the peripheral vascular system, away from the heart where periodical structural motion may not be prominent. Hence, in organs such as the brain or kidney, single channel OCT with structural gating technique [3] will not be sufficient for 4D imaging. Acquisition of the gating signal on the same SS-DOCT system is an attractive option and thus we are currently in the process of modifying existing systems to perform this function. Since most commercially available DAQ cards have multiple analog input channels, systems can be modified to capture both the cardiogram and the B-mode images using a two-interferometer setup. The use of booster semi-conductor optical amplifiers may be necessary if the laser output power is not sufficient.

During data acquisition there are a number of effects that can lead to degradation in the temporal resolution of the reconstructions. This degradation effect, appears as “jittering”, tends to increase as the duration of data acquisition increases. We attribute this degradation to: (a) environmental effects such as evaporation of water in V-groove can cause a drift of the specimen; (b) small variations of heart beat period due to anesthetics; (c) breathing motion of the tadpole; and (d) de-correlation between the two DOCT systems. The de-correlation is limited by the accuracy of the two computer system clocks and fluctuations in the RSOD and SS sweeping frequencies. These effects limit the extent to which frame rates can be increased without introducing a significant amount of jitter. We found that the best compromise between jitter and high frame rates occurred at approximately 200-300 fps, corresponding to an acquisition time of approximately 20-30 seconds with the current setup. Using a two-channel setup, one could limit the de-correlation effect and allow higher effective frame rates in the future.

The Doppler noise floor in the 12 fps real-time acquisition and display mode of the SS-DOCT used in this study can be improved by using a swept wavelength source with higher sweeping frequency to increase the A-scan density (i.e., A-scans/mm) for a given frame rate. In addition, frequency domain mode locking [13] will also improve the phase stability and subsequently the accuracy of Doppler shift estimation.

The spatial-temporal artefacts observed in the high frame rate movies can be removed by re-sampling the data in the direction parallel to the equal-temporal lines. An alternate approach is to stop the lateral scanning and simply acquire M-mode data in a point-by-point fashion in the x-y plane. The advantage of such an approach is that the Doppler noise floor will be reduced to the intrinsic noise level of the SS-DOCT without contribution from the lateral scanning optics. The trade-off would be the loss of real-time monitoring capability of any breathing motion or other artefacts, and further post-processing detection of such effects would be required.

5. Conclusion

In conclusion, we have demonstrated a Doppler optical cardiogram gating technique to increase the effective frame rate for DOCT systems. This method allowed detailed Doppler imaging of the blood flow in the aortic arches of Xenopus laevis embryos at 1000 fps with 512 lines per frame on a SS-DOCT system. This image gating technique also yielded 4D imaging visualization of the complex 3D motion of the embryonic heart, and the hemodynamics therein, at 45 fps. These techniques may be used to image the cardiovascular system development in wild type and transgenic animal models, where phenotypic determination of cardiac morphology and function are important for studying the underlying genetics.

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2.2 Addendum

Subsequently, after publishing this paper, the data acquisition system of the swept source DOCT imaging system was modified so that two channels of data could be acquired simultaneously. This had a number of benefits to the retrospective gating technique described in our manuscript: (1) It reduced the system complexity, by splitting the power from the laser in two separate interferometers, such that the Doppler optical cardiogram (DOC) and imaging could be done using the same system and (2) it allowed better synchronization between the DOC and the image acquisition, thereby reducing any jitter that was introduced by using two separate systems.

More recently, newer retrospective gating techniques have been demonstrated in the OCT literature based on the use of similarity measures between structural OCT images to rearrange asynchronously acquired data of embryonic hearts [50]. This technique allows the gating to take place without the use of a secondary channel to acquire the cardiogram. However, it should be noted that situations exist, where the structural images do not exhibit periodic motion but the blood flow within them is periodic. In these situations the DOC method we have proposed will outperform a purely structurally-based technique. On the other hand, the technique proposed by Gargesha et al. [50] may be reformulated and extended to use the corresponding Doppler data as well as the structural data to overcome this limitation. Regardless of the specific technique used, retrospectively gated OCT imaging techniques will continue to play an important role in 3D and 4D imaging of heart formation in embryos.
Chapter 3: Fourier domain mode locked laser for high speed phase stable imaging

The previous chapter demonstrated how periodic motion could be eliminated using retrospective gated image acquisition and processing. However, not all motion artefacts have a periodic nature. In the case of non-periodic motion an appropriate way to remove artefacts is to simply image faster. To this end a new type of OCT system is discussed, which has the potential to image orders of magnitude faster than conventional TD-OCT systems.

Spectral Domain optical coherence tomography (SD-OCT) is a relatively new optical imaging technique based on the concept of spectral interferometry that produces images very similar to TD-OCT. SD-DOCT, like TD-DOCT, can achieve near histological resolution of approximately 10 μm isotropically and flow sensitivity of ~500 μm/s in vivo [44, 45, 51]. SD-DOCT has a number of important advantages over TD-DOCT, including reduced system complexity, smaller footprint, improved SNR and faster A-scan scan rates, which is why it has largely superseded the previous generation TD-OCT approaches.

3.1 Theory and background

To understand the operation of a SD-OCT system, consider the complex amplitude that describes a monochromatic plane wave in one dimension,

\[ U = U_0 e^{-ik_0z} \]  \hspace{1cm} (3.1)

, where \( U_0 \) is the amplitude, \( k_0 = \frac{2\pi}{\lambda_0} \) is the wave number, and \( \lambda_0 \) is the wavelength of the light. The intensity measured at the detector of the interference of two such waves in an interferometer where the roundtrip path length difference in the sample and reference arms is \( 2\Delta z \), is

\[ I = \left| (U_1 + U_2) \right|^2 = \left| U_0 \left( e^{-ik_0z} + e^{-ik_0(z+2\Delta z)} \right) \right|^2 \]
\[ I = U_0^2 \left[ 1 + e^{i k_0^2 \Delta z} + e^{-i k_0^2 \Delta z} \right] \]
\[ = 2U_0^2 \left[ 1 + \cos(2k_0 \Delta z) \right] \] (3.2)

, where \( I_0 = |U_0|^2 \). If one of the mirrors in the interferometer is replaced with a continuum distribution of scattering particles with depth-dependent reflectivity, \( a(z') \), such as tissue, the signal measured is approximately:
\[ I \approx 2I_0 \left[ 1 + \int_0^\infty a(z') \cos(2k_0 nz') dz' \right] \] (3.3)

In the last expression we have neglected the mutual interference of waves scattered within the sample, since \( a(z) \ll 1 \), whereas the reflectivity of the mirror in the reference arm is 1. If this experiment is performed at many different \( k \)-values we get,
\[ I(k) \approx 2S(k) \left[ 1 + \int_0^\infty a(z') \cos(2knz') dz' \right] \] (3.4)

Here \( S(k) \) is the incident intensity of each \( k \)-value used (the swept source spectrum). The integral term can be expressed (using a symmetrical expansion of \( a(z') \) and moving to complex notation) in terms of the Fourier transform (\( FT \)) of \( a(z') \), therefore the expression can be rewritten as:
\[ I(k) \approx S(k) \left[ 2 + FT \{ a(z') \} \right] \] (3.5)

Finally, taking the inverse Fourier transform of \( I(k) \),
\[ FT^{-1} \{ I(k) \} \approx FT^{-1} \{ S(k) \} \otimes \left( 2\delta(z') + a(z') \right) \] (3.6)

In this expression, the inverse Fourier transform of the source spectrum now determines the point spread function (PSF), and hence the axial resolution of the imaging system is
\[ \delta z = \frac{2 \ln 2 \lambda_0^2}{\pi n \Delta \lambda} \] (3.7)

, where \( \lambda_0 \) is the center wavelength of the source, \( n \) is sample index of refraction and \( \Delta \lambda \) is the spectral bandwidth of the source, assuming a Gaussian shaped spectrum. This expression is equivalent to the expression for axial resolution in TD-OCT (Equation (1.1)). The term containing the delta function in Equation (3.6) only contributes a signal
at \( z' = 0 \); thus, as long as the sample is displaced slightly from this position it may be ignored. The last term is the convolution of the PSF with the depth resolved sample reflectivity (the signal of interest) or an A-scan.

In practice, the above theory can be implemented in two ways (1) a narrow band laser can be swept in time, using a tunable filter in the laser cavity, allowing the interference signal at each \( k \)-value to be measured by a single element time-resolved photodetector, or (2) a broadband light source can be used and spectrally resolved detection can be done via a multi-element spectrometer. This second implementation uses a diffraction grating to disperse the interference signal at many \( k \)-values over a multi-element charge couple device (CCD). We have chosen to pursue the time resolved scheme known as swept source (SS) OCT and we will restrict our discussion to this subclass of OCT system.

One of the practical issues in implementing a SS-OCT system is that it is not trivial to build a laser that sweeps linearly in \( k \). Typically, the data acquisition card samples the detector signal linearly in time, so a recalibration from time to \( k \)-space must be performed. This can be done by acquiring a known reference signal, which provides the necessary information to recalibrate the linear-in-time data to linear-in-\( k \) data. The most common way to do this is by generating an interferometric signal at a fixed path length delay and finding the maxima and minima of this signal. This corresponds to replacing the sample arm reflectivity, \( a(z') \), with the delta function, \( \delta(z' - z_0) \) in Equation (3.4) which reduces to

\[
I(k) = 2S(k) \cos(2knz_0)
\]  

(3.8)

The maxima and minima of this equation occur when \( k = m(\pi/2nz_0) \), where \( m \) is a positive integer. Thus, finding the maxima and minima of Equation (3.8) determines the location of equally spaced \( k \)-values regardless of the temporal dependence of the sweep. One of the limitations of this technique is that both the recalibration and actual sample signal must be acquired at higher than Nyquist sampling rates, such that the maxima and minima of the recalibration signal and corresponding points in the sample signal are accurately determined.
Another practical issue is the electronic detection bandwidth of the system. To obtain an estimate of this, the expression \( k = 2\pi \nu / c \) was substituted into Equation (3.8)

\[
I(\nu) = 2S(\nu)\cos\left(\frac{4\pi \nu}{c}\right)
\]

(3.9)

If the frequency is swept linearly in time, \( \nu(t) = \nu_0 - \nu_1 \cdot t \), then the highest temporal frequency that must be measured for a mirror at pathlength displacement \( z_0 \) is given by

\[
f_s = \frac{2\nu_1 z_0}{c}
\]

(3.10)

However, as stated, linear swept lasers in frequency are not straightforward to build, so Equation (3.10) is only a rough estimate of the necessary detection bandwidth for a given depth; to obtain a better estimate \( \nu_1 \) should be determined from the fastest part of any non-linear frequency sweep.

There are number of advantages that make SS-OCT a more attractive option than TD-OCT systems, the principal one being that SD-OCT systems provide a sensitivity improvement of 20 – 30 dB over TD-OCT systems [52]. This improvement is primarily due to the fact that each spectral component is acquired simultaneously for the entire A-scan in SS-OCT, while in TD-OCT only spectral components from a given depth are acquired at a time. For American National Standards Institute (ANSI) limited power this results in a large number of wasted photons. This can be thought of as a decreased integration time for each spectral component, and thus a lower sensitivity that is proportional to the number of depth points sampled. Another advantage of SS-OCT over TD-OCT systems is that the reference arm is fixed and the previously required rapid scanning optical delay line (RSOD) complexity is now shifted to the laser design. This removal of moving parts from the RSOD allows for more stable Doppler detection, free from vibrational artefacts. Lastly, as a direct consequence of the increased sensitivity, the A-scan rate of the system can be increased by an order of magnitude with only a marginal loss in SNR. To date the fastest single channel SS-OCT system constructed runs at 370 kHz and was developed by Huber et al. To achieve these extremely high scanning rates they have developed a technique called Fourier domain mode locking (FDML) [53, 54]. In a standard short cavity laser configuration, at any particular tunable filter position the lasing builds up from the amplified spontaneous
emission (ASE) of the gain medium. As the filter moves, in time, to the next position, lasing at the new wavelength must once again build up from the ASE background. To ensure maximum power output, the cavity length must be minimized to maximize the number of roundtrips through the SOA at each filter position. However, at extremely high laser scan rates, the cavity cannot be made short enough to achieve lasing due to the finite length of fiber optic components. Therefore, the FDML design was implemented to combat cavity length restrictions.

In a FDML laser configuration, the cavity length and tuning speed of the filter are controlled such that \( T_0(L/c)=1 \), where \( T_0 \) is the tuning period and \( c=2\times10^8 \text{ m/s} \) is the speed of light in fiber. In this configuration each laser sweep is a replica of the previous, since a given wavelength \( \lambda_0 \) returns to the filter exactly when the filter returns to a position corresponding to the wavelength \( \lambda_0 \). This eliminates the need for lasing to build up from the ASE background, and the laser is said to operate in a quasi-stationary regime. One particularly interesting aspect of FDML lasers is whether they have better characteristics for Doppler imaging. Since color Doppler detection of blood flow is based on analyzing phase shifts between adjacent A-scans, the mode-locked configuration may have an advantage over conventional swept lasers, where lasing always begins from the ASE background. To investigate this aspect of FDML lasers and to take advantage of the fast imaging rates they provide, a FDML laser was designed, built and characterized based on a rotating polygon filter that was first developed by Oh et.al.[55].

### 3.2 Construction of a polygon filter FDML laser source

To take advantage of the numerous benefits offered by SS-OCT, a FDML polygon laser with a maximum sweeping rate of 62 kHz was built. This laser was based on a ring cavity of length \( L \) that incorporates the components shown in Figure 3.1. The semiconductor optical amplifier (Covega BOA1017) provided the gain medium for the laser and the amplified spontaneous emission source to seed the lasing process. Photons travel from the SOA to the optical circulator, which then directs the photons to the second output that is connected to a tunable wavelength filter (TWF), discussed in detail below. A narrow bandwidth of input photons is selected and retro-reflected through the
filter and back to the circulator, which next channels the filtered spectrum to the third output and into a 50/50 output coupler. Lastly the remaining 50% of photons travel through a large length of fiber, which provides the necessary path length to achieve Fourier domain mode locking before they reenter the gain medium. Additionally, two polarization controllers are used to control the polarization of the light entering the SOA and TWF, as these are very sensitive to the input polarization state.

The tunable wavelength filter, shown in Figure 3.1, is a band-pass filter (filter width ≈ 0.2 nm) that sweeps across the near-infrared spectrum from 1265 nm-1375 nm. The fiber-optic collimator (OZ Optics, focal length of 10 mm, diameter of 2.2 mm) converts the photons from the fiberoptic cable into a collimated beam that illuminates the 830 lines/mm diffraction grating (Newport 53004BK) at an incident angle of \( \theta = 69° \). The two lenses with focal lengths of \( f_1 = 45 \text{ mm} \) and \( f_2 = 75 \text{ mm} \) (Thorlabs, AC254-075-C & AC254-040-C) were used to construct a telescope, which focuses the spectrally diverging light onto the surface of the 72 facet polygon mirror (Lincoln Laser, SA34). The spectrally resolved rays hit the polygon surface at different angles and are reflected to an end reflection mirror, where only rays at normal incidence to the end reflector are able to retro-reflect back through the optical system. In Figure 3.1 the red line indicates a wavelength that does not hit the end reflection mirror at normal incidence, while the green line indicates one that does. As the polygon turns, the angle the facet makes with the end reflection mirror changes, thereby determining the narrow bandwidth of wavelengths that are retro-reflected.
Figure 3.1: Schematic of the polygon based FDML laser. PC: Polarization Controller, SOA: Semi-Conductor Optical Amplifier, C: Collimator, 50/50: A 50% output coupler. Green line indicates a wavelength that hits the end reflection mirror at normal incidence, while the red line indicates one that does not.

The maximum sweep rate parameters for the laser in the mode locked configuration was a 62 kHz sweep rate \((T_0 = 16\mu s)\) and corresponding cavity length of \(L=3300\) m. The advantage of the mode locked polygon based laser over the MIT group’s resonant scanner based approach is two-fold: (1) the use of a resonant scanner induces highly non-linear wavelength scanning, which in turn makes correct sampling of the signal difficult and (2) Using a resonant scanner generates both forward (low to high wavelength) and reverse (high to low wavelength) spectrums, which typically have different noise profiles and complicate Doppler detection.
3.3 Critical OCT laser characterization measurements

To characterize the laser, measurements of (1) output power (2) bandwidth (3) coherence length and (4) phase noise were made, while the laser was operating in Fourier domain mode locked and in short cavity configurations.

_Laser Output Power Measurements_

Running a mode locked laser in a quasi-stationary regime will ensure SOA saturation, providing that cavity loss is not too high. This mode is in contrast with the short cavity configuration, where the output power is controlled by the number of round trips a photon can make before the mode hops to the next filter position. It is possible to saturate the SOA in this operational mode by sufficiently reducing the cavity length. However, due to the finite length of fiberoptic components, it is very difficult to do this in practice. For this reason it is expected that lasing will start at a lower injection current threshold and will achieve a higher output power in the mode locked configuration. Power measurements were made using a calibrated Thorlabs PM144 optical power meter that had an optical bandwidth of 700-1700 nm. Laser output power was measured as a function of injection current into the SOA and plotted on a linear scale (see Figure 3.2), which shows typical data obtained from the laser in both the short cavity (squares) and mode-locked (triangles) configurations. As predicted, the lasing threshold in the mode-locked configuration is approximately 100 mA compared to 250 mA in the short cavity configuration. A beneficial increase in output power of about 20 mW was also realized in the mode locked configuration.
Figure 3.2: Mode locked laser (triangles) and short cavity laser (squares) output power as a function of semi-conductor optical amplifier injection current. The mode locked configuration had a lower injection current threshold for lasing and achieved a higher laser output power than the short cavity configuration. (Reprinted with permission of the Optical Society of America)

Bandwidth Measurement
The total lasing bandwidth determines the axial PSF of the OCT system, as demonstrated by Equation (3.7). Thus measurement and optimization of this quantity throughout the laser alignment process is crucial. Bandwidth was measured using an optical spectrum analyzer (Ando AQ-6310B) in peak-hold mode, where the lasing spectrum is shown in Figure 3.3 and has a total lasing bandwidth (Free Spectral Range) $\Delta \lambda_{\text{FSR}} = 112.2$ nm and FWHM value of $\Delta \lambda_{\text{FWHM}} = 95.3$ nm. No significant difference in lasing bandwidth was observed between the FDML and short cavity laser at the maximum SOA injection current.
Coherence Length Measurement

In SS-OCT, the coherence length of the laser determines (1) the signal to noise roll-off of the system and (2) the decrease in axial resolution as the sample is moved away from the zero-plane of the system (zero path length difference between sample and reference arms of the interferometer). An increase in coherence length ultimately means that an increased amount of empty space can be traversed before the surface of the sample must be imaged (the ranging depth). This can improve imaging quality when trying to scan large areas of hollow organs such as the colon, where the distance from the surface of the tissue to the probe can vary by millimeters. To measure the coherence length of the laser, a 50/50 (50% of the laser input directed to each of the arms) Mach-Zender interferometer was built with mirrors in both arms, as shown in Figure 3.4. One of the mirrors was placed on a calibrated micrometer stage that could be moved up to 2.5 cm. This allowed for the introduction of a controlled path length difference between the sample and reference arms. The interference signal was acquired using 2.5 Gs/S oscilloscope, which was triggered using a fiber Bragg grating.
that produced a strong transient signal at a wavelength defined by the periodicity of the grating (in our case, 1270 nm). This signal provided a stable A-scan trigger at the beginning of each laser sweep. The interference signal was measured at various path length differences and analyzed by first re-sampling the signal, such that the data were equally spaced in $k$-space, and then Fourier transforming this data. The magnitude of the Fourier transform (a complex valued signal) of the signal from the mirror should resemble a peaked function with FWHM equal to the resolution of the system at that particular displacement. As the mirror in the sample arm was translated away from the zero-plane, a decrease in peak height and increase in peak width (decrease in axial resolution) is expected due to the finite coherence length of the laser. Data from this measurement are shown in Figure 3.5 for both the short cavity and mode locked configurations. The coherence length of lasers in the OCT field is usually characterized by the determining 7.5 dB drop in peak height. For our mode locked laser, this reduction in peak height occurred at approximately 8.5 mm, compared to 3.5 mm in the short cavity case, with a corresponding decrease in axial resolution of 8 μm to 15 μm over these ranges. These results are slightly better than the results reported by the MIT group for their FPI based mode locked laser [54].

**Figure 3.4:** Schematic of the optical configuration used to measure the coherence length of the short cavity and mode locked laser sources. A standard 50/50 fiber Mach Zender interferometer (MZI) was used to perform this measurement. PC: Polarization Controller, SOA: Semi-Conductor Optical Amplifier, C: Collimator, FBG: Fiber Bragg Grating, DB: Dual Balanced Detector, D: Detector.
**Figure 3.5:** Coherence length measurements for the short cavity and mode locked lasers. The short cavity laser shows a much sharper signal drop as a function of relative displacement compared to the mode locked system. A 7.5 dB drop after 3.5 mm for the short cavity laser compared to 8.5mm for the mode locked laser was observed (Reprinted with permission of the Optical Society of America).

**Phase Noise Measurement**

Ultimately, the smallest possible measurable phase shift between two adjacent A-scans is the phase noise of the laser. This can be characterized using the setup shown in Figure 3.6. The interferometer uses a stationary (m-mode imaging mode) collimated beam of light to illuminate a mirror in both the sample and reference arms. Data from this interferometer and the MZI recalibration interferometer are acquired by a 100 MS/s DAQ card for each channel. The raw data was re-sampled using the MZI recalibration signal as described in section 3.1, the signal from the sample mirror was isolated and the phase over several hundred A-scans was calculated via the N=2 phase estimator. External vibrations can be removed by subtracting the median phase shift from each pair of A-scans.
Figure 3.6: Schematic of the optical configuration used to perform phase noise measurements. A Mach Zehnder interferometer with two sample arms was used to measure the phase noise of the system. The secondary 1% sample arm provides a calibration signal for removing timing induced phase ramps. The MZI clock in the triggering and recalibration box provides the signal used to generate linear k data. PC: Polarization Controller, SOA: Semi-Conductor Optical Amplifier, C: Collimator, FBG: Fiber Bragg Grating, DB: Dual Balanced Detector, D: Detector.

In chapter 1 we noted that the SNR limited phase noise between two A-scans is equal to

\[ \sigma_{\Delta \phi} = \frac{1}{\sqrt{X_s}} \]  

(3.11)

, where \( X_s \) denotes the SNR of the reflector. However, there is an additional source of noise that must be accounted for, which comes from optical triggering of the DAQ card using the FBG. The trigger signal generated from the FBG arbitrarily falls between two samples of the DAQ card, causing a timing error to be introduced. After performing the Fourier transform, this timing error in the k-space acquisition translates to a phase ramp in the depth direction given by

\[ \Delta \phi_{ramp}(i) = i \Delta \phi_{timing} \]  

(3.12)

, where \( i \) is the depth index and \( \Delta \phi_{timing} \) is the phase error induced by the timing error at depth index 1.
To account for these timing induced phase ramps, a calibration arm was made by tapping 1% of the light from the sample arm and placing a mirror with SNR $X_c=30$ dB at a depth index of $Z_c=450$. Since the phase ramp builds up linearly as a function of depth, the ideal place to measure the ramp is at large depths. Additionally, using a 30dB mirror signal provides adequate SNR to ensure the majority of the phase shift is due to the phase ramp, while not creating any large imaging artefacts. Using the measured phase at depth index $Z_c$ we can obtain an estimate for $\Delta \phi_{\text{timing}}$.

$$\Delta \phi_{\text{timing}} = \frac{1}{Z_c} \Delta \phi_{\text{ramp}} (Z_c)$$

(3.13)

Using this estimate, the phase ramp can be removed from all points in the image by subtracting Equation (3.12) from the measured phase according to depth index $i$.

$$\Delta \phi (i) = \Delta \phi - i \Delta \phi_{\text{timing}}$$

(3.14)

The calibration process described by Equation (3.14) adds another term to the total phase noise floor such that the noise floor becomes

$$\sigma_{\Delta \phi}^2 = \frac{1}{X_s} + \left( \frac{Z_s}{X_c} \right)^2 \frac{1}{X_c}$$

(3.15)

Measurements of the calibrated phase noise for the short cavity and FDML lasers are shown in Figure 3.7 and show good agreement with theory. The dotted and dashed lines are the first and second terms in Equation (3.15), respectively, while the solid line represents the combination of the two terms. At low SNR both the short cavity and FDML laser show SNR limited phase noise, while at high SNR (above 45 dB) the FDML laser outperforms the short cavity configuration. These results are consistent with those previously published [51, 53]. However, the implications for microvascular imaging are not particularly encouraging, considering that typical biological samples exhibit SNR ranging from 20-35 dB. Thus, perhaps the most important benefits of a FDML laser are the long coherence length, high power and fast A-scan rates they provide.
Figure 3.7: Phase noise measurement for the short cavity and FDML lasers after removal of timing induced phase ramps. Up to about 45 dB both the short cavity and FDML lasers exhibit SNR limited phase noise. At higher SNR the FDML laser slightly outperforms the short cavity laser.

The results of this chapter (excluding the phase noise measurements) were published in a manuscript entitled


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High power wavelength linearly swept mode locked fiber laser for OCT imaging

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Abstract: We report a long coherence length, high power, and wide tuning range wavelength linearly swept fiber mode-locked laser based on polygon scanning filters. An output power of 52.6 mW with 112 nm wavelength tuning range at 62.6 kHz sweeping rate has been achieved. The coherence length is long enough to enable imaging over 8.1 mm depth when the sensitivity decreases by 8.7 dB (1/e2). The Fourier components are still distinguishable when the ranging depth exceeds 15 mm, which corresponds to 30 nm optical path difference in air. The parameters that are critical to OCT imaging quality such as polygon filter linewidth, the laser coherence length, output power, axial resolution and the Fourier sensitivity have been investigated theoretically and experimentally. Since the wavelength is swept linearly with time, an analytical approach has been developed for transforming the interference signal from equidistant spacing in wavelength to equidistant spacing in frequency. Axial resolution of 7.9 μm in air has been achieved experimentally that approaches the theoretical limit.

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References and links

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1. Introduction

Optical coherence tomography (OCT) [1] is finding widespread applications in biomedical imaging, with micrometer spatial resolution [2, 3] and its Doppler extension can image blood flow with velocity ranging from 0.02 to 100 mm/s [4]. High-speed wavelength-swept light sources have become available, and OCT techniques have been improved with Fourier domain measurements [5-11]. The wavelength tuning range determines the axial spatial resolution, while the time of a complete wavelength scan corresponds to an A-line data acquisition, therefore, the repetition rate of the light sources determines the imaging frame rate. Yun et al. experimentally reported a high-speed wavelength-swept laser source using a polygon-scanner-based wavelength filter [12]. Approximately 20 mW output power with repetition rates at 115 kHz based on polygon scanner [10] and 370 kHz using scan Fabry-Perot filters [13, 14] have also been achieved experimentally.

For effective lasing, photons are required to have sufficient resonant times within the laser cavity for building up the optical power and narrowing the spectral linewidth through mode competition before being coupled out. This typically requires the laser cavity to be short enough to reduce the optical round-trip time of the resonant photons when the wavelength is swept. However, it is very difficult in practice to build a very short fiber cavity since fiber components are typically tens of millimeters in length and need to be spliced together. This significantly limits the performance of high-speed, wavelength-swept laser fibers, imposing a trade-off between the wavelength sweep rates versus output power, coherence length and wavelength tuning range [11, 12]. An alternative approach to achieve sufficient resonance for a certain frequency component is to use a long fiber delay line. For a specific speed of the bandpass transmission filter, those frequency components are delayed and pass the filter at the exact time when the transmission of the filter is at the next spectral position. Therefore, the returned photons are synchronized to the transmission filter. This synchronization technique is called Fourier domain mode locking (FDML) [12-15] and generates short optical pulses in the frequency domain. It is similar to the conventional mode-locked lasers that generate short optical pulses in the time domain.

In this paper, we report on a long coherence length, high power and wide tuning range wavelength-swept mode-locked fiber laser for OCT imaging. An output power of 52.6 mW with 112 nm wavelength tuning range centered at 1320 nm at sweeping rate of 62.6 kHz has been achieved. To the best of our knowledge, this is the most powerful wavelength-swept
light source reported to date. The coherence length is long enough to enable ranging depth over 8.1 mm with only an 8.7 dB decrease in sensitivity and the Fourier components are still detectable when the ranging depth is over 15 mm. An analytical expression has been developed for transforming the interference signal from equidistant spacing in wavelength (λ-space) to equidistant spacing in frequency (k-space) for fast Fourier transform (FFT). An axial resolution of 7.9 μm in air or 5.8 μm in tissue (assuming the refractive index is 1.3) has been achieved, which approaches the theoretically limit. The laser output power with respect to the driving current, polygon rotation frequency, and axial resolutions have been investigated experimentally.

2. Theory for OCT signal processing

2.1 Coherence length of Laser sources

For a typical fiber Mach-Zehnder interferometer (MZI), as shown in Fig. 1(c), a laser beam is split into two through a 3 dB fiber coupler and each of these beams is then aligned with a fiber collimator and reflected by a mirror. The two reflected beams are re-coupled back into the fiber and merge at the 3 dB fiber coupler to produce an interference signal. The interference signals output from the circulator and the 3 dB coupler have an inverse phase and thus can be measured by a balanced detector with an enhanced sensitivity. The output of the balanced detector can be expressed as

\[ I \sim \frac{1}{\Delta \lambda_{\text{fwhm}}} \int_{-\infty}^{\infty} f(\delta \lambda) \cdot \cos \left( \frac{2\pi \delta \lambda}{\lambda} + \phi \right) d(\delta \lambda), \tag{1} \]

where \(2\pi\) is the optical path difference, \(\Delta \lambda_{\text{fwhm}}\) and \(f(\delta \lambda)\) are the instantaneous laser linewidth and its spectral profile, \(\lambda\) is the light wavelength in vacuum, \(\delta \lambda = \lambda - \lambda_0\) is the wavelength detuning, \(\lambda_0\) is the filter center wavelength and \(\phi\) is the initial phase. For a top-hat spectral profile, \(f(\delta \lambda) = 1\) when \(|\delta \lambda| \leq \Delta \lambda_{\text{fwhm}} / 2\) and \(f(\delta \lambda) = 0\) when \(|\delta \lambda| > \Delta \lambda_{\text{fwhm}} / 2\). Eq. (1) reduces to

\[ x(t) = a(\tau, t) \cos \left( \frac{4\pi}{\lambda_0 + \Delta \lambda_{\text{fwhm}} f_{\text{fwhm}}} \right), \tag{2} \]

where the DC component and the initial constant phase \(\phi\) have been neglected for simplicity, \(a(\tau, t) = a_0(t) \cdot \text{sinc}(\tau / d_0)\), \(d_0 = \frac{\lambda_0^2}{2\Delta \lambda_{\text{fwhm}}}\), \(\Delta \lambda_{\text{fwhm}}\) is the spectral tuning range and \(f_{\text{fwhm}}\) is the sweep frequency. Since \(\Delta \lambda_{\text{fwhm}} \ll \lambda\), the sinc function is a slow varying term that is superimposed on the first fast term that produces the interference fringes. If \(\tau = d_0\), the interference fringes disappear and the corresponding path difference \(2d_0 = \lambda^2 / \Delta \lambda_{\text{fwhm}}\) is called the coherence length (\(d_0\) is called coherence depth). For a filter with a Gaussian spectral profile \(\exp \left[-4 \ln(2) (\delta \lambda / \Delta \lambda_{\text{fwhm}})^2\right]\), this coherence depth is usually expressed as

\[ d_{\text{coherence}} = \frac{\lambda_0^2}{\pi \Delta \lambda_{\text{fwhm}}}, \]

which is about 12% smaller than \(d_0\) [17, 18].

2.2 k-space

When the frequency of the light source is not swept linearly with time, an accurate and reliable transformation of the interference output to equidistant spacing in frequency (k-space) is critical for FFT to enable a high axial resolution. For AD converter cards with a clock input for triggering, a MZI is usually employed [11, 12] and extra electronics are therefore required.

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to generate a TTL clocking signal. The timing jitter of the clocking signal can be a problem and would seriously degrade the axial resolution. For polygon scanner based wavelength-swept light sources, the wavelength varies highly linearly in time, which offers an analytical approach to convert the interference output to equidistant frequency signal for FFT. Defining a new time stream \( t' \) to replace the time variable \( t \) in Eq. (2) as

\[
t' = \frac{t}{1 - \frac{\Delta \lambda_{\text{range}}}{f_{\text{swep}}, \lambda_0}},
\]

(3)

The Eq. (3) reduces to

\[
x(t) = a(z,t) \cos(2\pi ft),
\]

(4)

where \( f = \frac{z}{\Delta z_0} f_{\text{swep}} \) is the frequency of the k-spaced signal, \( \Delta z_0 = \lambda_0^2 / (2 \Delta \lambda_{\text{range}}) \), the constant phase has been ignored for simplicity. To obtain an equal frequency spaced signal \( t = \frac{i}{N f_{\text{swep}}} \), \( i = 1, 2, \ldots, N \) requires only to re-map the signal with a new time stream of \( t' = \frac{t}{N f_{\text{swep}}} \), where \( N \) is the total number of samples of the signal, and \( i' \) that can be solved by Eq. (3) is usually not an integer and an interpretation operation may be required. Since this is an analytical calculation and interpretation, the processing time is negligible compared to the time required for Fourier transformation. We will demonstrate that this analytical technique can provide a signal with an accurate equidistant spacing in frequency, and therefore, the resolution of the point spread function (PSF) is in good agreement with theoretical calculation.

2.3 Fourier detectable range, sensitivity, and axial resolution

The discrete Fourier transform is commonly expressed as

\[
X(k) = \sum_{n=0}^{N-1} x(n) e^{-j 2\pi k n / N},
\]

\( k = 0, 1, 2, \ldots \). For a total acquisition time of \( T_{\text{swep}} = 1 / f_{\text{swep}} \), the Fourier frequency is given by \( F_k = k f_{\text{swep}} \), equal to the signal frequency \( f \) in Eq. (4). The depth is then obtained as \( z = k \Delta z_0 \). Clearly, \( \Delta z_0 \) represents the axial step, corresponding to the axial resolution.

Since the Fourier spectrum has two positive and negative Fourier frequency components, the maximum detectable axial range (\( z_{\text{max}} \)) corresponds to the maximum detectable Fourier frequency that can be obtained when these two opposite Fourier frequencies superimpose at \( k = N / 2 \) as \( z_{\text{max}} = N \Delta z_0 / 2 \). This maximum detectable range corresponds to the signal sampling rate of two samples every interference period, which is generally not sufficient to describe a periodical signal accurately, thus resulting in a low signal-to-noise ratio. For \( N = 1024 \), this Fourier detectable range is about 3.97 mm.

For Fourier transformation, the energy conservation law can be expressed as

\[
\sum_{n=0}^{N-1} |x(n)|^2 \Delta t = \frac{1}{N} \sum_{k=0}^{N-1} |X(k)|^2 .
\]

When the signal amplitude of Eq. (4) is a constant with respect to time \( t \) as \( a(z,t) = a_0(z) \), (the top-hat evolution profile), the peak of Fourier sensitivity (or strength) is thus obtained as \( |X(k)|^2 \propto a_0^2(z)(N/2)^2 \). This results in peak sensitivities of 94 and 54 dB, respectively for \( N = 10^7 \) and 1024 samples when the signal amplitude \( a_0(z) = 1.0 \). By measuring this peak sensitivity as a function of the depth \( z \), the profile of \( a_0(z) \) or the coherence length of the light source can be determined.
When a signal is shaped with an evolution profile, the signal can be simply treated as a shaping profile multiplying a signal with a top-hat profile. The Fourier transformation is therefore the convolution of the Fourier transforms of the shaping profile and the top-hat profile signal. For a typical Gaussian shaping profile as

\[ \text{Gaussine}(q,t) = \exp \left[ -4 \ln 2 \frac{t}{t_{\text{tophat}}} \right], \]

the normalized Fourier sensitivity is thus

\[ \left| X(z, \phi) \right|^2 = \exp \left[ -4 \ln 2 \frac{z_p}{u \Delta z_0} \right] \odot \text{sinc} \left( \frac{z_p - z}{\Delta z_0} \right), \tag{5} \]

where \( \odot \) indicates a convolution operation, \( u = 4 \ln 2 / \pi \), \( z_p \) is depth variable converted from the Fourier frequency \( \phi \), and \( q \) is the parameter to describe the width of the Gaussian shaping profile. The sinc function is the Fourier transform of signal with a top-hat profile. Note that when \( q \rightarrow \infty \), Eq. (5) reduces to top-hat profile as \( \text{sinc} \left( \frac{z_p - z}{\Delta z_0} \right) \). The axial resolution is thus the axial step \( \Delta z_0 \), given by Eq. (4). Simulation shows the side lobes of the sinc profile can be suppressed by the operation of the convolution at the expense of degrading the depth resolution, which is in good agreement with our experimental results.

3. Experiments and results

A broad-bandwidth semiconductor optical amplifier (Covega BOA1017) was used as a laser gain medium. A fiber-optic collimator (OZ Optics, focal length of 10 mm, diameter of 2.2 mm) was used to couple the light between the fiber and the polygon filter. Two achromatic lenses (Thorlabs, AC254-075-C & AC254-040-C) with focal lengths of \( f_1 = 75 \) mm and \( f_2 = 40 \) mm were used to construct the confocal telescope system. An 830 nm/mm diffraction grating (Newport 53004BK) was the dispersion component. A 72-facet polygon mirror (Lincoln Laser, SA34) was used to sweep the wavelength. By inserting a 3.33 km fiber delay line into a 7 m long ring laser cavity, we could alternate between mode-locked and short cavity lasers operation, respectively, as shown in Fig. 1(a).

The polygon based scanning filter is shown in Fig. 1(b). When a parallel beam is incident on the diffraction grating, the rays with the same wavelengths are diffracted by the grating to the same direction. The diffracted rays are then redirected by the confocal telescope and illuminate the polygon mirror and the end mirror. The end mirror reflects back the light to the polygon mirror and finally couples to the fiber through the fiber collimator to realize resonance. Clearly, only the beam with specific wavelength components at specific time can pass the polygon filter and return to the laser cavity. The maximum wavelength tuning range is the free spectral range (\( \Delta \lambda_{\text{FSR}} \)) that is determined by the number of the polygon facets (\( N \), focal lengths of the two lenses and the grating period (\( \Lambda \)) as \( \Delta \lambda_{\text{FSR}} = \frac{4 \pi}{N} \Lambda f_1 / f_2 \) \[10\].

Spinning the polygon mirror in clockwise direction produces a positive wavelength tuning in the configuration as shown in Fig. 1(b). The linewidth of the polygon filter was \(~0.2\) nm when the beam was incident at an angle of \( 69^\circ \). In order to synchronize the optical resonance to the polygon scanning filter, the optical round-trip time must match the time between two neighboring sweeping period or its harmonics. The sweeping frequency is then expressed as
\[ f_{\text{Sweep}} = M \frac{c}{n_{\text{eff}} L}, \quad (M=1,2,\ldots) \]

where \( L \) is the fiber physical length and \( n_{\text{eff}} \) is the effective refractive index of the fiber core, \( c \) is the light velocity in vacuum, and \( M \) is the order of the harmonic. In order to reduce the fiber loss and potential dispersion effect, the fundamental mode \((M=1)\) was used in our experiments. Obviously, when the sweeping frequency is tuned around the center mode-locked frequency, the returned photons will mismatch the exact filter wavelength. When this mismatched wavelength is out of the filter wavelength range, no returned photons can pass the filter and the laser is not lasing. The relationship between the sweeping frequency and the filter linewidth is then obtained as

\[ \delta \lambda = \Delta \lambda_{\text{FWHM}} \frac{\delta f}{f_0} \]

where \( f_0 \) is the center mode-locked frequency, \( \delta f \) is FWHM of the laser output power profile with respect to the detuning of frequency. For \( n_{\text{eff}} = 1.45 \), \( L = 3.3 \text{ km} \), Eq. (7) gives the mode-locked frequency as \( f_0 = 62.70 \text{ kHz} \), which is agreement with our experimental result of 62.57 kHz. Detuning the sweeping frequency from \( f_0 \) results in the decrease of the output power, as shown in Fig. 2. For \( \delta f = 0.11 \text{ kHz} \) and \( \Delta \lambda_{\text{FWHM}} = 112 \text{ nm} \), Eq. (7) gives the filter linewidth as \( \delta \lambda = 0.197 \text{ nm} \), which agrees well with the experimental value of 0.2 nm. The linewidth was measured at the circulator port 3 using an optical spectrum analyzer when the polygon is stationary, where the SOA acted as a wideband light source. Note that the free spectral range is used to represent the wavelength tuning range. This provides a useful way to determine the linewidth of spinning polygon filters. The asymmetry profile as shown in Fig. 2 indicates the wavelength sweeping direction. On average, higher power on the long wavelength side was observed. When the sweeping frequency of the polygon mirror is slightly faster than the
center mode-locked frequency $f_o$, the polygon filter will catch up with the wavelength in advance, and thus sweeping direction from short to long wavelength will result in a slow power decrease on average.

![Graph of output power vs. sweeping frequency](image)

Fig. 2. The frequency domain mode-locked (FDML) laser output power as a function of the sweeping frequency, showing $f_o=62.57$ kHz and $df=0.11$ kHz. Squares are experiment data, line is their connection. The asymmetry profile indicates sweeping direction, from short to long wavelengths.

The fiber dispersion effect can be neglected in our experiments. For $L=3.3$ km fiber with $\sigma=3.1$ ps/nm/km dispersion coefficient at 1300 nm band [18], the group delay for $\Delta\lambda$ spectral width is expressed as $dt = dt.\Delta\lambda$, 2.05 ps and 1.15 ns, respectively for instantaneous filter linewidth of 0.2 nm and wavelength tuning range of 112 nm, corresponding to the filter wavelength mismatch of $1.4\times10^{-3}$ nm and $8.0\times10^{-3}$ nm at a 62 kHz ($f$) sweeping frequency ($\Delta\lambda_{\text{corr}}/df$). This is about two orders of magnitude lower than the filter linewidth and hence can be neglected. Note that the second order dispersion with coefficient of $0.085$ ps/km/nm² has the same order magnitude effect compared with the first order.
The spectrum of the FDM laser, showing sweeping range of Δλ_{sweep}=112.2 nm and full width at half maximum of 95.3 nm.

The FDM laser spectrum measured using an optical spectrum analyzer (Ando AQ-6310B) in peak-hold mode is shown in Fig. 3. 112.2 nm tuning range (Δλ_{sweep}) centered at 1320 nm was obtained, which agrees well with the theoretical calculation of 111.7 nm. The full width at half maximum width is approximately 95 nm.

The laser output power with respect to the SOA driving current is shown in Fig. 4. The lasing thresholds for the modelocked and short cavity lasers are ~100 mA and ~250 mA, respectively. The slope efficiencies for the both laser configurations are almost the same, ~0.134 mW/mA. When the polygon mirror is stationary, the short cavity gives slightly more output power compared to that of the long fiber mode-locked cavity, since the presence of 3.3 km fiber will introduce an additional loss. However, when the polygon is spinning, the mode-locked laser has considerably higher output power and longer coherence length due to the synchronized resonance. Up to 52.6 mW high power has been obtained. Such a large output power level is attractive for OCT, enabling potentially faster imaging or multiple-OCT-probe operation.
Figure 4. The output power of the laser as a function of the SOA driving current, showing that the
current thresholds for mode locked (triangles) and short cavity lasers (squares) are ~100 mA
and ~250 mA, respectively. The slopes for both configurations are the same within
experimental error, ~0.134 mW/mA.

Figure 1(c) illustrates the MZI for the measurement of the point spread functions (PSFs)
with respect to the reflection depths. The reflection depth can be adjusted by moving a mirror
mounted on a translation stage. A balanced detector and an oscilloscope (Tektronix
TDS5054B) with 2.5 GS/s sampling rate (500 MHz bandwidth) were used to capture the
interference signal. At the depth position of 1.65 mm, the interference signal is shown in Fig.
5. The insert is the zoom-in of the signal to shown the interference fringes.

When this interference signal is shaped to a top-hat and Gaussian(1/e²) evolution
profiles, respectively, the theoretical and experimental PSFs at this depth are shown in Fig.
6(a) and (b). The insert figures in Fig. 6(a) and (b) are used to indicate the signal evolution
profiles. The interference frequency in the insert figures is reduced for visual purposes. The
FWHM at this depth for top-hat profile is about 7.9 μm, which closes to the 7.8 μm, the
theoretical derived limit for a light source with a tuning range of 112.2 nm centered at 1320
nm wavelength. For the Gaussian shaping evolution profile, the signal-to-noise ratio can be
improved due to the suppression of the side lobes while the axial resolution degrades to 13.4
μm. The mechanism of this resolution degradation results from the reduced effective tuning
range. For a shaping profile of Gaussian(0.5,2), experimental results show the axial resolution
is ~10.0 μm. Experimental results are in good agreement with the theoretical calculation.
Fig. 5. The interference signal captured by an oscilloscope at a depth of 1.65 mm, corresponding to a path difference of 3.3 mm. The insert is the zoom-in of the interference fringes.

Fig. 6. The experimental and theoretical PSFs for signal with evolution profiles of (a) Top-hat, (b) Gaussine(1/e²t). Points are experimental data and dashed curves are their connections. Solid curves are theoretical calculations, showing a 7.9 µm and 13.4 µm FWHM for top-hat and Gaussine(1/e²t) profiles.

The coherence length of the laser source can be investigated by measuring the peak Fourier sensitivities as a function of the reflection depths. The PSFs at different depths for FDML at the signal sampling rate of 2.5 GS/s is shown in Fig. 7(a). An OCT ranging depth of 8.1 mm, corresponding to 16.2 mm optical path difference in air, with only an 8.7 dB (1/e²) decrease in sensitivity has been achieved in our experiments. Note that the depth is ~7.3 mm for a 7.5 dB sensitivity decrease. The Fourier components are still distinguishable when the ranging depth exceeds 15 mm.
When the FDML laser is switched to a short cavity fiber ring laser by removing the 3.3 km long fiber delay line, the resulting PSFs at different depths are shown in Fig. 7(b). The ranging depth reduces to 3.4 mm when the sensitivity decreases by 8.7 dB at sweeping frequency of 42.9 kHz.

We used an oscilloscope (TDS505B) with signal sampling rate of 2.5 GS/s to capture the interference signal. The samples for an A-scan was $\sim 4 \times 10^4$, which is large enough to analyse tens of millimeters in ranging depth for a sweeping frequency of 62.6 kHz. When the signal sampling rate reduces to 100 MS/s, the samples reduce to ~1600, corresponding to a maximum Fourier measurable range of ~6.2 mm. When the ranging depth closes to this depth, the signal-noise ratio will be degraded dramatically.

4. Conclusion

We have demonstrated a long coherence length, high power, wide spectral range wavelength-swept fiber mode-locked laser based on a polygon scanning filter. An output power of 52.6 mW with 112 nm wavelength tuning range centered at 1320 nm, 62.6 kHz sweeping rate and 8.1 mm OCT ranging depth with 8.7 dB or $1/e^2$ decrease in sensitivity have been achieved from a single semiconductor optical gain medium and 3.3 km fiber cavity. The Fourier components are still distinguishable at the reflection depth of 15 mm. For a 7 m long short-cavity, the corresponding power and depth drop to 35 mW and 3.4 mm, respectively when the sweeping frequency is 42.9 kHz. The laser thresholds for mode-locked and short-cavity are $\sim 100$ mA and $\sim 250$ mA while the slope efficiencies are $\sim 0.134$ mW/mA. An analytical expression has been developed for calculating of the polygon filter linewidth from the measurement of the power profile width with respect to the sweeping frequency. The asymmetry of this power profile indicates the wavelength sweeping direction. By taking the merit of linear wavelength tuning, an analytical method has been demonstrated to provide an accurate equidistant k-spaced signal. Axial resolution of 7.9 μm in air, approaching the theoretical limit, has also been achieved.
Chapter 4: Speckle variance OCT

As discussed and demonstrated in the previous chapters, the problem of motion artifacts in 3D vascular reconstructions can be largely mitigated through either the use of fast imaging systems and/or retrospective gating. The other two issues discussed in the introductory chapter were (1) the angle dependence of phase resolved (color Doppler) imaging and (2) the finite phase noise floor, which requires a different approach. In fact, the use of faster lasers for phase resolved imaging is somewhat counterproductive, as standard measurement techniques rely on measuring A-scan to A-scan changes in phase. As the time between consecutive A-scans becomes smaller (inversely proportional to the A-scan rates of OCT lasers) there is less time for the phase difference to build up. In the best-case scenario, where the phase noise floor remains constant as the A-scan rate of the laser increases, there is a linear increase in the minimum detectable velocity. This can be overcome by a proportional increase in the packet length (number of A-scans used in the calculation) used in the Kasai (or other) velocity estimator. However, this results in a reduction of the imaging frame rate, diminishing advantages gained by moving to a faster laser.

An alternative technique to phase resolved imaging first applied to TD-OCT imaging systems is Power Doppler imaging[43]. This technique is based on filtering out the tissue component (known as the clutter signal in ultrasound) of the slow time signal power spectrum using a high pass filter (wall filter) and subsequently displaying the remaining signal intensity. More recently, a technique known as optical angiography (OAG) has been developed for frequency domain OCT. This technique is similar to PD-OCT except that the filtering of the tissue clutter from the slow time power spectrum occurs in the frequency domain via a Hilbert transform [45, 56]. It should be noted that these standard time domain PD-OCT techniques can be applied to frequency domain OCT after the axial inverse Fourier transform from k-space to depth has been applied. The greatest benefit of power Doppler and OAG imaging, when compared to color Doppler imaging and other phase sensitive techniques, is that averaging multiple lines and frames from the same location (a technique known as persistence in ultrasonography) increases the system sensitivity to smaller vessels[42].
Power Doppler imaging is regarded as a phase insensitive technique as it displays the energy in the spectrum above a cutoff frequency and does not make use of the mean frequency, or any higher order moments, of the signal. However, at very low blood flow velocities (below the phase noise floor of the system) and/or Doppler imaging angles approaching 90°, the flow signal still becomes buried in the clutter signal and is subsequently removed in the filtering process. This poses a challenge when attempting to image the microcirculation of tumors, which can consist of tortuous blood vessel formation, where the angle of incidence continuously changes within the imaging region and vessels may have intermittent flow patterns.

In an effort to overcome the limitations of both phase resolved and power Doppler imaging techniques, a novel inter-frame OCT fluid contrast algorithm was developed, which is both phase insensitive and angle independent, even at a 90° Doppler angle. We termed this technique speckle variance (SV) OCT.

Portions of the work presented in this chapter resulted in two publications that are attached as an appendix to this chapter. In our initial publication, the SV algorithm was described, flow phantom measurements were performed and in vivo measurements were validated against the gold standard for intravital microvascular imaging, confocal fluorescence microscopy.


In the second publication, simple models for speckle variance contrast and speckle decorrelation were utilized to improve vascular contrast in low clutter situations and to determine appropriate imaging frame rates to mitigate the effects of bulk tissue motion, while performing in vivo B-mode speckle variance imaging, for the first time, in high clutter situations.

However, much of the work in these publications and in this chapter relies on having an extremely stable tissue volume while performing 3D imaging. Therefore the discussion of the SV technique begins with the description of the animal model and associated hardware used to ensure the stable imaging environment.

4.1 The dorsal skinfold window chamber model

Mouse window chamber models (WCM) provide an optical window through which one can examine tumor angiogenesis in exquisite detail. Until now, the WCM has been primarily used with multi-photon confocal microscopy (MPCM) [57]. However, MPCM suffers from a number of drawbacks that makes it impractical for large preclinical studies (a) microscopy time, for example scanning a 5mm x 5mm region can take over one hour, (b) fluorescent dyes leak out of tumor vasculature making longitudinal imaging difficult and (c) limited depth of penetration (<400um). On the other hand, the SV-OCT algorithm allows the same size region (5mm x 5mm) to be imaged in approximately 5 minutes, to a depth of 2mm and without the use of exogenous contrast agents. The inter-frame nature of the algorithm demands a stationary sample, free of bulk motion artefacts (in the absence of extremely high imaging frame rates). Thus, the dorsal skin-fold window chamber model, shown in Figure 4.1, provided a stationary sample and became a crucial part of the imaging platform that was developed.
Figure 4.1: The Dorsal skin-fold window chamber model implanted in a female NcrNu female nude mouse.

Figure 4.2 displays the titanium hardware components (Research Instruments Inc. Durham, NC) that make up the window chamber in their separate and assembled forms. The surgery was performed by first anesthetizing a male or female athymic nude mouse (NCRNU-M, Taconic) with a ketamine–xylazine (90-10 mg/kg) mixture. A 10 mm diameter region of skin was removed from the dorsal area of the animal. The remaining flap of skin was then placed between the titanium plates shown in Figure 4.2 (a) and (b) forming the saddle-like structure shown in (c). The exposed fascia and vasculature of the remaining tissue was protected through the use of a standard 12mm diameter 250 μm thick coverslip. When the surgery was performed under sterile conditions the window chamber’s integrity could be maintained for up to 1 month. All procedures were carried out with institutional approval at the Princess Margaret Hospital, Toronto, Canada.
Figure 4.2: Photograph of (a) front and (b) back of window chamber titanium hardware; (c) shows the two plates in an assembled configuration. The central circular aperture is 8mm in diameter.

Once the chamber had been implanted and the animal had recovered from surgery, OCT imaging was performed by anesthetizing the animal and fixing it to the removable light weight aluminum plate, shown in Figure 4.3, using the protruding screws on the back of the chamber. The metal plate was then clipped into the heated imaging platform, shown in Figure 4.3, which was held at 37°C. The whole imaging platform then slides underneath the scanning optics of the OCT system, and was then ready for imaging as shown in Figure 4.4. Typically a red aiming laser (650 nm), coupled into the scanning optics of the OCT system, was used for precise localization of the imaging region.

Figure 4.3: Photograph of the heated OCT imaging platform, which is held at 37°C. The metal plate is detachable and bolts on to the screws shown on in Figure 4.1. The cord to the right interfaces to the heating element controller.

Figure 4.4: Photograph of anesthetized mouse on the heated imaging platform underneath the OCT imaging scan head. Various components are labeled.
When performing structural OCT imaging of the WCM, a number of clearly delineated layers are visible, as shown in Figure 4.5. The blood vessels in the skin are located in two separate layers. First, there is a layer of fine ~7 µm diameter capillaries in the fascia, which is located directly under the cover glass. A set of larger blood vessels is found directly beneath the muscle layer in the subcutaneous fat; these vessels can be as large as 200 µm.

When implanting tumors within the window chamber, the optimal location for implantation is directly underneath the fascia. Approximately 200,000 cells were typically implanted into the center of the chamber immediately after surgery. Two cell lines, thus far have been implanted in the WCM, the human renal clear cell carcinoma 786-O cell line and the 9L rat gliosarcoma cell line.

![Figure 4.5: Schematic of the various layers that make up the skin in the dorsal skin-fold window chamber model, and corresponding OCT structural image. (Scale bar represents 250 µm)](image)

**4.2 Speckle variance processing**

Speckle variance imaging is a decorrelative measurement technique that enables visualization of slow moving blood, independent of Doppler angle. It was first applied to high frequency ultrasound systems (HFUS) to visualize flow dynamics in the Xenopus Laevis tadpole, a model commonly used in developmental biology [58]. The results of these studies were quite impressive; an image from the original paper is shown in Figure 4.6 (reprinted with permission). The image shows exquisite visualization of vasculature in the spinal column (S), gills (G) and the developing toes (T).
Figure 4.6: HFUS speckle flow processing imaging of the developing hind leg region of the tadpole, showing the toes (T), gut (G), and spinal vessels (S), rendered within the 3D structural image (slice thickness = 50 μm and scale bar represents 1 mm). Note the toe vessels with diameters less than 100 μm. (Adapted from [58])

The algorithm for generating speckle variance images for both HFUS and OCT, in its most basic form, requires calculating the variance of pixels from a set of N B-mode images (N is referred to as the gate length), acquired from the same spatial location using the equation,

\[
SV_{ijk} = \frac{1}{N} \sum_{i=1}^{N} (I_{ijk} - \frac{1}{N} \sum_{i=1}^{N} I_{ijk})^2
\]  

(4.1)

Here i, j and k are indices for the frame, transverse, and axial pixels, respectively, and I is the corresponding pixel intensity value. For clarity, a schematic representation of the data set and pixel indices is shown in Figure 4.7.

Figure 4.7: Schematic representation of an acquired speckle variance data set of N frames and corresponding indices used to label the frame (i), transverse pixel location (j) and the axial pixel location (k).
For experiments described in this chapter the custom built SS-OCT system described in Chapter 3 (Figure 4.8) was used. Briefly, the system utilized a variable A-scan rate 36-67 kHz FDML fiber-ring laser source incorporating a polygon-based tunable filter with a sweeping range of 112 nm centered at 1310 nm, -6 dB ranging depth of 6 mm in air, an axial resolution of \(~7\) μm in tissue, and average output power of 40-50mW. The total cavity length used in the FDML configuration of the laser ranged from 3.3 to 4.5 km. A fiber Bragg grating provided the A-scan trigger and OCT signal recalibration was performed, as described in Chapter 3.

![Figure 4.8 Schematic of FDML SS-OCT system. SOA: Semi-conductor optical amplifier, PC: Polarization controller, C: Collimator, FBG: Fiber Bragg grating, MZI: Mach-Zender interferometer, D: Detector, DB: Dual balanced detector.](image)

A flow phantom study was initially performed to evaluate the relationship between speckle variance, flow velocity and Doppler angle. Figure 4.9 shows Doppler and speckle variance images of a 600 μm inner diameter polymer tube filled with 0.5% Intralipid (as an optical scatterer) driven by a syringe pump. The tube was embedded in Agarose gel mixed with Intralipid such that the fluid and gel had similar scattering properties. SS-OCT images (2000 × 512 pixels) were acquired at 4 different Doppler angles (75°, 80°, 85°, 89.5°) with the pump off or on (24 mm/sec peak velocity, assuming laminar flow). Doppler images were processed using phase-based color Doppler signal processing techniques, as discussed in Chapter 1, with an ensemble length of 16 A-scans. Figure 4.9 demonstrates that the speckle variance has little
dependence on Doppler angle or flow velocity and can even distinguish the Intralipid fluid from the surrounding gel at zero bulk flow rate. This effect is believed to be due to the Brownian motion of individual Intralipid particles (this topic is discussed in detail in Section 4.5).

Figure 4.9(a) Doppler phase shift measured for stationary Intralipid within a 600 μm tube at an 80° Doppler angle. (b-e) Doppler phase shift measured from Intralipid moving through the tube at a rate of 12 ml/hour (24 mm/s peak flow velocity) for Doppler angles of 75, 80, 85, 89.5°, respectively. (f-g) Corresponding normalized (to fixed value for all images) speckle variance images.

In this regime of complete decorrelation, the temporal statistics of pixel intensities from regions of blood flow are Rayleigh distributed[41], whereas regions of stationary tissue, assuming no bulk tissue clutter, give rise to Gaussian statistics

\[ g(x) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \]  

, with mean and standard deviation \( \mu \) and \( \sigma \), respectively.

The Rayleigh probability distribution is parameterized by a single variable \( \sigma \), and is defined as

\[ f(x) = \frac{x}{\sigma^2} e^{-\frac{x^2}{2\sigma^2}} \]  

, with mean = \( \sigma (\pi/2)^{1/2} \) and variance = \( \sigma^2(4-\pi)/2 \). Examples of both distributions acquired from solid silicone gel phantom, (Gaussian) and Intralipid solutions (Rayleigh) are shown in Figure 4.10.
One way to visualize the shift from the correlative regime (Gaussian) to the completely decorrelative regime (Rayleigh) is to use the ratio of the mean to standard deviation (which is fixed for to a value of 1.859 for a Rayleigh distribution) of a discretely sampled signal as a measure of the decorrelation. To demonstrate this shift, M-mode imaging of a dilute suspension of 1µm polystyrene spheres in water ($\mu_s$=1 mm$^{-1}$) was performed. The M-mode image contained 8000 A-scans, which were acquired in 0.22 s (A-scan rate of 36 kHz). Eight subsets of 32 A-scans were selected from this set of 8000 scans with A-scan spacing (lags) corresponding to 1,2,4,8,16,32,64 and 128 A-scans. Next, the pixels at 100 µm depth were isolated from each subset, generating a 1D signal for analysis. The mean and standard deviation of each signal was calculated and the ratio between the two was plotted as a function of the lag. The result is shown in Figure 4.11 and demonstrates that, for large lags, the distribution does indeed converge to that of a Rayleigh distribution of Equation (4.3). From this simple phantom result, we can safely assume that complete decorrelation of the speckle pattern occurs when imaging blood flow in vivo at frame rates below 20 fps. A more rigorous discussion of decorrelation is presented in section 4.5.
Figure 4.11: Ratio of signal mean to standard deviation for a set of signals obtained by sampling an M-mode image (acquired at 36 kHz) of 1 µm microspheres in solution at various A-scan lags. The graph demonstrates the transition from the correlative regime to the completely decorrelative regime, which exhibits Rayleigh statistics.

Our initial application of the speckle variance technique to in vivo imaging was first demonstrated in the dorsal skin-fold window chamber model. The preparation and anatomy of the animal model as well as the associated tumor models used are described in section 4.1. Here, we simply show and discuss some of the earliest images and issues that initially arose when performing 3D in vivo imaging, as several of the flow phantom and pre-optimized SV-OCT in vivo imaging studies occurred in parallel.

As previously discussed, the speckle variance algorithm uses $N$ frames acquired from the same location. However, in the original implementation of the algorithm, multiple frames from exactly the same location could not be performed when acquiring 3D volumes, due to constraints of the original data acquisition system. Therefore, a high density stack of 1600 B-mode images was acquired over a total scan range of 2.5 mm. A schematic representation of this scan pattern is shown in Figure 4.12, and used $N=3$ to generate the first 3D speckle variance images. The obvious drawback of this approach is that the stationary speckle pattern also begins to decorrelate and so using
large $N$ was not feasible. Improvements to the scanning protocols are discussed in Section 4.3.

**Figure 4.12:** Schematic representation of the scanning pattern originally used to generate 3D speckle variance images. The scanning protocol consists of acquiring a dense stack of B-mode images with interframe spacing of 1-2 μm.

To validate the SV-OCT technique, *in vivo* imaging experiments were performed and included a direct comparison of speckle variance imaging to the gold standard, intravital fluorescence confocal microscopy, in the dorsal skin fold window chamber model as demonstrated in Figure 4.13a-b. Window chamber implantation, confocal microscopy and SS-OCT imaging were carried out under continuous ketamine-xylazine anesthesia, while the mice were maintained at 37°C. Fluorescence confocal imaging (LSM 510 Meta NLO, Zeiss, excitation 488 nm, collection 530 ± 15 nm bandpass) was performed using 5 mg kg$^{-1}$ of 500 kD fluorescein labeled dextran via tail vein injection. A 5× (NA=0.25) objective was used for imaging a 1.8 ×1.8 mm$^2$ region of the window, from which a z-stack of 10 images was acquired in approximately 5 minutes, with each image having a 40 μm depth of focus and step size of 20 μm between images. 3D speckle variance OCT (1000 × 2000 × 512 pixels) was performed in the same region, where the total imaging time of the 3D volume was approximately one minute (limited by data acquisition and transfer). For both the confocal and OCT image stacks, maximum intensity projection was performed in the depth direction to obtain *en face* vascular maps for comparison, as shown in Figure 4.13c and d, respectively. The smallest vessels detectable by speckle variance imaging were ~ 25 μm in diameter. The
capillary bed, consisting of microvessels ~ 7μm in diameter as detected by the fluorescence confocal microscopy, was below the current sensitivity/resolution of the OCT system (~15 μm). An optimized SV-OCT imaging platform, capable of detecting the capillary bed, is discussed in section 4.3.

Figure 4.13(a) Dorsal skin-fold window chamber model (b) White light microscopy of entire window. The white box represents the approximate location of confocal and OCT imaging. (c) Maximum-intensity projection image of a fluorescence confocal z-stack obtained using 500 kD Fluorocein labeled dextran (1.8×1.8 mm²). (d) Speckle variance OCT en face projection image of vasculature, without the use of any external contrast agent (1.8×1.8 mm²).

Another important milestone in the development of the speckle variance technique was the application of SV-OCT to image tumor-induced angiogenesis. Figure 4.14 shows a SV-OCT microvasculature projection map of a xenograft 5 days after implantation within the WCM. The xenograft exhibits a higher microvascular density than the normal microvascular phenotype shown in Figure 4.13.
Figure 4.14: One of the first speckle variance microvascular maps from a tumor. The image is of the renal clear cell carcinoma 786-O tumor cell line 5 days after implantation in the window chamber model (~200,000 cells implanted). The tumor clearly has a higher microvascular density than the previous images shown of the normal vascular phenotype in the dorsal skin-fold window chamber model (See Figure 4.12). (Scale bar represents 500 μm)

Although implied throughout this chapter and shown using flow phantom experiments, the in vivo improvement of speckle variance over standard OCT vascular imaging techniques, was not explicitly demonstrated. In Figure 4.15, we show a comparison of a Power Doppler OCT projection image and the corresponding speckle variance image. The Power Doppler image was generated by acquiring a total of 1000 B-Mode images each with 2500 A-scans. The filter cutoff was set to π/20 radians to attenuate the clutter signal. The same data, although they were not optimal for SV, were used to generate a speckle variance image using \( N=3 \). These images demonstrate the performance benefits of SV-OCT over the Power Doppler imaging technique, when attempting to visualize the vasculature in the dorsal skin-fold window chamber model. However, we should note that for B-mode vascular imaging in high clutter situations (large motion on the order of the frame rate), intra-frame Power Doppler techniques may be superior to inter-frame speckle variance OCT as they are generally less affected by tissue motion.
Figure 4.15: Comparison of power Doppler OCT and speckle variance OCT imaging in the dorsal skin-fold window chamber model. The speckle variance algorithm outperforms the power Doppler technique, which is only able to detect the largest artery.

Figure 4.16 illustrates one of the challenges encountered with speckle variance OCT. In this figure, a dense array of blood vessels is clearly visible. However, underneath each blood vessel (which should appear approximately circular or elliptical) a streaking or shadowing artefact is observed. This shadowing occurs due to the scattering of photons first with highly forward scattering blood before undergoing a second interaction in a stationary region of tissue below the vessel. In effect, when making comparative measurements between A-scans, the photon acquires an artificial phase shift from the first interaction. Clearly, this shadowing will lead to an overestimation of vascular area. Hence, in section 4.4, a signal processing technique is discussed, that can be used to partially remove this artifact.

Figure 4.17 shows a number of different physiological artefacts we encounter when using speckle variance OCT and should always be considered when examining speckle variance images. In sub-image (a) there are large vertical streaks due to bulk tissue motion; although care is taken to stabilize the animal before imaging, large spontaneous movements by the animal can still cause these effects. In (b), smaller high-contrast vertical streaks centered on the blood vessel are seen; these streaks are possibly caused by very localized muscle spasms around blood vessels, but the underlying cause for this has still not been determined. However, it is hypothesized that animal
hydration and anesthetics may play a role. Lastly, image (c) shows a speckle variance image from an animal with a large amount of inflammation within the window chamber; although vessels are still visible, the contrast is reduced due to the high water content in the tissue, which leads to an increased mobility of scatterers.

Figure 4.16: Speckle variance OCT B-mode image showing the shadowing artifact beneath blood vessels, which is due to the highly forward scattering properties of red blood cells. (Scale bar represents 250 µm)

Figure 4.17: Speckle variance OCT projection images depicting some of the physiological artefacts that are encountered. (a) Large high intensity vertical streaks due bulk motion of the animal. (b) Localized low intensity vertical streaks may be due to muscle spasms. (c) Low contrast of blood vessels due to a large amount of inflammation. (Scale bar represents 200 µm)
4.3 Optimization of scanning parameters for different clutter scenarios

The scanning protocol used to acquire the 3D speckle variance data sets shown in the previous section consisted of taking a high-density stack of B-mode images with inter-frame spacing of 1-2 μm. This inter-frame spacing caused a partial decorrelation of static structures, which increased the variance background, thereby reducing the contrast of blood vessels. This limitation was due in part to the original data acquisition system that was used to acquire OCT data. Eventually an approach was designed and implemented that circumvented this problem. This reduced the number of actual positions sampled, while minimizing decorrelation from static structures by acquiring many frames at each sample position. A schematic of the new scanning protocol is shown in Figure 4.18 (see Figure 4.12 for old scanning protocol).

![Figure 4.18: Schematic representation of new scanning pattern that enables acquisition of multiple frames (example of N=8 depicted) from the same location before moving to the next B-mode imaging plane (see Figure 4.12 for old scanning protocol).](image)

The implementation of the new scanning pattern enabled us to increase the number of frames used in the variance calculation arbitrarily. It also allowed investigation of how the SV scanning pattern influenced the contrast of regions undergoing bulk flow/Brownian motion relative to stationary targets. It has been shown that, in the regime of complete decorrelation, when the time between acquired frames is large, the intensity values of a pixel within a fluid are Rayleigh distributed, whereas the intensity values of a pixel within a stationary solid are Gaussian distributed in time. This leads to a difference in the magnitude of calculated variance and is the source of contrast between solids and fluids. For typical imaging speeds of 20 frames per second, it was
also demonstrated that complete decorrelation occurs between frames, even for stationary Intralipid solution. However, the contrast between solids and fluids is also affected by the accuracy of the variance estimate for these distributions. We, therefore, define the SV signal-to-noise (SNR) between a “fluid” pixel and “solid” pixel, both with equal time averaged intensity, \( I_0 \), as

\[
SV_{SNR}(N, I_0) = \frac{SV_{\text{Fluid}}(N, I_0) - SV_{\text{Solid}}(N, I_0)}{\sqrt{\sigma^2_{\text{Fluid}}(N, I_0) + \sigma^2_{\text{Solid}}(N, I_0)}}
\] (4.4)

where \( SV_{\text{Fluid}} \) and \( SV_{\text{Solid}} \) are the raw SV calculated from Equation 4.1, and \( \sigma^2_{\text{Fluid}} \) and \( \sigma^2_{\text{Solid}} \) are the variance of \( SV_{\text{Fluid}} \) and \( SV_{\text{Solid}} \), respectively.

To determine the optimal gate length \( N \) in situations where bulk tissue motion is low, the stationary solid gel and 10% stationary liquid Intralipid solution were imaged using gate lengths of \( N = 2, 4, 8, 16 \) and 32 at a single B-mode imaging plane. Imaging parameters were 800 A-scans over a 5-mm-wide region (160 A-scans/mm) using an NA=0.06 objective with beam radius \( W_0 = 6.5 \mu m \). The SV contrast SNR was calculated over 1000 pixels at 16 different depth indexes, each with decreasing structural SNR (4-20 dB). The intralipid exhibited an increase in SV SNR as a function of structural signal SNR and gate length \( N \), albeit with diminishing returns at larger \( N \) (Figure 4.19a). Therefore, in this low-motion scenario, a long gate length \( (N = 8-32) \) should be used. However, a large amount of averaging is not feasible with high bulk tissue motion (BTM). To simulate increasing BTM, we induced a transverse step between each B-mode frame used in the variance calculation, while scanning the solid stationary gel (160 A-scans/mm). The mean SV for Intralipid and for the solid target with induced transverse steps of 1 to 25 \( \mu m \) between frames was calculated from 10 \( \times \) 600 pixels regions (at the focus of the lens) with approximately equal mean SNR values using \( N = 2 \) (Figure 4.19b). For an increased step size, diminishing contrast between the stationary gel and the Intralipid target was observed, with a 50% decrease in variance at a displacement corresponding to the beam waist radius (~6.5 \( \mu m \)). The results were compared to a theoretical model used to describe decorrelation resulting from beam
movement and amounts to calculating the overlap integral between two Gaussian beams [47, 58]. The SV data was fit to the function,

\[
SV \propto 1 - e^{-\left(\frac{1}{(\alpha f)^2}\right)}
\]

(4.5)

where \(dx\) is the step displacement (ranged from 1-25 µm). The results were in good agreement with the theoretical model at large displacements, and at small displacements the dominant contribution to the SV was from white noise. To account for these effects, the white noise (\(\sigma^2_{\text{white}}\)) was measured in a region near the bottom of the image.

**Figure 4.19:** (a) SV SNR measured from Intralipid and silicone gel phantoms doped with titanium dioxide for various temporal averaging gates (\(N=2,4,8,16,32\)). (b) SV measured from Intralipid compared to the silicone phantom, demonstrating the effect of spatial shifts between frames (\(N=2\)) and theory.

The correct choice of SV-OCT imaging parameters is thus crucial, and was demonstrated in two corresponding *in-vivo* models: the dorsal window chamber model (low BTM) and the human nail root (high BTM). All animal procedures were performed under ketamine-xylazine anesthetic and approved by the Princess Margaret Hospital Animal Care Committee. In the first scenario, we used the dorsal window chamber model. 1600 positions over a 5 × 5 mm² region were recorded; for each position, a gate of \(N = 8\) images with 800 A-scans per frame was used to keep the imaging time under 10 minutes, while maintaining good SV SNR. Confocal fluorescence microscopy (CFM) was performed for comparison. CFM imaging (LSM 510 MetaNLO, Zeiss) was performed immediately after I.V. injection of 5 mg kg\(^{-1}\) of 500 kD fluorescein-labeled
dextran. A 5× (NA = 0.25) objective was used for imaging the 5 ×5 mm² region of the window chamber, from which a z-stack of 10 images was acquired. Each imaged x-y section had a 40 μm depth of focus at a step size of 20 μm. The resultant projection images (over 220 μm depth) are shown in Figures 4.20a and b, with magnified regions demonstrated in c and d, respectively. It is apparent that the SV technique can detect the capillaries due to the use of improved scanning pattern and N = 8 gate length, a significant improvement over our earlier work shown in Figure 4.13 (previously, a continuously scanned high density 3D image stack was used with N = 3). The contrast of the smaller vessels in the SV-OCT image is lower than in the CFM image, but still clearly detectable. However, the capillaries in the SV-OCT image appear blurred due to the transverse resolution of the system (~13 μm), as we chose to sacrifice transverse resolution for a larger depth of field by using low NA optics.

![Figure 4.20: Normal microvascular imaging in the dorsal skin fold window chamber model, a low BTM situation (a) Fluorescence image; (b) Corresponding SV image with gate length N=8; (c) magnified region of interest from fluorescence data; (d) magnified region of interest from SV data. Scale bars represent 1 mm in a, b and 200 μm in c and d.](image)

To further demonstrate the benefits of optimized SV-OCT imaging parameters, a 9L gliosarcoma tumor was imaged 9 days after implantation in the window chamber. Orthogonal slices through the structural data are shown in Figure 4.21a. The corresponding SV vascular map is shown in Figure 4.21b, where the vasculature has been color-coded according to its depth in the tissue. This image processing technique
is discussed in detail in Section 4.4. Suspected infiltrating tumor nests, not obvious in the structural image, are detected in the SV-OCT image (indicated by blue arrows in Figure 4.21b). Figures 4.21c-e demonstrate how the contrast of smaller vessels improves as the gate length is increased from 2 to 8.

**Figure 4.21:** (a) Orthogonal slices through a 9L gliosarcoma tumor implanted within the window chamber, with the main tumor appearing as the low intensity region. (b) Corresponding microvascular projection image for the tumor, with gate length $N=8$, blue arrows indicate the location of suspected infiltrating tumor nests. The color indicates relative depth of the vessels, with yellow indicating superficial (closest to the coverslip of the window chamber) and grey indicating deeply seated vasculature. (c-e) Magnified region ($750\times750$ µm²) demonstrating the effects of increased gate length ($N=2,4,8$). Scale bar = 250 µm

To test the performance of SV in a high motion *in-vivo* scenario, the vasculature in the non-stabilized human nail root (Figure 4.22a) was imaged. In this situation only B-mode imaging was performed, as the high motion between frames distorts 3D imaging. Using a frame rate of 100 fps and $N=2$ gate length minimized the tissue motion between frames to less than the beam radius ($W_0=6.5$ µm), but structural features were present when using this short gate length. To improve the image, 3 consecutive SV images were averaged together (i direction in Figure 4.18) and this result was overlaid on the structural image shown in Figure 4.22a. The SV-OCT was also compared to color Doppler image processing (Figure 4.22b). The Kasai velocity estimator was used with
an ensemble length of 16, along with histogram rejection to remove BTM artefacts[48]. The SV approach is superior for detecting microvasculature when compared to color Doppler, due to its sensitivity and angle independence; however, it suffers much more from blood vessel shadowing artefacts as described in section 4.2.


designed for a high BTM situation (a) Structural image of human nail root with SV data overlay. (b) Structural image from the same location with color Doppler image overlay (The scan head was tilted by 10° relative to the surface of the finger, providing a Doppler angle of ~80°). Field of view is 2×2 mm². Scale bar = 500 µm

4.4 Deshadowing datasets and depth encoded visualization

The issue of shadowing beneath blood vessels poses a limitation for future work, which will attempt to generate 3D vascular metrics from the speckle variance datasets. It is also one of the primary reasons why only projection images of the vasculature have been shown. However, this projection technique removes information associated with the depth of a given blood vessel. To eliminate these shadowing effects, a step-down exponential filter, which at least partially removes the shadowing effect while preserving the overall vascular structure[59] was implemented. This encoding scheme, which displays depth as color, thereby preserves the depth information of blood vessels in 2D vascular maps.

The deshadowing is accomplished by starting at the top surface of the tissue and working in an axial direction, where the speckle variance signal of the current point is attenuated by a factor proportional to the sum of deshadowed speckle variance pixels above it. Mathematically, this process can be expressed as
Here, $x$ denotes the frame, $y$ the lateral location and $z$ the depth in each image and $\gamma$ is a proportionality constant that controls the rate of attenuation. Figure 4.23 shows a B-mode speckle variance image before and after deshadowing; although there is an overall attenuation of the speckle variance intensity in the deshadowed image, it demonstrates significantly less shadowing than the original.

\[ SV_{DS}(x, y, z) = SV(x, y, z) \times e^{-\frac{1}{\gamma} \sum_{i=1}^{N} SV_{DS}(x, y, i)} \]  

(4.6)

**Figure 4.23:** Speckle variance OCT B-mode images of tumor microvasculature (a) without deshadowing (b) with deshadowing. (Scale bar represents 1 mm)

To qualitatively demonstrate that there is no significant loss of vascular information, projection images for both a shadowed and deshadowed 3D vascular dataset are shown in Figure 4.24.
Figure 4.24: Projection images of 3D speckle variance datasets that (a) have not been deshadowed and (b) have been deshadowed. (Scale bar represents 1mm)

With the shadowing artifact attenuated, a color depth-encoding scheme to preserve depth information in 2D vascular maps was implemented. This process yielded the information-rich vascular maps shown in Figure 4.25. The display process requires several steps, where each image in the stack is first encoded with a RGB color depending on its depth in the stack. Secondly, the transparency or alpha channel for each pixel in the stack is set to its speckle variance intensity. The RGB and alpha channels are then combined into a stack of RGBα PNG images. These PNG images are then layered one upon another from the bottom up using Amira™ software (Visualization Toolkit, version 5.1).
Figure 4.25: Depth encoded color projection image of six different 9L gliosarcoma tumors imaged 9 days after implantation in the dorsal skin-fold window chamber model. Features closest to the coverslip are encoded with green-yellow, while deeper features are encoded with orange-red. The transparency of a pixel is determined by the speckle variance intensity. The images show significant amount of intra and inter-tumor microvascular heterogeneity.

4.5 Measurements of speckle decorrelation times induced by Brownian motion

The decorrelation of the speckle pattern gives rise to the contrast mechanism in SV-OCT images. We have previously stated that, when imaging at 20 fps, complete speckle pattern decorrelation is achieved between frames, even when imaging stationary intralipid (no bulk flow). We attributed this effect to the Brownian motion of the Intralipid particles. In this section, we set out to show that this effect is indeed due to the Brownian motion of particles. It should be noted that, in order to achieve maximum contrast for morphological imaging of vasculature, the frame rate must be selected to allow for complete inter-frame decorrelation of the speckle pattern in blood, while also
choosing a sufficiently high frame rate such that the speckle pattern in stationary tissue
does not decorrelate appreciably. Thus, the Brownian motion decorrelation times
presented in this section provide an approximate upper bound for decorrelation when
imaging blood flow in vivo (i.e., the decorrelation times for blood flow in vivo will be
shorter than the decorrelation due to Brownian motion).

Brownian motion describes the “random walk” process of particles in suspension as a
function of time at a particular thermodynamic temperature $T$. In 1905, Einstein made
the important realization that the same forces that cause these random Brownian
motions would create drag forces if the particle was pushed in a particular direction.
Using statistical mechanics, Einstein was able to show that the mean squared
displacement of a particle undergoing Brownian motion after a time $t$ is given by

$$\langle r^2 \rangle = 6k_BT\mu t \quad (4.7)$$

where $k_B$ is the Boltzmann constant, $T$ is the temperature and $\mu$ is the mobility of the
particle undergoing Brownian motion. The mobility coefficient relates the drift speed of
the particle in thermodynamic equilibrium with its surroundings to the force, $F$, being
applied to the particle

$$v_{drift} = \mu F \quad (4.8)$$

The mobility coefficient can be calculated analytically from fluid mechanics in the special
case of a small spherical particle moving slowly in a viscous fluid

$$\mu_B = \frac{1}{6\pi\eta R} \quad (4.9)$$

where $\eta$ is the viscosity of the solution and $R$ is the radius of the particle. For this
special situation the inverse of the mobility coefficient is sometimes called the Stokes
drag coefficient. Einstein also made the important connection that the diffusion
coefficient, $D$, can be related to the thermodynamic temperature through,

$$D = \mu k_B T \quad (4.10)$$

Thus, for a small spherical particle undergoing Brownian motion we can write its mean
squared displacement after a time $t$ as
\[ \langle r^2 \rangle = 6D_B t \]  
(4.11)

where \( D_B \) is

\[ D_B = \frac{k_B T}{6\pi \eta r} \]  
(4.12)

and is referred to as the Einstein-Stokes relation for the diffusion of small spherical particles.

In our OCT measurements we want to relate the rate at which the speckle pattern decorrelates when imaging particles undergoing Brownian motion to some physical parameter of the system. Speckle is generated from the constructive or destructive interference of scattered light from the scatterers within a given voxel. In order for the signal from that voxel to change appreciably, one would expect that the scatterers within that volume must reorder themselves on a distance scale of \( \sim \lambda_0/2n \), where \( \lambda_0 \) is the wavelength of the incident light in vacuum and \( n \) is the index of refraction of the medium, which results in a round trip scattering path length difference \( \lambda_0/n \). Thus, to determine a characteristic timescale over which the speckle pattern will decay when imaging particles undergoing Brownian motion, we substitute \( \langle r^2 \rangle = (\lambda_0/2n)^2 \) in equation 4.11:

\[ \frac{\lambda_0^2}{4n^2} = 6D_B \tau \]  
(4.13)

where \( \tau \) is the characteristic decay time. If we now use the wavevector notation \( k = 4\pi n/\lambda_0 \) and rearrange for the decay time we get

\[ \tau = \frac{\pi^2}{6k^2 D_B} \]  
(4.14)

The above expression is simply a phenomenological approximation to the decorrelation time. In actuality, the laser speckle decorrelation time for a weakly scattering solution of particles undergoing Brownian has been rigorously shown to be equal to

\[ \tau = \frac{1}{2k^2 D_B} \]  
(4.15)

and the characteristic shape for the decay has been shown to be exponential [60]. Previous work in the TD-OCT field has validated this expression experimentally for
depth-resolved measurements[61, 62]. In the highly scattering regime, where each photon scatters more than once before being detected, the decorrelation time becomes

$$\tau = \frac{1}{2\beta k^2 D_B} \quad (4.16)$$

Here \(\beta = s/l^*\), where \(s\) is the optical path length to the point of measurement in the medium and \(l^*\) is the photon random walk length defined as \(l^* = l_s/(1-g)\), \(l_s\) is the photon scattering length and \(g\) is the coefficient of anisotropy[62]. To facilitate our experiments we chose to work in the weakly scattering regime, with the understanding that, in a highly scattering regime such as that of biological tissues or highly concentrated solutions of Intralipid, the decorrelation time must be necessarily smaller as per the \(\beta\) factor in Equation (4.16).

The Brownian motion experiments comprised OCT intensity measurements at a depth of 50 µm within 4 different viscosity glycerol-water solutions (0%, 30%, 50% and 70% glycerol by weight) doped with 1 µm polystyrene microspheres. The 4 solutions had viscosities of 0.000968, .00235, .00567 and 0.0196 Pa•s at 21.5°C. These values were calculated from an empirical model developed by Cheng [63]. The scattering length for each solution was calculated by first estimating the index of refraction of glycerol, water and polystyrene at 1.3µm using the empirically based Sellmeiere equations, with \(n_{\text{glycerol}}=1.4614, n_{\text{water}}=1.3207, n_{\text{polystyrene}}=1.5613\) [64]. For glycerol the index of refraction varies linearly (as it does for most organic materials dissolved in water) with the mass concentration of the dissolved substance[65]. Thus, we estimated the index of refraction of each of the solution in a straightforward manner, which yielded \(n_0=1.3207, n_{30}=1.3507, n_{50}=1.3707, n_{70}=1.3907\). Once the index of refraction of each solution was known, we used a Mie scattering calculator [66] to obtain an estimate of the scattering length for the polystyrene microspheres in each solution \(l_0=0.4081\) mm, \(l_{30}=0.4456\) mm, \(l_{50}=0.4742\) mm and \(l_{70}=0.5059\) mm (corresponding scattering coefficients \(\mu_0=2.4502\) mm\(^{-1}\), \(\mu_{30}=2.244\) mm\(^{-1}\), \(\mu_{50}=2.1086\) mm\(^{-1}\), \(\mu_{70}=1.9767\) mm\(^{-1}\)). The exact value of the scattering length does not play a significant role in our experiments since measurements were made at a depth much smaller than the scattering length, thus ensuring we remain in the singly-scattered regime.
For each solution the OCT intensity signal was sampled at a rate of 420 Hz for approximately 25 seconds. These intensity signals are plotted for each of the 4 different viscosity solutions in the M-mode display of Figure 4.26; qualitative differences due to the different viscosities are easily discernible. To calculate the decorrelation time, the 1/e fall off of the autocorrelation function was measured. The decorrelation time is determined by first calculating the power spectrum of the normalized intensity signal and then taking real part (since the original intensity signal is purely real) of the inverse Fourier transform of the power spectrum.

The autocorrelation function for each of the solutions is plotted in Figure 4.27 and demonstrates the characteristic exponential decay expected for particles undergoing Brownian motion. The autocorrelation functions were fit with 2-parameter exponential functions ($A \cdot \exp(-b \cdot t)$, $R^2$ values > 0.995), and the fitted curves were subsequently used to determine the 1/e fall off. Figure 4.28 plots experimental and theoretical decorrelation times for each of the 4 viscosity solutions. Good agreement was observed, with all experimental values within 10% of the theoretical values. Error bars on experimental values are from the errors obtained in the fitting of the exponential decay.

For microspheres undergoing Brownian motion in water, we find experimentally a decorrelation time of $\tau = 0.0062$ s (in the highly scattering regime, this would be reduced by a factor of $\beta = s/l^*$. This decorrelation time corresponds to a frame rate of 160 fps, a factor of eight greater than our 20 fps frame rate, giving credibility to the hypothesis that we were in fact observing complete decorrelation due to Brownian motion between frames. These decorrelation times also provide an upper bound for speckle decorrelation rates of highly scattering flowing blood encountered in vivo. The experiments presented here set the framework for future measurements of decorrelation times in bulk flow and in in vivo models.
Figure 4.26: OCT intensity signal sampled at a rate of 420 Hz at a depth of 50 µm within four different viscosity glycerol – water solutions doped with 1µm polystyrene microspheres at 21.5°C.

Figure 4.27: Autocorrelation function for the 4 different viscosity solutions doped with 1µm polystyrene microspheres. Autocorrelation functions exhibit exponential decay, which is characteristic of Brownian motion. Higher viscosity solutions exhibit a slower exponential decay.
This chapter has outlined the development of a 3D microvasculature imaging technique termed speckle variance OCT. SV-OCT has a number of advantages over traditional Doppler OCT-based imaging and fluorescence confocal microscopy, namely:

(1) Doppler angle Independence

(2) High sensitivity (capability of visualizing Brownian motion)

(3) Fast (capable of imaging 5 mm × 5 mm ROI in 5 minutes)

(4) Intrinsic contrast (No contrast agents needed)

(5) Relatively deep imaging (up to 2mm in tissue)
These characteristics make SV-OCT an attractive tool for longitudinal monitoring of microvascular changes due to tumor angiogenesis and/or response to vascular targeted therapies in preclinical models. These in vivo applications are precisely the topic of the following chapter.
Speckle variance detection of microvasculature using swept-source optical coherence tomography

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We report on imaging of microcirculation by calculating the speckle variance of optical coherence tomography (OCT) structural images acquired using a Fourier domain mode-locked swept-wavelength laser. The algorithm calculates interframe speckle variance in two-dimensional and three-dimensional OCT data sets and shows little dependence to the Doppler angle ranging from 75° to 90°. We demonstrate in vivo detection of blood flow in vessels as small as 25 μm in diameter in a dorsal skinfold window chamber model with direct comparison with intravital fluorescence confocal microscopy. This technique can visualize vessel-size-dependent vascular shutdown and transient vascular occlusion during Visudyne photodynamic therapy and may provide opportunities for studying therapeutic effects of antivascular treatments without on exogenous contrast agent. © 2008 Optical Society of America


Optical coherence tomography (OCT) [1] is a high-resolution (1–10 μm) modality used for minimally invasive imaging in anatomical sites, such as the retina, gastrointestinal tract, airways, and (coronary) vasculature [2]. Various OCT blood flow detection techniques [3–11] have been developed with velocity sensitivity ranging from 50–500 μm/s, depending on the minimum detectable frequency or phase shift and the Doppler angle. As microvasculature can be tortuous, the angular dependency of these techniques may lead to incomplete vascular maps in vivo. Fingler et al. [12] demonstrated a phase variance technique for detecting fluid mobility and transverse flow. Speckle variance techniques based on structural image intensity have been used in tumor microvascular imaging with high-frequency ultrasound [13,14]. Spectral speckle analysis has also been attempted in OCT [15]. However, its performance is hindered by the computational complexity and slow frame rate in the initial demonstration. Recent developments in swept-wavelength lasers [16] and the application of Fourier domain mode locking (FDML) [17] can provide an imaging frame rate up to 370 kHz, which may yield a sufficient imaging speed for real-time microvascular imaging using speckle-based techniques.

In this Letter we report a simple and computationally efficient microvascular detection algorithm based on interframe speckle variance processing of structural image intensity using an FDML swept-source optical coherence tomography (SS-OCT) system and demonstrate its performance in a flow phantom and an animal model. As shown in Fig. 1, the SS-OCT system uses a 45–67 kHz FDML fiber-ring laser source incorporating a polygon-based tunable filter [7] with a sweeping range of 112 nm centered at 1310 nm, −6 dB ranging depth of 6 mm in air (corresponding to a coherence length of 12 mm), axial resolution of ~8 μm in tissue, and average output power of 48 mW. The total cavity length used in FDML the laser ranges from 3.3 to 4.5 km. A fiber Bragg grating provides the A-scan trigger and OCT signal recalibration was performed as previously described in [18]. Interframe speckle variance images (SVj,k) of the structural OCT intensity (Ij,k) are calculated across N=3 B-mode images as

\[ SV_{j,k} = \frac{1}{N} \sum_{i=1}^{N} (I_{j,k} - I_{mean})^2, \]

where j and k are lateral and depth indices of the B-mode images and i denotes the B-mode slice index with I_{mean} as the average over the same set of pixels.

Fig. 1. Schematic of the FDML SS-OCT system. SOA, semiconductor optical amplifier; PC, polarization controller; C, collimator; FBG, fiber Bragg grating; MZI, Mach-Zehnder interferometer; D, detector; DB, dual balanced detector.

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A flow phantom study was performed to evaluate the relationship among speckle variance, flow velocity, and the Doppler angle. Figure 2 shows Doppler and speckle variance images of a 600 µm inner diameter polymer tube filled with 0.5% Intralipid driven by a syringe pump. The tube was embedded in agarose gel mixed with Intralipid such that the fluid and gel had similar scattering properties. SS-OCT images (2000 × 512 pixels) were acquired at four different Doppler angles (75°, 80°, 85°, and 90.5°) with the pump off or on (24 mm/s peak velocity, assuming laminar flow). Doppler images were processed using phase-based color Doppler signal processing techniques [6] with an ensemble length of 16 A-scans. For speckle variance imaging, four adjacent A-scans were averaged together to improve the signal-to-noise ratio of the structural image [5,15], leading to a decrease in the speckle variance noise floor. Figure 2 demonstrates that the speckle variance has little dependence on the Doppler angle or flow velocity and can even distinguish the Intralipid fluid from the surrounding gel at a zero bulk flow rate.

We directly compared speckle variance imaging with intravital fluorescence confocal microscopy using a dorsal skinfold window chamber model (WCW) in athymic nude mice (NCrNU-M, Taconic) [19] as shown in Figs. 3a and 3b, where the exposed skin flap and cutaneous microvasculature were protected by a glass coverslip window. Surgery, confocal microscopy, and SS-OCT imaging were carried out under ketamine-xylazene anesthesia with the mice maintained at body temperature. All procedures were carried out with institutional approval at the Princess Margaret Hospital, Toronto, Canada. Fluorescence confocal imaging (LSM 510 Meta NLO, Zeiss, excitation 488 nm, collection 530±15 nm bandpass) was performed using 5 mg kg⁻¹ of 500 kD fluorescein labeled dextran via tail vein injection. A 5× (NA = 0.25) objective was used for imaging a 1.8 mm × 1.8 mm region of the window from which a z stack of ten images was acquired within 10 min of tail vein injection, each having a 40 µm depth of focus and step size of 20 µm between images. Three-dimensional (3D) (1000×2000×512 pixels) speckle variance OCT was performed in the same region; total imaging time of the 3D volume was approximately 1 min (limited by data acquisition and transfer). For both the confocal and OCT image stacks, maximum intensity projection was performed in the depth direction to obtain en face vascular maps as shown in Figs. 3c and 3d, respectively. The smallest vessels detectable by speckle variance imaging were ~25 µm in diameter. The capillary bed, consisting of microvessels ~5 µm in diameter as detected by the fluorescence confocal microscopy, was below the lateral resolution of the OCT system (15 µm).

Speckle variance OCT imaging can be used to monitor antivascular treatment effects dynamically. In Fig. 4, we demonstrate detection of microvascular changes induced by Visudyne photodynamic therapy (PDT) by imaging before, during, and immediately posttreatment. The PDT treatment parameters were Visudyne 2 mg/kg, light exposure 10 min at 690 nm, with a fluence rate of ~42 mW/cm². Rapid shutdown of the smaller blood vessels within the treatment region was observed approximately 1 min after light
Fig. 4. (Color online) Speckle variance OCT imaging of Visudyne-mediated PDT within a 1 mm × 1 mm region of the dorsal skinfold window chamber mouse model (fluence rate=42 mW/cm², total fluence=25 J/cm², treatment time=10 min). a. Vasculature prior to laser irradiation. b. One minute after start of laser irradiation. c. Total shutdown of right branch. d. Reperfusion of right branch with imaging artifact. e. Reperfusion of right branch without imaging artifact. f. 10 min postind of laser irradiation showing reperfusion, but main vessels still appear to be constricted.

exposure began (Fig. 4b, t=1 min). A transient localized total occlusion of the larger vessels occurred between t=5 min 40 s and 6 min 35 s, with a partial reperfusion event at 6 min 27 s (Figs. 4c–4e). At the end of the PDT light irradiation (Fig. 4g), there was persistent vascular shutdown of the smaller vessels, and the larger vessels had an approximately 30% reduction of diameter as measured by speckle variance OCT.

The main advantage of the speckle variance processing technique compared with conventional Doppler OCT is its simplicity, since it can be implemented in real time to provide additional Doppler angle-independent microvascular information with little additional computational complexity. Being based on intrinsic contrast, this technique also has a potential benefit over fluorescence microscopy, especially when imaging leaky neovasculature, where background saturation can occur rapidly owing to abnormal vessel permeability and extravasation of fluorescent markers. Like phase-variance-based techniques [12], speckle variance detection also suffers from multiple scattering induced artifacts leading to artificial speckle variance contrast beneath the blood vessels. Another key disadvantage is the effect of interframe bulk tissue motion, which can dominate the speckle variance. However, higher-frame-rate (>1000 fps) FDMIL OCT systems are being developed for volumetric imaging that may remove artifacts owing to physiological motion. At these very high frame rates, speckle decorrelation between frames may not be complete, thus leading to a decrease in speckle variance contrast. However, it may also be possible to quantitatively relate the amount of decorrelation to the flow velocity within the vessel. For such systems, interframe speckle variance may be an attractive technique to detect, quantify, and monitor effects of vascular targeted therapies, such as PDT and anti-vascular drugs in animal models beyond the geometric confines of the WCM.

In summary, we have shown interframe speckle variance detection of microcirculation using an FDMIL polygon-based SS-OCT system in a flow phantom and compared its imaging performance with intravital fluorescence confocal microscopy techniques in an in vivo WCM. We demonstrated that speckle variance OCT could detect vessel size dependent vascular shutdown and transient vessel occlusion during PDT.

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References
Optimized speckle variance OCT imaging of microvasculature

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We optimize speckle variance optical coherence tomography (SV-OCT) imaging of microvasculature in high and low bulk tissue motion scenarios. To achieve a significant level of image contrast, frame rates must be optimized such that tissue displacement between frames is less than the beam radius. We demonstrate that higher accuracy estimates of speckle variance can enhance the detection of capillaries. These findings are illustrated in vivo by imaging the dorsal window chamber model (low bulk motion). We also show SV-OCT imaging of the nonstabilized finger (high bulk motion), using optimized imaging parameters, demonstrating better vessel detection than Doppler OCT. © 2010 Optical Society of America

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Over the past decade, two types of algorithms and techniques for microvascular imaging using optical coherence tomography (OCT) have been developed, those that quantify blood flow/velocity, and those that visualize microvasculature but generally provide no quantitative flow information. Techniques in the first category, such as color Doppler imaging, are based on measuring phase shifts induced by moving scatterers [1–3]. In addition, Barton et al. have shown that speckle spatial frequency analysis in OCT intensity images can extract flow rates [4]. The second category was developed to visualize, but not quantify, microvascular flow. They include speckle variance (SV) [5] and power doppler OCT imaging [2,6]. Unlike the spatial frequency analysis method [4], speckle variance OCT (svOCT) identifies microvasculature by calculating the interface intensity variance of structural images, where contrast is based on different time-varying properties of fluid (blood) versus solid tissue components. Despite promising results [5], interferome calculation is seriously affected by bulk tissue motion (BTM) if care is not taken to optimize acquisition parameters. In this Letter, we characterize svOCT image acquisition parameters under two different bulk sample motion regimes in phantoms and then use these results to perform optimized svOCT in two corresponding in vivo imaging scenarios.

SV is calculated from the structural OCT signal by

\[ SV_{ijk} = \frac{1}{N_{i-1}} \sum_{j=1}^{N} \left( I_{ijk} - \frac{1}{N_{i-1}} \sum_{j=1}^{N} I_{ijk} \right)^2, \]

where the gate length specifies the number of frames used in the variance calculation [N in Eq. (1)], and i, j, and k are indices for the frame, transverse, and axial pixels, respectively. Two parameters must be chosen to optimize the SV calculation in different tissue motion situations—the gate length N and frame rate or field of view. To determine these parameters we performed a phantom study using intralipid solution and a solid silicone gel containing titanium dioxide.

SV contrast is due to different time dependent scattering properties of fluids and solids. In the regime of complete decorrelation, when the time between acquired frames is large, the intensity values of a pixel within a fluid are Rayleigh distributed, whereas the intensity values of a pixel within a stationary solid are Gaussian distributed in time [7]. This leads to a difference in the magnitude of calculated variance and is the source of contrast between solids and fluids. For typical imaging speeds of 20–100 frames per second, we have previously demonstrated that complete decorrelation occurs between frames, even for stationary intralipid solution [5]. Furthermore, the contrast between solids and fluids is also affected by the accuracy of the variance estimate for these distributions. We define the SV signal-to-noise ratio (SNR) between a “fluid” pixel and a “solid” pixel, both with equal time averaged intensity I_o as

\[ SV_{SNR}(N,I_o) = \frac{SV_{Fluid}(N,I_o) - SV_{Solid}(N,I_o)}{\sqrt{\sigma^2_{Fluid}(N,I_o) + \sigma^2_{Solid}(N,I_o)}}. \]

where SV_{Fluid} and SV_{Solid} are the raw SV calculated from Eq. (1) and \( \sigma^2_{Fluid} \) and \( \sigma^2_{Solid} \) are the variances of SV_{Fluid} and SV_{Solid}, respectively.

To determine the optimal gate length in situations where BTM is low, the stationary solid gel and 10% stationary liquid intralipid solution were imaged using gate lengths of N=2, 4, 8, 16, and 32 at a single B-mode imaging plane. All experiments used a 36 kHz swept source OCT system similar to that described previously [5]. Briefly, the system utilized a swept laser source based on a polygon filter configuration with coherence length of 6 mm. The axial imaging range was 3 mm and transverse imaging parameters were 890 A-scans over a 5-mm-wide region (160 A-scans/mm) using an NA=0.06 objective with beam radius v0=6.5 μm. The SV contrast SNR was
calculated over 1000 pixels. The intralipid exhibited an increase in SV SNR as a function of structural signal SNR and gate length, albeit with diminishing returns at larger N (Fig. 1(a)). Therefore, in this low-motion scenario, a long gate length (N = 8–32) should be used.

However, a large amount of averaging is not feasible with high BTM. To simulate increasing BTM, we induced a transverse step between each B-mode frame used in the variance calculation; while scanning the solid stationary gel (160 A-scans/mm). The mean SV for intralipid and for the solid target with induced transverse steps of 1 to 25 μm between frames was calculated from 10 × 600 pixels regions with approximately equal mean SNR values using N = 2 (Fig. 1(b)). For an increased step size, diminishing contrast between the stationary gel and the intralipid target was observed, with a 50% decrease in variance at a displacement corresponding to the beam waist radius (∼6.5 μm). The results were compared to a theoretical model used to describe decorrelation resulting from beam movement [8,9]. The SV data was fit to the function, \( SV = \frac{1}{\sqrt{N}} \times (dx/\alpha)^2 \), where dx is the step displacement (ranged from 1–25 μm). The results were in good agreement with the theoretical model at large displacements, and at small displacements the dominant contribution to the SV was from white noise. To account for these effects, the white noise (\( \sigma^{\text{white}} \)) was measured in a region near the bottom of the image.

The correct choice of SV OCT imaging parameters is crucial, and was demonstrated in simulating in vivo models: the dorsal window chamber model (low BTM) and the human nail root (high BTM). All animal procedures were performed under ketamine–xylazine anesthetic and approved by the Princess Margaret Hospital Animal Care Committee. In the first scenario, we used the dorsal window chamber model in a NCrNu (Taconic) female nude mice [5]. 1600 positions over a 5 × 5 mm² region were recorded; for each position, a gate of N = 8 images with 800 A-scans per frame was used to keep the imaging time under 10 min, while maintaining good SV SNR. Confocal fluorescence microscopy (CFM) was performed as a comparison. CFM imaging (LSM 510 MetaNLO, Zeiss) was performed immediately after intravenous injection of 5 mg kg⁻¹ of 500 kD fluorescein-labeled dextran. A ×5 (NA = 0.25) objective was used for imaging the 5 × 5 mm² region of the window chamber, from which a z stack of 10 images was acquired. Each imaged x-y section had a 40 μm depth of focus at a step size of 20 μm. The resultant projection (over 220 μm depth) images are shown in Figs. 2(a) and 2(b), with magnified regions demonstrated in Figs. 2(c) and 2(d), respectively. It is apparent that the SV technique can detect the capillaries, a significant improvement over our previously published work [5] due to the use of improved scanning pattern and N = 8 gate length (previously, a continuously scanned high density three-dimensional (3D) image stack was used with N = 3). The contrast of the smaller vessels in the sV OCT image is lower than in the CFM image, but still clearly detectable. However, the capillaries in sV OCT image appear blurred due to the transverse resolution of the system (∼13 μm), as we chose to sacrifice transverse resolution for a larger depth of field. To further demonstrate the benefits of optimized sV OCT imaging parameters, a 9L gliosarcoma tumor was imaged 9 days after implantation in the window chamber. Orthogonal slices through the structural data are shown in Fig. 3(a). The corresponding SV vascular map is shown in Fig. 3(b), where the vasculature has been color coded according to its depth in the tissue [10]. Satellite metastasis, not obvious in the structural image, are detected in the sV OCT image [indicated by blue arrows in Fig. 3(b)]. Figures 3(c)–3(e) demonstrate how the contrast of smaller vessels improves as the gate length is increased from 2 to 8. Although the amount of data acquired to generate the image in Fig. 3(b) is large, it is significantly less than the storage requirements for phase sensitive techniques such as intensity modulated phase variance [10] and optical angiography [11].

To test the performance of SV in a high motion in

![Fig. 1. (a) SV SNR measured from intralipid and silicone gel phantoms doped with titanium dioxide for various temporal averaging gates (N=2, 4, 8, 16, 32). (b) SV measured from intralipid compared to the silicone phantom demonstrating the effect of spatial shifts between frames (N=2) and theory.](image1)

![Fig. 2. (Color online) In vivo imaging in a low BTM situation: (a) fluorescence image; (b) corresponding SV image with gate length N=8; (c) magnified region of interest from fluorescence data; (d) magnified region of interest from SV data. Scale bars represent 1 mm in (a) and (b) and 200 μm in (c) and (d).](image2)
**Fig. 3.** (Color online) (a) Orthogonal slices through a 9L gliosarcoma tumor (b) implanted within the window chamber, with the main tumor appearing as the low intensity region. (b) Corresponding microvascular projection image for the tumor, with gate length \( N = 8 \), arrows indicate the location of satellite metastasis. The color indicates relative depth of the vessels, with brighter shades indicating superficial (closest to the coverslip of the window chamber) and gray indicating deeply seated vasculature. (c)-(e) Magnified region (750 x 750 \( \mu m^2 \)) demonstrating the effects of increased gate length (\( N = 2, 4, 8 \)). Scale is bar=250 \( \mu m \).

In vivo scenario, we imaged the vasculature in the non-stabilized human nail root [Fig. 4(a)]. In this situation we performed only B-mode imaging. Using a frame rate of 100 fps and \( N = 2 \) gate length, minimized the tissue motion between frames to less than the beam radius, but structural features were present when using this short gate length. To improve the image, we averaged three consecutive SV images and displayed this result on the structural image shown in Fig. 4(a). We also compare the svOCT to color Doppler imaging processing [Fig. 4(b)].

The Kasai velocity estimator was used with an ensemble length of 16, along with histogram rejection to remove BTM artifacts [2]. The SV approach is superior for detecting microvasculature when compared to color Doppler, due to its sensitivity and angle independence; however, it suffers from blood vessel shadowing artifacts [12,13].

To summarize, svOCT is a highly sensitive endogenous-contrast microvascular imaging technique that performs best in situations with low tissue motion, where large gate lengths can be used. Optimization of svOCT imaging parameters led to a significant improvement in capillary detection. In situations where tissue motion is high, the field of view or frame rate during acquisition must be optimized to keep inter-frame displacements to less than the beam waist radius. Finally, the lower computational complexity and data storage requirements of svOCT, relative to phase sensitive microvascular imaging techniques adds further utility to the technique.

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**References**

Chapter 5: In vivo applications of techniques developed

The techniques developed in chapters 2-4 include the implementation of color Doppler algorithms and speckle variance imaging on swept source OCT systems. This chapter highlights the contributions I have made using these techniques to biological problems of interest. In particular, the application of 3D Doppler OCT and speckle variance OCT to monitoring the vascular targeted photodynamic therapy and a technique which degrades HIF-α in an effort to disrupt angiogenesis in preclinical models.

5.1 Monitoring two photon PDT response using 3D swept source DOCT

Recently, it has been suggested that two-photon photosensitizers be used to achieve an even higher spatial localization of the PDT effect[67]. As the two photon excitation scales quadratically with light intensity, only photosensitizers located very close to the focus of a femtosecond pulsed laser are excited. This leads to excellent 3D control of the treatment volume. In collaboration with Collins et al. DOCT was used to help validate the first PDT drug designed for two-photon excitation in vivo [68]. This 2-photon photosensitizer was based on a porphyrin dimer that had a 2-photon absorption peak at 916 nm and exhibited a two photon cross-section 300-1500 times higher than conventional PDT drugs verteporfin and photofrin.

2D and 3D Doppler OCT were used to monitor vascular shutdown pre and post two-photon excitation of the photosensitizer in the Dorsal skin-fold window chamber model. In these studies optical coherence tomography(OCT) imaging was performed using a 24-kHz swept source OCT system (OCM1300SS, Thorlabs) with an isotropic spatial resolution of approximately 10 µm. 3D image stacks were acquired before and after two-photon light exposure in a 1.0 mm³ cubic volume of interest. The irradiated regions were selected using the real-time colour Doppler mode to identify blood vessels. Both structural and Doppler images were collected and processed for peak-blood-flow velocity estimation and 3D rendering. The Doppler background noise was first minimized using a histogram rejection algorithm, after which peak blood flow velocity estimates were made [48]. For 3D visualizations a consecutive series of segmented
blood-vessel images was used to reconstruct the vessel surfaces, which were subsequently rendered using image processing software (Amira 4.1.1, Mercury Computer Systems).

For confocal microscopy a 5× (NA 0.25) objective lens was used to in conjunction with a laser scanning microscope (LSM 510 Meta NLO, Zeiss) through the window chamber with either 920 nm, 300 fs, 90 MHz light from a Ti:sapphire laser (Chameleon, Coherent), 488 nm from an argon ion laser, or 543 nm from a helium-neon laser. A dorsal window chamber mouse was anesthetised with 80 mg kg⁻¹ ketamine and 13 mg kg⁻¹ xylazine with its body temperature maintained using a 30 °C stage heater. The mouse was injected, via the tail vein, with 200 μL of 5 % dextrose containing 15 mg kg⁻¹ of 2,000,000 MW tetramethylrhodamine-labelled dextran (λ_{ex} 543 nm, λ_{em} 565–615 nm, emission shown in red).

The control group, shown in Figure 5.1, 180 mg kg⁻¹ of Visudyne™ (containing 3.25 mg kg⁻¹ of verteporfin) was administered intravenously. The artery was identified and the focal region selected using a 920 nm light (< 3.0 mW) and a 83 × 83 μm region was irradiated (39 mW) as four series of vertical stacks consisting of 5 images, each 10 μm apart through the depth of the vessel. The irradiated region is denoted by the white box in Figure 5.1 at 20 min after the two-photon light dose, 5 mg kg⁻¹ of 464,000 MW dextran labelled with fluorescein (FITC-dextran, Sigma), was injected via the tail vein in 100 μL of 5 % dextrose and imaged 15 min later (λ_{ex} 488 nm, λ_{em} long pass 505 nm, emission shown in green). The second FITC-dextran blood tracer demonstrated bright fluorescence throughout the vasculature, including the irradiated section of the artery, indicating continuing vessel function at the treatment site. This was confirmed by OCT imaging.

For the two-photon PDT treatment group shown in Figure 5.2, mice bearing dorsal window chambers were administered 10 mg kg⁻¹ of the two-photon photosensitizer diluted from a 10 mM stock in dimethylsulfoxide. A selected artery was targeted with 920-nm light (<3 mW) guided by the striations caused by blood flow visible in the transmission image. An 83 × 83 × 40 μm volume was irradiated four times (39 mW) as a stack of five planes, 10 mm apart, each plane consisting of 512× 512 pixels, with a dwell time of 0.8 ms per pixel. All optical imaging techniques demonstrated highly localized vascular shutdown of vessels.
Figure 5.1: Example confocal and optical coherence tomography (OCT) images (a–e) before and (f–j) after two-photon irradiation with verteporfin and 2,000,000 MW tetramethylrhodamine labelled dextran (Molecular Probes). Confocal transmission (a, f) fluorescence (b,g) and superimposed (c, h) images of vascular tracers are demonstrated, scale bar = 200 μm. Three-dimensional images of blood flow (d, i) overlaid on pre-treatment stereomicroscope images) produced by Doppler OCT imaging are included, and the Doppler flow profiles at the irradiation site are segmented and superimposed onto the structural OCT images (e, j). (Reprinted with permission, [68])

Figure 5.2: In vivo two-photon blood-vessel closure with two photon photosensitizer (a) Pre-treatment confocal (×5, 0.25 NA) transmission (left panel, λ<sub>ex</sub>=543 nm), TRITC–dextran fluorescence (middle panel, λ<sub>ex</sub>= 543 nm, λ<sub>em</sub>= 565–615 nm) and superimposed (right panel) images (scale bar, 200 μm). (b) Images immediately after TPE-PDT with 2-photon photosensitizer (scale bar, 200 μm). (c) Expanded image of irradiated area (scale bar, 100 μm). (d) Pre-treatment stereomicroscope image of the entire dorsal window chamber (scale bar, 1.0 mm). (e) Post-treatment image (scale bar, 200 μm). (f,g) 3D-rendered images of blood flow produced by Doppler OCT imaging pre-treatment (f) and post-treatment (g) (scale bar, 400 μm); the images are overlaid on the pre-treatment stereomicroscope image. The blood flow in the targeted artery is from left to right. The white boxes (a-c, e, f, g) indicate the irradiated region. (Reprinted with permission, [68])
Doppler OCT B-mode images were used to quantify the extent of vascular shutdown in both the control arms and two-photon treatment arm of the study. Table 5.1 shows the blood flow velocity in the vessels pre and post irradiation. The treatment mouse showed a 70 fold decrease in blood flow velocity, whereas the reference vessel and control arms demonstrated variations in blood flow by less than a factor of 2.

<table>
<thead>
<tr>
<th>Photosensitiser</th>
<th>Pre-irradiation (μm s⁻¹)</th>
<th>Post-irradiation (μm s⁻¹)</th>
<th>Pre-irradiation (μm s⁻¹)</th>
<th>Post-irradiation (μm s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated vessel</td>
<td>7000</td>
<td>&lt;100</td>
<td>1800</td>
<td>1000</td>
</tr>
<tr>
<td>Reference vessel</td>
<td>2200</td>
<td>3000</td>
<td>530</td>
<td>890</td>
</tr>
<tr>
<td>control</td>
<td>700</td>
<td>380</td>
<td>150</td>
<td>120</td>
</tr>
</tbody>
</table>

**Table 5.1**: Peak blood flow velocities pre- and post-irradiation, for both the irradiated and neighbouring non-irradiated vessel, determined by Doppler optical coherence tomography. Reported values are based on using an autocorrelation (Kasai) velocity estimator. Variations in pre- and post-irradiation peak blood flow velocity are as large as a factor of two for the reference vessels, and the irradiated vessels of the animals injected with tetramethylrhodamine labelled dextran with or without verteporfin. In contrast, the irradiated vessel of the animal administered the two-photon photosensitizer shows a decrease of more than 70-fold from 7000 μm s⁻¹ to below the noise baseline (~100 μm s⁻¹). (Reprinted with permission, [68])

2D and 3D DOCT, in conjunction with confocal fluorescence microscopy, played a crucial role in the first *in vivo* demonstration of the potential of PDT drugs specifically designed for two-photon excitation and this research was published in collaboration with Harry Anderson’s group in the following manuscript,

5.2 Application of speckle variance OCT monitoring of photodynamic therapy and targeted degradation of HIF-α

The results of the last section demonstrated how 3D DOCT could be used to monitor single-vessel targeted therapies. However, the majority of anti-angiogenic and anti-vascular therapies, including standard PDT generally target a much larger region. The limited field and sensitivity of DOCT make it impractical to monitor these widefield changes. Thus, in this section we describe our collaborative efforts to apply SV-OCT to monitoring vascular-targeted therapies over large regions of interest and with high sensitivity. In particular, vascular changes due to standard single photon PDT [69], and suppression of angiogenesis using bioengineered variants of the von Hippel–Lindau (VHL) protein delivered through an adenoviral delivery system [21]. The VHL variant, unlike the naturally occurring form, is able to enzymatically degrade the HIF-α molecule in the absence of oxygen. The preclinical experiments described below, were also performed in the dorsal skin fold window chamber model.

Figure 5.3 shows images generated in a simple experiment that was performed to demonstrate the feasibility of using SV-OCT to visualizing vascular changes induced by 1-photon PDT, both acutely and in the chronic phase of the response. Mice bearing the window chamber were administered a dose of 1.4 µM kg$^{-1}$ verteporfin. Fifteen minutes after photosensitizer administration, an ~1.5 mm diameter region was targeted with a 690 nm laser light. A total light dose of 100 J cm$^{-2}$ was delivered over 10 minutes. OCT images taken before and immediately after PDT exhibit some localized vascular shutdown. This is in stark contrast to the 24 hour time point when widespread vascular shutdown was observed. This result was published in collaboration with Brian Wilsons group in the following manuscript,

Figure 5.3: Example image of verteporfin (1.4 µm kg$^{-1}$) broad beam PDT at 690 nm, spot size = 1.5 mm in diameter in the center of the image, 100 J cm$^{-2}$fluence delivered over 10 min) in the window chamber model. The 24 h time point vascular response image shows vascular shutdown of numerous vessels. (Reprinted with permission, [69])

The second study looked at using bioengineered variants of the VHL protein’s ability to inhibit angiogenesis in vivo through the enzymatic degradation of HIF-α in an oxygen independent manner. The tumor cell line used in these experiments was renal clear cell carcinoma RCC-786-O, which stably expressed the DS-red2 fluorescent protein. Two days after WCM surgery on immune-deficient SCID mice approximately $7 \times 10^5$ cells were implanted into the dermis. The tumor was allowed to develop for two days and was then infected with one of three different adenoviral vectors: (1) Ad-EGFP which transduces the tumor cells with eGFP fluorescent protein gene, (2) Ad-EGFP-T7-VHL which transduces the tumor cells with the eGFP gene and the normal VHL gene, which should enzymatically degrade HIF-α under normoxic conditions, (3) Ad-EGFP-T7-HPACGV which transduces the tumor cells with the eGFP gene and the VHL variant gene, which should enzymatically degrade HIF-α under hypoxic conditions. The adenoviral infections were performed by intratumoural injections of Ad-EGFP, Ad-EGFP-T7-VHL or Ad-EGFP-T7-HPACGV using $2 \times 10^8$ infectious units (ifu). Ad-EGFP treatment had negligible effect on the growth of tumours, therefore large amounts of angiogenesis within the tumour mass was observed (Figure 5.4A). Ad-EGFP-T7-VHL treatment had a modest negative effect on tumour growth, with some angiogenesis occurring within the tumour as compared to the Ad-EGFP treated group (Figure 5.4B). In contrast, Ad-EGFP-T7-HPACGV treatment appeared to inhibit tumour angiogenesis and showed tumour regression, especially in the central core region by day 8 post-implantation and most noticeably by day 10, the final day of the assay (Figure 5.4C).
Figure 5.4: Ad-EGFP-T7-HPACGV treatment inhibits human CCRCC tumour xenograft angiogenesis in a dorsal skin-fold window chamber model. 786-dsRed cells were implanted into dorsal skin-fold window chambers in SCID mice. Tumours were intratumourally injected with (A) Ad-EGFP on day 2 post-implantation; (B) Ad-EGFP-T7-VHL on day 3 post-implantation; (C) Ad-EGFP-T7-HPACGV on day 8 post-implantation. Tumours were visualized by red fluorescence microscopy and positivity of adenoviral infection was monitored by green fluorescence microscopy. Tumour angiogenesis was visualized by white-light microscopy and SV–OCT. Four mice received treatments per recombinant adenovirus. Representative images are shown from each treatment group. (Reprinted with permission, [21])

These results were published in collaboration with Michael Ohh’s group in the following manuscript,

Chapter 6: Conclusions

6.1 Summary

The motivation for this thesis was to develop high resolution 3D microvascular imaging techniques capable of imaging angiogenesis longitudinally. However, we realized early on that standard Doppler OCT-based imaging techniques suffered from 3 major limitations, namely:

1. Motion artefacts cause difficulty in 3D reconstruction, such as discontinuities and high background phase noise.
2. The angle dependence of Doppler detection makes it difficult to image highly tortuous vessel structures.
3. Finite velocity noise floor of conventional Doppler OCT techniques makes it difficult to image below ~500 µm/s.

Thus, the overall hypothesis of this thesis was that mitigation of these particular limitations would improve our ability to generate high resolution 3D microvascular maps in vivo.

Chapter 2 dealt with implementation of Doppler OCT algorithm on a swept source OCT system and the development of a retrospectively-gated OCT system. The feasibility of negating periodic motion artefacts was demonstrated through retrospectively-gated high speed 2D (~1000 fps) and 4D imaging in the xenopus laevis heart at 45 volumes per second.

In Chapter 3, a Fourier domain mode locked laser with a maximum A-scan rate of 65 kHz was constructed and characterized. The FDML laser had SNR limited phase noise up to approximately 55 dB, a slight improvement over the standard short cavity configuration. Nevertheless the high speed of the laser source was also important for the mitigation of non-periodic motion artefacts.

Chapter 4 tackled the finite velocity noise floor and angle dependence of color Doppler based algorithms, for which our solution was the development and optimization of the
speckle variance technique. We demonstrated this technique in the dorsal skin fold window chamber model and validated it using flow phantom experiments and intravital fluorescence confocal microscopy.

Application of some of the developed techniques to biologically motivated problems of interest involving anti-vascular (photodynamic therapy) and anti-angiogenic therapies (targeted degradation of HIF-α) was pursued on an ongoing basis during the technical development. For example, the Doppler algorithm implemented on SS-OCT system in chapter 2 was used to help validate the first PDT drug specifically designed for two-photon activation.

The 3D microvascular toolkit of techniques developed in this thesis sets the groundwork for helping answer many interesting scientific questions in tumor biology, developmental biology and regenerative medicine where the microvasculature plays a key role.

6.2 Future work

Parts of the work described in the previous chapter summarizes the application of speckle variance OCT to monitoring vascular targeted therapies. However, the images shown only provide anecdotal evidence of vascular changes occurring over time. The major focus of the future work involving speckle variance OCT should be in the area of quantification of the 3D datasets. In particular, the implementation of algorithms to calculate important metrics such as vascular volume, vascular length, vessel tortuosity and branch point density.

I have begun some preliminary work to develop algorithms to estimate vascular volume and vascular length. Vascular volume is straightforward to calculate and simple requires thresholding the 3D dataset and counting the remaining non-zero voxels. Vascular length is a much more difficult quantity to extract from complex 3D vascular networks such as those shown in Figure 6.1. To calculate vascular length, the centerline of each vessel in the dataset must first be extracted. One way to do this is to fit super ellipsoids (3d ellipsoidal shapes) to the vascular network, as demonstrated previously in confocal
microscopy and OCT [59]. I chose to try a different method in a preliminary attempt to do this, where a 3D grayscale skeletonization technique [70] was applied to the datasets (Gorgon interactive molecular modelling system). This algorithm attempted to generate a one pixel thick skeleton from the 3D data, while maintaining the topology of the network. Results from this skeletonization algorithm are shown in Figure 5.7 (c) and (d). From these skeletons, an estimate of vascular length can be made by counting pixels in a region of interest. For example, in Figure 5.7 the normal tissue had calculated vascular metrics (in regions defined by the green boxes) of vascular length = 18.75 mm and vascular volume = 0.027 mm³, while the 9L gliosarcoma tumor vascular metrics were vascular length = 46.53 mm and vascular volume = 0.057 mm³. At first glance the skeletonization technique seems very useful for characterizing the microvascular network. However, it must be noted that the skeletonization technique tends to fragment vessels, which makes estimation of parameters such as branch point density and tortuosity inaccurate. Therefore, methods that minimize these discontinuities in our skeletonized datasets will also need to be investigated.
Figure 6.1: Depth-encoded speckle variance images and corresponding microvascular skeletonization of (a,c) normal and (b,d) 9L gliosarcoma tumor microvasculature. Normal tissue had calculated vascular metrics of vascular length = 18.75 mm and vascular volume = 0.027 mm$^3$, while the tumor vascular metrics were vascular length = 46.53 mm and vascular volume = 0.057 mm$^3$. (Scale bar represents 1mm)

In order to facilitate quantitative comparison of vascular networks between different tumors it will also be necessary to calculate the vascular metrics per unit tumor volume. Most tumors can be segmented fairly easily by hand from image subsets of the 3D structural OCT dataset. Once the subset has been segmented, interpolation techniques can be used to obtain the intermediary boundary locations. Figure 6.2 shows the results of one such segmentation that was performed. The volumetric information is displayed as a thickness map at each point in the tumor tissue. Good geometric correspondence between the tumor map and the vascular map is observed.
Figure 6.2: (a) Tumor thickness map and (b) corresponding microvascular SV-OCT map. Tumor volume can be calculated from the thickness, to enable calculation of volume-normalized metrics for inter-animal comparisons of vascular changes. (Scale bar represents 1mm)

Another important aspect of SV-OCT that should be investigated in the future is dynamic imaging of transient processes such as those seen during PDT. Since typical widefield imaging times are on the order of only 1-5 minutes with SV-OCT, this method may prove useful as a high resolution technique for monitoring the transient fluctuations in the vascular network during PDT or other vascularity targeted therapies. Figure 6.3 shows a time lapsed sequence of SV-OCT images of normal tissue within the window chamber undergoing a verteporfin based PDT treatment. Investigation of how transient changes during PDT relate to singlet oxygen production, vascular shutdown and tissue necrosis in both normal and tumor tissues will be extremely interesting and may be a potential predictor for treatment outcome [20]. Another potentially interesting transient phenomenon, important in tumor biology, is the imaging of cycling hypoxia, which happens on the time scale of a few cycles per hour [71]. If SV-OCT is able to visualize these changes, then it may be able to shed light on how to optimize oxygen delivery to the tumor when performing oxygen-dependent therapies such as PDT and radiation therapy.

One of the benefits but also drawbacks of the SV technique is that, at currently available frame rates, it is completely insensitive to velocity. In section 4.5 we demonstrated that
the decorrelation due to the Brownian motion of microspheres in water occurred in about 0.006 seconds (singly scattered regime). Thus, in this model system when imaging at frame rates up to 165 fps, we cannot distinguish bulk flow from Brownian motion using the SV algorithm. If we begin to increase the frame rate above this threshold it should be possible to distinguish pure Brownian motion to the situation where the fluid is also undergoing a bulk flow. If the same idea can be extended to the in vivo blood flow situation it may similarly, be possible to distinguish moving and non-moving blood. A number of important differences exist between our model and the in vivo situation which must be first investigated (1) the red blood cell takes up a significant portion of the imaging voxel, (2) the red blood cell is asymmetric so that there may be an additional decorrelation times associated with its rotational motion (3) whole blood is a viscoelastic fluid (the red blood cells deform and orient themselves as function of the shear rate). All of these factors will influence the time over which stationary and flowing blood will decorrelate. However, through careful experimentation it may possible to distinguish them and perhaps even extract quantitative flow velocity information. This advance could have important consequences for the measurement of functional perfusion.
Figure 6.3: Dynamic SV-OCT imaging of microvascular fluctuation during photodynamic therapy. Each image takes approximately 1.5 minutes to acquire. Images show transient vascular shutdown during PDT and reperfusion post PDT. Treatment parameters included a Verteporfin™ dose = 1.4 μm kg⁻¹, PDT spot size = 1.5 mm in diameter in the center of the image, 100 J cm⁻²fluence delivered over 10 minutes in the WCM at 690 nm.
Finally, the potential of applying speckle variance OCT to clinical imaging applications should and will be investigated. In section 4.3 we described a simple proof of principle experiment in which we performed B-mode SV-OCT imaging of the unstablerlized human nail root (Figure 4.22). Motivated by this result a study will be performed within the next two years that will test the applicability of the SV-OCT algorithm in the clinical setting. The study will focus on monitoring complications in the rectum induced by radiation therapy to the prostate, with the eventual goal of helping guide interventions. The imaging will be done via an OCT proctoscope, designed and built by Bahar Davoudi, shown in Figure 6.4. Since the rectum is very accessible, the OCT proctoscope can be relatively large and commercial bulk optics can be utilized for creating the scanning objective. The proctoscope also takes advantage of the same scanning galvonometers used throughout this thesis (for preclinical applications, and shown in Figure 4.4) to perform 3D scanning. Based on our previous results in section 4.3, on SV imaging in the human nail root, it should be possible, at the very least, to generate B-mode speckle variance images in the rectum provided the imaging frame rate and field of view are chosen appropriately. 3D microvascular maps of the rectum can potentially also be created, however, image realignment algorithms may need to be developed to remove the effects of bulk tissue motion in the processed 3D vascular map.
Figure 6.4: Schematic of the OCT proctoscope (Courtesy of Bahar Davoudi). The probe utilizes commercially available bulk optics and scanning galvanometers to scan a 3D section of the rectum.

There are also a number of other clinical applications where SV imaging may be applied, since OCT is a fiberoptic-enabled technology, making it extremely adaptable to catheter based imaging. High speed catheter-based imaging probes for OCT have developed significantly over the past decade and are now able to image cylindrical volumes around the catheter as long as 6 cm in a matter of seconds [72]. The high frame rates (>100 fps) provided by these probes will be essential to generating the speckle variance signal as discussed in Chapter 4. These catheter probes have previously been used intravascularly, interstitially and endoscopically for a diverse array of problems, including, assessment of coronary plaques/stents and assessment of dysplastic conditions in the colon, esophagus and lung [37, 72, 73]. Adding the high resolution vascular imaging capability of SV-OCT to any and all of these applications may lead to a direct increase in clinical benefit to the patient.
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