The Potential of Optical Coherence Tomography for Intravascular Imaging of Chronic Total Occlusions

By

Nigel R. Munce

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy in the Graduate Department of Medical Biophysics at the University of Toronto

© Copyright by Nigel Robert Munce, 2009
Abstract

This thesis presents the first work, to our knowledge, to evaluate the potential of Optical Coherence Tomography (OCT) as an intravascular imaging modality to characterize and guide interventions on chronic total occlusions (CTOs) in arteries. An ex vivo imaging study using OCT is presented that characterizes various pathologies associated with peripheral CTOs and illustrates the ability to differentiate between the vessel wall and the occluded lumen. We also found that, while OCT could image approximately 1mm through tissue, it was effective for imaging deeper through clarified microchannels seen within the occluded lumen. While others had reported observing such microchannels within the lumen before, little was known about the global architecture of these channels. This motivated a study of the global morphology of microchannels in occlusions using micro computed tomography (microCT). In this microCT study, we found that microchannels within the occluded lumen of the artery appeared to be continuous over several millimeters. However, these channels also exited the artery frequently, suggesting the need for some form of imaging guidance. As a potential intravascular imaging set-up,
a forward-viewing OCT catheter was built. This catheter uses a novel scanning mechanism that combines high voltage and a dissipative polymer to achieve fast compact actuation. Doppler OCT results are presented using this catheter to image flow in the forward direction. Doppler OCT imaging of microchannels \textit{in vivo} is also shown in a surgically exposed occluded artery \textit{in situ}.
Acknowledgements

This work would not have been possible without the help and support of several people and the support of several agencies. In addition to this support, I was tremendously fortunate to work with people who made this experience incredibly fun.

Firstly, I would like to acknowledge Graham Wright, my co-supervisor, for taking a chance on me with a high risk project and for being able to bring together so many experts that are interested in the problem of arterial occlusions. I have been given a tremendous amount of resources to pursue this problem and I am truly grateful for this opportunity.

I would also like to thank my co-supervisor Alex Vitkin, for his patience in reading over my countless revisions that start out as unpolished pieces and gradually become understandable works that I am proud of with his corrections.

I would also like to acknowledge the help of Victor Yang for constantly coming up with new ideas to try and aggressively pushing many projects. Victor has also been an inspiration for me in pursuing both medical studies and research.

I also wish to thanks Brian Wilson, a committee member, for his continuing reminders to me to question, to be curious and to never stop loving the science.

Brad Strauss has also been instrumental in the work presented here. He has provided tremendous amounts of input on the clinical problem and helped frame my work within the difficulties that interventional cardiologists face. He has also taken considerable time in answering my many questions.

I would also like to acknowledge my tremendous appreciation to Gary Tearney for agreeing to come to Toronto to serve as an external examiner for my final PhD defense. Dr. Tearney’s presence was an incredible honor and made the day very special.
I am also grateful to my many friends at Princess Margaret Hospital for their help, friendship, and laughter. Special thanks to Beau Standish for his instruction in how to get the back tires of our Shelby Mustang in San Jose to spin out. I am forever ruined. Also to Adrian Mariampillai whose many get-rich schemes (algae/truffle growing, arbitrage/sports betting, part-time financial consultant) will one day work out. I sincerely appreciate all the help that you guys have given me. Also to the many others who made each day fun: Tony, Ralph, Moriyama-san, Jarvi and Big-E.

I was also fortunate to have another group of amazing friends at Sunnybrook Hospital in the cardiac imaging group. General Leung and Kevan Anderson were amazing partners-in-crime who provided tremendous help in all things related to MRI, and signal processing. They also taught me such useful tips as: how to cool beer with liquid nitrogen without breaking the bottle (use a plastic bottle) and the finer points of infection control. Mihaela Pop was instrumental with her help with high speed imaging and for the many conversations that we shared over coffee. I also wish to acknowledge John Graham for the most cutting Scottish wit this side of the Atlantic.

I have also been incredibly lucky to have the support of a wonderful family. My parents: Muma, Bert; my sister and brother-in-law: Sare & Margus, my brother: Matt; and my mother-in-law: Susanna who have all helped me tremendously.

Most importantly, I want to thank my most wonderful wife, Emily for being with me through all of this and for being my most trusted friend. Em has helped me at every step of the way: she has gone through just about every presentation, commented on every journal image, listened to all my frustrations and kept me going when nothing worked. She is an amazing person who is both a tough-as-nails practical clinician and gentle caring person. I am a very lucky guy to have her as my wife.
Funding for this project was provided by Canadian Foundation for Innovation, the National Science and Engineering Research Council of Canada and the Ontario Centres of Excellence.
# Table of Contents

**Chapter 1: Chronic Total Occlusions and the Need for Forward Viewing Image Guidance**

1.1 Chapter Overview .......................... 1
1.2 Introduction to Atherosclerosis .......... 1
1.3 Progression of Atherosclerosis to Complete Occlusion ................. 4
1.4 Definition of a Chronic Total Occlusion ......................... 4
1.5 Vascular Pathology of a Chronic Total Occlusion ..................... 5
1.6 Clinical Presentation and Motivation for Restoring Blood Flow in a Chronic Total Occlusion ....................... 8
1.7 Background of Intravascular Angioplasty ........................... 10
1.8 Clinical Efficacy of Angioplasty and Motivation for Intravascular Recanalization of the Occluded Artery ...................... 10
1.9 Conventional and Experimental Intravascular Therapy in the Context of Chronic Total Occlusions ...................... 13
1.10 Guidance in the Context of Chronic Total Occlusions .............. 16
1.11 Challenges and Requirements for Forward Viewing Catheters .......... 17
1.12 The State of Forward-Viewing Catheter Based Imaging ................ 18
    1.12.1 Progress in the Development of Forward-Viewing Intravascular Ultrasound .................. 18
    1.12.2 Forward-Viewing Intravascular Magnetic Resonance Imaging .................. 19
    1.12.3 Angioscopy ................................ 20
1.13 Optical Coherence Tomography .................. 20
1.14 Motivation for Forward-Viewing OCT for Chronic Total Occlusions .................. 26
1.15 Thesis Aims and Overview .................. 27
Chapter 2 – Ex Vivo Characterization of CTOs using OCT

2.1 Chapter Overview

2.2 Ex Vivo Imaging of Chronic Total Occlusions with Optical Coherence Tomography

2.3 Reprint: Ex Vivo Imaging of Chronic Total Occlusions using Optical Coherence Tomography

2.4 Addendum

2.5 Conclusions

Chapter 3 - Continuity and Global Morphology of Microchannels in Chronic Total Occlusions

3.1 Introduction

3.2 Motivation for 3-D Vascular Morphology Study of Chronic Total Occlusions

3.3 Preprint: Micro Computed Tomography of Chronic Total Occlusions: Patterns of Vascular Formation

3.4 Addendum to this Work – Using \( \mu \)CT to Monitor Intravascular Therapy

3.5 Addendum to this Work – Preliminary Investigation into Tissue Hypoxia in CTOs

3.6 Conclusions

Chapter 4 – Forward-Viewing Intravascular OCT Probe Development

4.1 Chapter Overview

4.2 Motivation and Technical Requirements for an Intravascular Forward-Viewing Catheter
Chapter 5 – Doppler Optical Coherence Tomography Imaging in Chronic Total Occlusions

5.1 Chapter Overview

5.2 Doppler Optical Coherence Tomography

5.3 Motivation for Forward-Viewing Intravascular Doppler OCT

5.4 Forward-Viewing Doppler OCT: Experimental Set-Up

5.5 Forward-Viewing Doppler OCT: Imaging Results

5.6 Experimental Limitations

5.7 Design of Experiments to Test in vivo Doppler OCT Imaging of Occluded Arteries

5.8 Doppler OCT in vivo Imaging Results

5.9 Limitations of these Experiments

5.10 Future in vivo Imaging of Arterial Occlusions in Animal Models
# Chapter 6- Concluding Remarks

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Accomplishments of this Work</td>
<td>106</td>
</tr>
<tr>
<td>6.2</td>
<td>Relevance to Interventional Cardiology</td>
<td>107</td>
</tr>
<tr>
<td>6.3</td>
<td>Future Work: Combined Imaging and Intervention</td>
<td>108</td>
</tr>
<tr>
<td>6.4</td>
<td>Future Work: Modifying Medical Therapy towards Natural Recanalization of Arterial Occlusions</td>
<td>109</td>
</tr>
<tr>
<td>6.5</td>
<td>Conclusions</td>
<td>109</td>
</tr>
</tbody>
</table>

## References

111
### List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Histological Progression of Arterial Disease</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>Angiogram of a Coronary Artery Occlusion</td>
<td>5</td>
</tr>
<tr>
<td>1.3</td>
<td>Microvessels in an Arterial Occlusion</td>
<td>6</td>
</tr>
<tr>
<td>1.4</td>
<td>Collateral Arteries in an Occlusion</td>
<td>7</td>
</tr>
<tr>
<td>1.5</td>
<td>The Principal Arteries of the Heart</td>
<td>13</td>
</tr>
<tr>
<td>1.6</td>
<td>Angular Field of View Required in a Forward-Viewing Catheter</td>
<td>18</td>
</tr>
<tr>
<td>1.7</td>
<td>Basic Michelson Interferometer for OCT</td>
<td>21</td>
</tr>
<tr>
<td>1.8</td>
<td>Focusing Parameters of a Gaussian Beam</td>
<td>23</td>
</tr>
<tr>
<td>1.9</td>
<td>Time and Fourier Domain Signal Processing Steps</td>
<td>25</td>
</tr>
<tr>
<td>2.1</td>
<td>3-D OCT Image of an \textit{ex vivo} CTO with a Microchannel Passing Through the Lumen of the Occlusion</td>
<td>39</td>
</tr>
<tr>
<td>2.2</td>
<td>3-D OCT Image of an \textit{ex vivo} CTO with a Microchannel Exiting out of the Lumen of the Occlusion</td>
<td>40</td>
</tr>
<tr>
<td>3.1</td>
<td>Serial Histology of a 6 Week Old Arterial Occlusion</td>
<td>43</td>
</tr>
<tr>
<td>3.2</td>
<td>MicroCT Images of VEGF Treated Occluded Arteries</td>
<td>67</td>
</tr>
<tr>
<td>3.3</td>
<td>Patent Arterial Segment Stained for Hypoxia with PIMO</td>
<td>66</td>
</tr>
<tr>
<td>3.4</td>
<td>Occluded Artery Segment Stained for Hypoxia</td>
<td>67</td>
</tr>
<tr>
<td>3.5</td>
<td>Central Section of Occluded Artery Stained for Hypoxia</td>
<td>68</td>
</tr>
<tr>
<td>3.6</td>
<td>12 Week Old Occlusion Stained for Hypoxia</td>
<td>68</td>
</tr>
<tr>
<td>4.1</td>
<td>Review of Cantilever-Based Forward-Viewing OCT Probes</td>
<td>72</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>5.11</td>
<td>Doppler OCT Image of Central Microchannel in 2 Week Occlusion</td>
<td>100</td>
</tr>
<tr>
<td>5.12</td>
<td>OCT Image of 6 Week Old Occlusion</td>
<td>101</td>
</tr>
<tr>
<td>5.13</td>
<td>Thrombus Filled Artery with Potential Observation of Vasa Vasorum</td>
<td>102</td>
</tr>
<tr>
<td>5.14</td>
<td>3-D Reconstruction of <em>in vivo</em> Occluded Artery Showing Motion Effects</td>
<td>104</td>
</tr>
<tr>
<td>6.1</td>
<td>Proposed Design Incorporating Forward-Viewing OCT with Laser Ablation</td>
<td>108</td>
</tr>
</tbody>
</table>
Publications from this thesis:

**First author publications:**

1. Munce NR, Yang VX, Standish BA, Qiang B, Butany J, Courtney BK, Graham JJ, Dick AJ, Strauss BH, Wright GA, Vitkin IA.


Micro Computed Tomography of Chronic Total Occlusions: Patterns of Vascular Formation. *Circulation Research* (in preparation)

**Second Author Publications:**


2.
Lee KK, Munce NR, Shoa T, Charron LG, Wright G, Madden JD, Yang VX.

*Sensors and Actuators A (in review)*

**Third Author Publications**

1.

Natural History of Experimental Arterial Chronic Total Occlusions.
*Journal of the American College of Cardiology (in press)*

2.

Investigation of micro-ultrasound for microvessel imaging in a model of chronic total occlusion.
## Glossary of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad</td>
<td>Adventitia</td>
</tr>
<tr>
<td>C</td>
<td>Calcification</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary Artery Bypass Graft(Surgery)</td>
</tr>
<tr>
<td>CMUT</td>
<td>Capacitive Micromachined Ultrasound Transducer</td>
</tr>
<tr>
<td>CTA</td>
<td>Computed Tomography Angiography</td>
</tr>
<tr>
<td>CTO</td>
<td>Chronic Total Occlusion</td>
</tr>
<tr>
<td>DOCT</td>
<td>Doppler Optical Coherence Tomography</td>
</tr>
<tr>
<td>F</td>
<td>Fat</td>
</tr>
<tr>
<td>FC</td>
<td>Fibrous Cap</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast Fourier Transform</td>
</tr>
<tr>
<td>FFT&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>Inverse Fast Fourier Transform</td>
</tr>
<tr>
<td>GRIN</td>
<td>Graded Index of Refraction (lens)</td>
</tr>
<tr>
<td>HV</td>
<td>High Voltage</td>
</tr>
<tr>
<td>ID</td>
<td>Inner Diameter</td>
</tr>
<tr>
<td>IEL</td>
<td>Internal Elastic Lamina</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>IVUS</td>
<td>Intravascular Ultrasound</td>
</tr>
<tr>
<td>LAD</td>
<td>Left Anterior Descending (Artery)</td>
</tr>
<tr>
<td>LC</td>
<td>Loose Connective (tissue)</td>
</tr>
<tr>
<td>M</td>
<td>Media</td>
</tr>
<tr>
<td>MC</td>
<td>Microchannel</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MEMS</td>
<td>Microelectromechanical System</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MT</td>
<td>Medical Therapy</td>
</tr>
<tr>
<td>MZI</td>
<td>Mach-Zehnder Interferometer</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical Aperture</td>
</tr>
<tr>
<td>NC</td>
<td>Necrotic Core</td>
</tr>
<tr>
<td>OCT</td>
<td>Optical Coherence Tomography</td>
</tr>
<tr>
<td>OD</td>
<td>Outer Diameter</td>
</tr>
<tr>
<td>OL</td>
<td>Occluded Lumen</td>
</tr>
<tr>
<td>PCI</td>
<td>Percutaneous Coronary Intervention</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PIMO</td>
<td>Pimonidazole</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethyl Methacrylate</td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth Muscle Cell</td>
</tr>
<tr>
<td>STAR</td>
<td>Subintimal Tracking And Reentry</td>
</tr>
<tr>
<td>TIMI</td>
<td>Thrombolysis In Myocardial Infarction</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
<tr>
<td>Vv</td>
<td>Vasa Vasorum</td>
</tr>
<tr>
<td>µCT</td>
<td>Micro-Computed Tomography</td>
</tr>
</tbody>
</table>
“The history of surgery is the history of its tools.”

-Lars Leksell (inventor of the Gamma Knife)
Chapter 1 – Introduction to Chronic Total Occlusions and the Need for Intravascular Image Guidance in Intravascular Interventions

1.1 Chapter Overview

A brief introduction to atherosclerosis and its progression in the context of occlusive vascular disease is given. The clinical motivation for opening chronic total occlusions (CTOs) is discussed primarily in the context of coronary artery occlusions. Standard methodologies in minimally invasive intravascular therapy are introduced. The pathophysiology of complete arterial occlusions is reviewed. The clinical motivation for imaging tools is discussed in terms of interventional guidance. An overview of current devices and techniques specific for opening occlusions is presented. While these devices present many possible ways to force a passage through the occlusion, little is available in terms of guidance, and therefore opening the lesion without damaging the vessel wall remains challenging. Thus a brief review of possible guidance mechanisms is presented with a particular focus on forward-viewing intravascular imaging methodologies. Optical coherence tomography is introduced and the motivation for the development of a forward-looking optical coherence tomography imaging catheter as a tool for guiding procedures involving chronic total occlusions is detailed. Overall projects goals are discussed and finally the structure of the thesis is outlined.

1.2 Atherosclerosis

Atherosclerosis is derived from the Greek words for gruel or paste and hardening [1]. Within the context of a chronic total occlusion of a coronary artery, it is the coagulation of blood in response to the rupture of the “gruel or fat” within the arterial wall and the subsequent aging of this thrombus that leads to a complex occlusion.

The build-up of fatty deposits within the intimal layer of the arterial wall is initiated by the natural migration of fat from the bloodstream into the intima. This gradual process may be accelerated by endothelial dysfunction caused by factors such as smoking [2] and hypertension[3]. This lipid accumulation in the arterial wall may be oxidized by
chemicals in the bloodstream, resulting in the immune system recognizing them as foreign[4]. This immune response results in macrophage migration into the fatty deposit within the arterial wall. The macrophages subsequently begin to absorb the fat to such an extent that they appear foamy and are referred to as foam cells[5]. In addition to macrophages, T-Lymphocytes also migrate into the fatty vessel wall; these cells release cytokines which encourage inflammatory processes as well as causing smooth muscle cells to migrate from the muscular layer of the vessel wall to the area overlying the fatty deposit[6]. These smooth muscle cells produce collagen and fibrin to form a protective cap over the fatty deposit[7]. Typically, plaques of this type grow outwardly rather than inwardly, hence preserving the blood flow of the artery[8].

Inflammation also results in the promotion of angiogenesis[9], forming tiny vessels within the plaque, which may themselves bleed. An example of such vessels is identified in Figure 1b with a dotted red circle. This intraplaque hemorrhage results in accumulation of free phospholipids, cholesterol and hemoglobin within the arterial wall from the membrane of the lysed red blood cells[10]. These molecules increase the size of the “necrotic core” of the plaque and cause further inflammation which degrades the fibrous cap leading to an increased likelihood of plaque rupture. From pathological studies, people who had suffered from acute coronary events were found to have an overlying fibrous cap thicknesses of 65 μm or less[11]. An example of such a thinly capped plaque is shown in Fig 1c. Such lesions are commonly referred to as “vulnerable plaque”. If such a plaque should rupture, the prothrombotic contents of the plaque become exposed to blood resulting in a thrombus at the site of the rupture (as is shown in Fig 1d).
The presence of calcium is also a key element in vascular disease. Although controversial, the most widely held belief in terms of the presence of calcification within the lesion is that a subpopulation of smooth muscle cells (SMCs) or macrophages undergo transformation in the presence of oxidative stress, high serum phosphate levels, and bone morphogenic protein, causing them to form nodules of bone[12]. These macrophages or SMCs may migrate into the plaque or thrombus of a lesion and form microcalcifications.
1.3 Progression of Atherosclerosis to a complete occlusion

The formation of a chronic occlusion within the coronary arteries is widely believed to be initiated with the rupture of a plaque resulting in thrombus formation. Unlike an acute heart attack, however, in which the downstream heart muscle is immediately cutoff from the blood supply, a CTO is widely believed to have developed from a significant stenosis such that the muscle has already been somewhat preconditioned to receiving less blood supply and alternate paths, or collateral vessels, which supply this muscle, have had time to develop.

The organization of thrombus is initiated by an immune response which transforms local leukocytes into fibroblasts and results in the emergence of myofibroblasts within the thrombus [13]. These cells are responsible for the fibrous nature of the occlusion and are also capable of promoting contraction and alignment of collagen fibers[14]. Thus the thrombus becomes more fibrotic as it ages within the artery.

1.4 Definition of a CTO

Coronary angiography is a procedure in which a catheter is inserted into a peripheral artery (typically the femoral artery), guided under x-ray imaging into the heart, and used to inject radio-opaque dye to image the arteries of the heart. This injection is performed under continuous x-ray imaging to make a movie of the propagation of this dye within the arterial tree. A CTO is by definition an artery that appears occluded under x-ray angiography which is older than three months [15]. While difficult to determine exactly how old the lesion is, the age of the lesion is typically estimated from the time from a clinical event such as an acute infarction or the onset of chest pain even when the patient is resting. The flow grade of a lesion under x-ray angiography is classified as either TIMI (Thrombolysis In Myocardial Infarction) flow level zero (no flow), one (minimal flow), two (partial flow) or three (normal). A CTO is defined as a lesion with TIMI flow grade of 0 or 1. In a CTO with a flow level of one, the operator is able to visualize the distal arterial segment but unable to see contrast within the lesion itself. This type of lesion is sometimes referred to as a functional occlusion because dye is able to propagate through it. An example of such an occlusion where the distal end is visible is shown in Figure 1.2.
In a CTO with flow level zero, on the other hand, no distal opacification, or presence of dye, is seen.

Figure 1.2 A coronary angiogram of an occlusion is shown in the right coronary artery of a patient prior to intervention in a). A magnified image of the area contained within the red rectangle in a) is shown in b). The Proximal entrance and distal end are identified and the space between these two points is the occlusion. In c) the artery is shown after an intervention in which an angioplasty balloon was used to dilate the lesion. Images are courtesy of Dr. Alexander Dick of Sunnybrook Health Sciences Centre.

1.5 Vascular Pathology of a CTO

A chronic total occlusion is composed of elements of both a ruptured vulnerable plaque and an organized thrombus. Srivatsa et al. report that younger (< 1 year) occlusive lesions had higher lipid and cholesterol content, and fibro-calcific lesions appeared with greater frequency as the lesions age[16]. Disruption of the internal elastic lamina, an elastic border between the intima and media, is also typically seen in CTOs[17]. In addition to these elements, microchannels are observed within the occlusion under pathology [16, 18]. It is widely believed that there are four types of neovascularature that play key roles in the evolution of a CTO: the vessels associated with the inflammation in the intimal layer, the vasa vasorum, collateral vessels, and recanalization channels.
Figure 1.3 An illustration of different types of microvessels within occluded arteries. The slide in a) is a Masson’s trichrome stained section that shows recanalization channels within the occluded lumen labeled as MV. The internal elastic lamina, labeled IEL, is only clearly visible between the 3 and 5 o’clock positions in the artery. The muscular medial layer of the artery is labeled M and the outer adventitial layer is labeled Ad. Vessels within the adventitia are identified as vasa vasorum and are labeled Vv. Image a) is an elastic trichrome stained slide while b) is an elastic van Gieson stained slide. The straight hollow arrow identifies vessels that may have originated from the intima. The solid curved arrow identifies recanalization channels, while the curved hollow arrow represents vasa vasorum. Scale bars in both images represent 1 mm. Image a) was reproduced from [19] and image b) was reproduced from [16].

As previously mentioned, angiogenesis within the intimal layer of the artery creates vessels that play a major role in the plaque’s potential to rupture. Vessels within the intima are seen in histology of CTOs and are thought to originate prior to arterial occlusion as a result of inflammation in the initial arterial disease. These vessels have been correlated in CTOs with the amount of intraplaque cellular inflammation[16]. A possible example of such vessels is shown in Figure 1.3 b) as vessels that are to close the medial layer (identified with a straight hollow arrow).

Also important in CTOs is the presence of the vasa vasorum (Vv). This group of vessels provides blood to the outer layers of the medial and adventitial layers of the artery. Studies of micro computed tomography of injured porcine arteries have described two types of Vv: a first order Vv that connects to the main lumen at regular intervals and runs longitudinally along the artery; and a second order Vv that branches off from the first order structure and runs circumferentially around the vessel[20]. The density of the second order Vv has been observed to increase upon intravascular balloon injury of the artery. Within CTOs, Srivatsa et al. report seeing well developed Vv channels within the media that frequently cross to the adventitia[16]. Similar to the relationship observed with the intimal microvasculature, the number and size of microvessels in the medial wall and
adventitial wall was closely associated with cellular inflammation. An example of Vv within a CTO is shown in Figure 1.3.

Collateral, or supporting vessels, serve as a back-up vascular network for the primary artery. It is thought that a pre-existing network of arterioles surrounding the artery undergoes extensive remodeling as a result of the hypoxic state of the tissue around the arterioles and in response to a change in the pressure through them. Additionally individual vessels within the Vv may become large enough to serve as a collateral vessel. These vessels provide a “detour” around the disease site allowing the tissue downstream to survive. An illustration of these collateral vessels is shown in the angiogram of a 12 week old femoral artery occlusion in a rabbit shown in Figure 1.4.

![Figure 1.4](image) An angiogram of an occluded rabbit femoral artery illustrates collateral vessels between the proximal and distal end of the CTO. These vessels appear as highly tortuous paths between the two ends. Scale bar represents 1cm.

Finally, it has been widely reported that the occluded lumen of a chronic total occlusion possesses recanalization channels [15, 16]. Examples of such channels are identified in Fig 1.3a as channels labeled MV. These channels have been shown to have an endothelial lining similar to a blood vessel [16, 21] and therefore the terms microvessel and microchannel are used interchangeably in this thesis. These channels are thought to be the most relevant to intravascular therapy as they may provide a “path of least resistance” within the lesion for a device or a pharmacological agent to follow[19]. Srivatsa et al.
report that 49% of CTOs are less than 98% occluded and that 59% of all CTOs possess channels >250 μm. The authors state that communication of these recanalization channels with the intimal plaque neovasculature is rare. However, no information is available regarding how continuous these channels are, what the three-dimensional morphology of the channels looks like, or to what extent these channels communicate with the medial wall and vasa vasorum. These questions provide the motivation for the work presented in Chapter 3.

1.6 Clinical Presentation and Motivation for Restoring Blood Flow in a CTO

A patient with a coronary CTO typically presents with chest pain (angina) that occurs in a predictable fashion (stable angina) [15]. In these patients, myocardial ischemia can be induced upon stressing the heart either through exercise or through chemical means. These patients are then sent for a coronary angiogram which often reveals the presence of an occlusion. It is estimated that a coronary occlusion is identified in 30-50% of patients undergoing coronary angiography [22, 23]. In most cases, the patient is then referred to bypass surgery as cardiologists have typically regarded occlusive disease as higher risk and more difficult to treat than diseased arteries that are open under coronary angiography.

The “gold standard” for treating an occluded artery has been coronary artery bypass graft (CABG) surgery, popularly known as “open heart” surgery. Typically, CABG has involved a sternotomy, which divides the sternum, in order to expose the heart. The heart is then cannulated in order to attach it to a cardiopulmonary bypass pump (commonly referred to as a heart-lung machine) which oxygenates the blood and pumps it back through the circulation. The heart is then stopped using a cold (4 °C) infusion of a solution primarily composed of dextrose and potassium chloride (cardioplegia). Depending on the number of bypasses needed the surgeon will harvest veins from the leg and/or arms. The procedure then involves creating an artificial anastomosis between the aorta and the coronary artery distal to the disease site. The left internal mammary or left internal thoracic artery may also be used to bypass the left anterior descending (LAD) artery of the heart by simply connecting the distal end of the bypass artery to a site on the LAD distal to
the lesion. These arterial bypasses typically remain functional much longer than the vein bypass grafts as the artery is accustomed to high pressure and pulsatile flow.

Despite its invasiveness, CABG has been shown to highly effective in reducing chest pain[24]. Additionally, because the procedure bypasses the entire vessel length proximal to the lesion it provides protection to downstream muscle against future events; thus it has also been shown to confer a survival benefit over medical therapy in patients with stable angina[25]; this benefit however was not statistically significant. Challenges in CABG are the limited lifetime of the venous grafts, due to their increased susceptibility to atherosclerosis when used to carry blood at higher flow rates than experienced in their native state. Vessel wall calcification of the native arteries also poses a technical challenge for the surgeons, as it can make it very difficult to connect a bypass.

Bypass surgery recovery is also a challenge for the patient. It typically involves 5-7 days in the cardiac intensive care unit of the hospital. After a resting period of three months, to allow the anastomoses to heal, patients undergo a cardiac rehabilitation regime to recondition the heart. While most patients return to their daily routine after approximately 6 months, surgery-related pain may persist for more than a year following the procedure [26, 27].

In addition to pain associated with the surgery, CABG using a cardiopulmonary bypass pump has also been associated with an increased risk of stroke and cognitive dysfunction. The incidence of stroke following surgery is approximately 3% [28] and cognitive impairment is reported in 40% of patients [29]. These complications were assumed to result primarily from the formation of micro-emboli in the cardio-pulmonary bypass pump; thus, new surgical techniques that involve heart stabilizers to allow for operating on the beating heart have been introduced (off-pump techniques) to address these concerns. Interestingly, while significant differences between patients who had undergone conventional on-pump versus off-pump coronary bypass surgery in neurological function were found at the three month time point (21 versus 29%) no differences were seen after
the one year time point (30.8 versus 33.1%) [30] suggesting other mechanisms besides micro-emboli formed in the pump may be at work.

Thus, CABG is a highly effective procedure for treating patients with coronary artery disease; however, both acute and chronic complications from the procedure are significant. It is thus not surprising that, although patients do want an intervention performed, a minimally invasive approach is preferred[31]. Procedures that address these complications in a minimally invasive manner are therefore highly desirable.

1.7 Background of Intravascular Angioplasty

Conventional intravascular therapy arguably began in 1929 when Dr. Werner Forssman was able to insert a catheter in his own antecubital vein and guide it under x-ray into his own atrium. In 1958, Dr. Mason Sones, demonstrated that x-ray opaque dye could be used to show the path that the arteries follow and that the injection of x-ray opaque dye could be used to diagnose disease of the vasculature. In 1974, Dr. Andreas Gruentzig used a specialized balloon catheter for dilating a narrowed artery in the peripheral vasculature and in 1977 the first coronary angioplasty was performed on an awake patient[32].

Today, angioplasty uses flexible guide wires strung through the patient’s vasculature by the cardiologist as guides for the delivery of subsequent devices. Typically, the balloon catheter fits over the wire and simply follows the path the wire takes. The balloon is also combined with a flexible stent that provides support for the newly opened artery. Typically this procedure is called percutaneous coronary intervention (PCI).

1.8 Clinical Efficacy of Angioplasty and Motivation for Intravascular Recanalization of the Occluded Artery

PCI of stenotic (narrowed but not occluded) arteries using balloon angioplasty and stenting has become incredibly common: over six hundred thousand procedures were
performed in the United States in 2004 [33]. This fact is in part due to the wide prevalence of cardiovascular disease (www.who.int/topics/cardiovascular_diseases/en) as well as patients’ preference for minimally invasive interventions as treatment. In the acute setting, where a patient has had a myocardial infarction (MI) (12 hours or less prior to intervention), PCI has been shown to be clinically effective in terms of increasing survival [34, 35]. In patients with stable angina, PCI has been shown to be effective at reducing symptoms and improving quality of life when used in addition to optimal medical therapy (MT)[36]. In patients with stable angina, however, no additional improvement is seen in mortality or risk of future MI when PCI is used in combination with optimal MT alone is seen [37]. Interestingly, however, in patients with stable angina and diabetes [38] or on hemodialysis [39] an improvement in life expectancy is seen with angioplasty as compared to MT alone. This difference, in diabetic patients, may be due to the noted impairment of collateral vessel formation in coronary arteries [40].

In light of the outcomes of angioplasty in patients with non-occlusive arterial disease, it is thus not surprising that the primary motivation for treating CTOs with PCI is relief of the symptoms of angina [15]. Examining patients with occluded arteries, the TOAST-GISE trial comparing successful versus non-successful angioplasty of CTOs did show a significant difference in terms of freedom from angina in the patient group in which the cardiologist was able to successfully dilate the occlusion using an angioplasty balloon [41]. Whether additional benefits are also conferred has been the subject of recent investigations. Recent trials of PCI in addition to MT versus MT alone in cases of non-acute (3–28 days) occluded arteries have shown a trend towards improved left ventricular output in the PCI group[42]. This improvement, however, has not translated into an overall improved mortality in patients undergoing PCI in addition to MT alone [43]. The discrepancy is likely due to increased re-infarction rates seen in the PCI population due to complications associated with intervention such as distal embolism formation and reduction in collateral vasculature following PCI. It is important to note, however, that evidence of severe ischemia within the infarct zone was an exclusion criteria for the OAT/TOSCA study and that treatment of silent ischemia by PCI has been shown to confer an increased survival advantage[44]. Therefore it is believed that the state of the
myocardium supplied by the occluded artery plays a large role in determining whether or not it is beneficial to revascularize the occlusion. Studies using nuclear imaging reveal that 83% of patients with a CTO have reversible ischemia on exercise or chemically induced stress[45]. Whether or not revascularization with PCI in these patients translates into improved mortality remains a subject of ongoing investigations.

Thus revascularization of diseased arteries by either surgery or PCI is desirable in patients with angina that cannot be treated medically and/or patients with downstream ischemic myocardium. While a minimally invasive approach is attractive due to lower risk of complications and a significant decrease in trauma and discomfort for the patient, there are several important exclusion criteria. If the patient has multiple as opposed to single vessel disease, surgical bypass is preferred over PCI due to the high risk of complete heart failure should the multiple sites treated with angioplasty all undergo restenosis.

Traditionally, surgical bypass has also been preferred over PCI in cases in which the lesion is located within the left main artery. The left main artery supplies both the left anterior descending and left circumflex arteries, which supply the left ventricle of the heart. Thus, the patency of this artery is critical to the life of the patient and therefore PCI has traditionally been considered riskier as the balloon temporarily occludes the artery during dilation- putting a large section of the myocardium at risk during a procedure. PCI has also been associated with a loss of collateral vessels following treatment of CTOs [46], a problem that may not be as severe with bypass surgery. Recent trials however, have been showing comparable risk to adverse effects in PCI as compared to surgery in the patients with significant stenosis of the left main artery [47].
One of the most prominent reasons for referral to bypass surgery over PCI is the presence of an occlusion [48, 49]. This trend is due to the fact that CTOs have traditionally been associated with higher complication rates, longer procedural times, lower success rates, and a greater chance of restenosis (re-narrowing)[18]. However, the advent of drug-eluting stents, which have been shown to dramatically reduce restenosis rates in CTOs [50, 51], has led interventional cardiologists to look for better ways to safely treat these lesions.

1.9 Conventional and Experimental Intravascular Therapy in the context of CTO

While procedural success with PCI in coronary CTOs is estimated at 75 % [52], a large majority of CTOs seen under angiography are simply not attempted due to perceived technical challenges. This perception is reflected in the fact that while CTOs are seen on approximately 33% of all angiograms, they account for only ~10% of all PCIs performed [53]. Procedural failure in PCI of CTOs is typically due to the inability to cross the lesion with the guidewire[54, 55]. It has been noted that this difficulty increases with lesion age [54], as it is suspected that the occlusive material becomes more fibro-calcific over time.
This difficulty has led to the development of a wide range of CTO-specific products designed to aid in providing a path through the lesion. Stiffer guidewires with fine tapered tips designed for entering microchannels at the proximal entrance of the occlusion have been produced [56]. Devices that ablate the occlusion with excimer laser [57], radio frequency[58, 59] and ultrasound energy[60] have been demonstrated. These devices have shown limited effectiveness in small studies. Typically they demonstrate, a 70% success rate in patients who have failed traditional guidewire crossing [61]. While recent embodiments have been miniaturized considerably, these devices have traditionally been excessively bulky, thus limiting their clinical acceptance. Mechanical devices have also been implemented to aid in crossing the lesion. Most prominent among these is the Frontrunner® blunt dissection catheter. This device uses retracting jaws to dissect the tissue within the lesion, creating a path that is likely the one of least resistance through the occlusion. Success rates with this device have been reported at 77%[62]. Unfortunately, in practice, the Frontrunner frequently enters tissue spaces between the intima and media creating so called “false-lumens”. This tendency has necessitated the creation of an additional device to re-enter into the “true lumen” and has limited the device’s approval to the peripheral vasculature. Another mechanical device, the Tornus catheter also uses rotational force to “burrow” through the lesion[63]. While this device has been marketed as primarily a method for crossing a CTO, it is also used in cases where the interventionalist has been able to guide a wire through the lesion but is unable to push the balloon catheter along the wire running through the lesion. In these cases, the Tornus follows the guidewire’s path but creates a larger opening for the balloon catheter. Recently, a rapidly vibrating tip catheter has also been produced [64] as well as a guidewire that performs blunt dissection in an “inch worm” like fashion. Unfortunately these devices are typically not studied in large patient groups at multiple centres, making critical evaluation difficult.

A wide variety of specialized guidewire techniques have also emerged for crossing CTOs. One such technique is the sub-intimal tracking and reentry (STAR) approach in which the physician intentionally directs a hydrophilic guidewire towards the subintimal space, and then attempts to bring the guidewire back into the lumen of the artery[65]. The
The motivation behind this approach is that it is easier to pass the guidewire through the vessel wall than the occluded lumen. The balloon and stent are expanded within this subintimal space to create an effect a minimally invasive bypass between the proximal and distal ends of the occlusion. Unfortunately, this procedure is incredibly risky as it is difficult to re-enter the artery from the subintimal space. There is also a significant chance of causing a coronary bleed during dilation of this space. While some investigators have demonstrated the use of intravascular ultrasound to guide re-entry into the lumen [66] and one company produces an intravascular ultrasound array for Doppler blood flow imaging to guide this re-entry, these procedures remain both excessively risky and technically complex for the average interventionalist. Retrograde approaches, in which the physician approaches the distal entrance of the CTO by sidebranches have also been demonstrated[67]. This technique is sometimes attempted when the conventional antegrade (proximal) approach fails.

Pharmacological approaches to facilitating CTO crossing through either softening the collagen matrix or dilating the channels in the CTO have also been attempted. Strauss et al. were able to show reduced wire crossing times in a rabbit model of CTO treated with collagenase[68]. Similarly O’Neill et al. found an improvement in guidewire crossing in patients after administering intracoronary fibrinolytics[69]. Both of these studies required long incubation times (72 and 8 hours, respectively) in order to demonstrate an effect, thus hindering the widespread acceptance of this approach. Recently, direct injection of contrast and nitroglycerin into the occlusion has also been shown[70]. The authors aimed to dilate the microchannels within an occlusion to ease guidewire crossing. This technique however has a high probability of dissecting the artery if too high a pressure is used.

Thus, although a wide variety of devices and techniques exist to aid in crossing an occlusion with a guidewire, no safe reliable method has been presented. The common weakness in most of the techniques is a lack of guidance for the procedure.
1.10 Guidance in the context of CTOs

Two principal types of guidance for CTO crossing have been proposed. One method envisions a “roadmap” style image taken in a non-invasive manner just prior to or during the intervention. This roadmap would hopefully give the physician an idea as to the complexity of the lesion and potential difficulties such as branching points or the presence of calcium. Computed Tomographic Angiography (CTA) has been proposed for such a purpose with CTOs [71]. Additionally, three dimensional roadmap images formed from multiple projections from a conventional two-dimensional x-ray imaging system have recently been shown[72]. The inherent difficulty however with such systems is the development of tools which are able to register where the guidewire is located within the three dimensional map. Efforts in tracking the guidewire tip position and orientation with MRI [73-75] and electromagnetic fields [76] have been demonstrated; however, the three-dimensional positioning accuracy is currently insufficient for interventions in CTOs.

Several intravascular guidance means have also been presented for guiding treatment of chronic total occlusions. Leon et al presented a catheter-based single-point fluorescence technique to distinguish between occlusive material and the vessel wall [77]. This measurement was coupled with a pulsed dye laser for ablation. This device aimed to provide a “stop / go” style feedback. Similarly, a single-line near-infrared reflectance probe coupled to a radiofrequency ablation catheter has been produced and marketed [58, 61] as a device for crossing CTOs. The infrared reflectance probe uses the interference properties of a broadband light source to provide a depth-resolved line map in the tissue approximately 1 mm ahead of the probe. Again, this device gives a “stop / go” style feedback to the operator based on the different reflectance properties of the occlusive material and the vessel wall. No attempt is made to form an image. This device has been shown to be able to cross 54% of CTOs that failed previous guidewire crossing attempts [61]. It has however not been widely accepted due to the lack of mechanical flexibility of the device and the extended time that the device adds to the procedure.

A limited number of clinicians have also reported on the use of side-viewing intravascular ultrasound (IVUS) to provide a step-by-step guide during the procedure. The
IVUS transducer is positioned several mm behind the guidewire and is used to determine whether or not the guide wire remains within the true lumen of the vessel[78]. Side-viewing ultrasound has also been reported as a means to guide re-entry into the lumen of the artery when the wire has gone into the space between the intima and medial layers of the artery. It has also been reported to be used to image the entrance of an occlusion from a branching artery[79]. These niche techniques, however, still remain the domain of the specialist.

1.11 Challenges and Requirements for Forward-Viewing Catheters

A technique capable of providing safe effective guidance during treatment of a CTO that is easy to use for the average interventionalist thus remains an unrealized goal. One concept that has been put forth to address this need is that of a forward-viewing catheter. While it has been recognized that a forward-looking imaging catheter is highly desirable, technical challenges have prevented the production of a suitable device. One challenge is packaging a catheter in a size on the order of a millimeter so that it is able to fit within the coronary arteries which range from 4 mm in the left main artery to 1.9 mm in the distal segment of the left anterior descending artery in patients with normal coronary arteries[80]. The probe must also be somewhat flexible if it is to be able to navigate to the coronary arteries; typically a maximal rigid length of 4 mm is considered acceptable. The probe should also have a sufficient field of view in order to visualize the both sides of the arterial wall simultaneously (given a working distance of ~5mm within a 3 mm diameter artery this translates into a viewing angle of ~33 degrees – as shown in Figure 1.6 below). Finally, the probe needs to be amenable to mass production as devices used in cardiology are typically single use only in order prevent infection.
1.12 The State of Forward-Viewing Catheter-based Imaging

We review efforts in ultrasound, magnetic resonance, and optical imaging modalities to develop suitable forward-viewing catheters.

1.12.1 Progress in Development of Forward-Viewing Intravascular Ultrasound

A forward-looking ultrasound catheter that provides depth resolved tomographic intravascular images has been long desired by the interventional cardiology community[81]. Ultrasound imaging is attractive because of its high resolution (50-200 μm), and its ability to image several millimeters to centimeters deep in tissue. Several approaches to forward-viewing intravascular ultrasound have been implemented; however, none of these has been widely accepted.

Mechanical methods were first proposed in the mid-90s but were hindered by the large size of the probes. Evans et al. demonstrated a mechanism which translated the rotational motion of a standard torque cable into a 90 degree forward scanning angle[81]. The authors were able to incorporate a 20 MHz transducer in order to achieve 120 μm axial by 220 μm lateral resolution in a 4-mm catheter. This work was later adapted to allow for three-dimensional imaging[82]. Back et al. described a catheter that is mechanically rotated in a forward-looking spiral pattern [83]. Liang and Hu demonstrated a rotating mirror to reflect ultrasound energy in a forward scanning pattern in a compact 1.6-mm catheter [84]. Gatzoulis et al. demonstrated a “push and pull” wire mechanism to achieve forward scanning coupled with rotational motion to achieve three dimensional imaging in a stiff 3.8-mm diameter tube[85]. These authors were also able to demonstrate
velocity-sensitive Doppler imaging with their forward looking catheter[86]. The slow frame rate, of their scanning mechanism however, limited this imaging to situations of constant flow.

Capacitive micromachined ultrasound transducers (CMUTs) have also been proposed for forward-looking catheter-based imaging [87, 88]. These transducers are produced using standard lithographic techniques making them amenable to both miniaturization and mass production. CMUTs can also be easily fashioned into a ring assembly such that an array could fit onto the end of a catheter. This geometry is particularly promising for interventional approaches as it allows seamless combination of imaging with an interventional device. While CMUT catheters have produced three dimensional images, this technology is still hindered by insufficient signal strength for soft tissue imaging. Finally, the use of optical detection of ultrasound energy has also been proposed by O’Donnell et al. as a means of achieving a miniaturized forward looking imaging catheter[89]. In this set-up, ultrasound energy is incident on an etalon which is placed at the end of a spatially coherent optical fiber bundle. A Michelson interferometer combined with a system which scans the proximal end of the fiber bundle is used to sense ultrasound-induced displacements in the etalon through each individual fiber in the bundle. While this idea shows promise, it has yet to demonstrate suitable sensitivity for intravascular imaging.

1.12.2 Forward-looking Intravascular Magnetic Resonance Imaging

While the majority of work in intravascular magnetic resonance imaging (MRI) has focused on the development of side-viewing probes for vessel wall characterization, Anderson et al. have recently described an arrangement of orthogonally wound coils to achieve a high resolution forward-looking catheter[90]. It has yet to be determined however if this system possesses the necessary temporal and spatial resolution for interventional guidance.
1.12.3 Angioscopy

Angioscopy is an imaging technique that uses fiber optic white light illumination coupled with fiber bundle detection to image arterial surfaces. Angioscopy was the first intravascular optical technique applied \textit{in vivo} to attempt to identify vulnerable plaques. Angioscopy identifies fibrous tissue as white and more lipid rich lesions as yellow. Efforts have been underway to quantify the “yellowness” of the lesion and correlate this metric to vulnerability to rupture\cite{91}. Angioscopy also played a key role in confirming the fact that a neointima does not form on a drug eluting stent up to 2 years after deployment \cite{92, 93}. The application of angioscopy to image guidance in CTOs has also been reported\cite{94}. However, bulky instrumentation, very pixilated images, and the need to create an optically clear field by using an occluding balloon and saline flush have limited the widespread acceptance of angioscopy in a clinical setting.

1.13 Optical Coherence Tomography

Optical coherence tomography (OCT) relies on the interference of two light waves in an interferometer to form a depth-resolved reflectance map of an object under investigation. To describe its simplest embodiment, it is helpful to examine a Michelson interferometer. As shown in Figure 1.7, an interferometer consists of a light source incident upon a beam splitter, which in turn directs the light to both a sample and a reference arm and also recombines the reflected light before the detector.
In a time-domain setup, the reference mirror is translated such that it matches the path length at a given depth in the sample arm. As the reference arm moves, the resulting signal at the detector is a convolution between the reflectivity of the sample at a path length equal to that in the reference arm and that at the mirror in the reference arm. For a Gaussian spectrum, the axial resolution of the system, $\delta z_{FWHM}$, is determined by optical bandwidth of the light source:

$$\delta z_{FWHM} = \frac{2 \ln 2 \lambda_o^2}{\pi \Delta \lambda},$$  \hspace{1cm} \text{Equation 1}$$

where $\lambda_o$ is the central wavelength of the light source, and $\Delta \lambda$ is the bandwidth of the source in the case of time domain OCT (or the sweeping range, in the case of Fourier domain OCT). Axial resolution can be thought of as the ability to resolve layers at different depths.
From Eqn. 1, we see that the axial resolution in OCT is determined by the properties of the light source rather than the focusing optics in the sample arm. Thus, OCT has the advantage of uncoupling lateral and axial resolution. Given a typical central wavelength of 1310 nm and bandwidths of 60 nm, an axial resolution of ~15 μm is achievable. From an intravascular device perspective, this coherence gating also allows the depth scanning to be performed outside of the catheter, greatly simplifying the design of the distal end of the probe. A two dimensional image may be formed by combining this depth scanning with lateral translation of the sample beam.

Lateral resolution is the ability to resolve two points that are at the same depth. It is defined by the beam waist of the focused optical beam and is governed by the focusing properties of Gaussian beams. The size of the beam waist, $w_o$, at the focus is given by:

$$w_o = \frac{w_p f}{\sqrt{z_1^2 + \frac{\pi^4 w_p^4}{\lambda^2}}}$$

Equation 2

with $w_p$ representing the beam waist exiting the fiber optic, $z_1$ being the distance from the fiber optic aperture to the focal plane of the lens, and $f$ being the focal length of the lens. As one moves away from the beam waist, the beam width increases as a function of $z$, the distance from the beam waist according to:

$$w(z) = w_o \sqrt{1 + \left(\frac{z}{R}\right)^2}$$

Equation 3

In the above equation, $R$ represents the Rayleigh range, or depth of focus, of the beam. This concept is illustrated in Figure 1.8.
Recently a new embodiment of OCT has been developed that spectrally resolves the interference signal from an interferometer with a stationary reference arm. This type of OCT is termed “Fourier domain” OCT as it is based on the fact that the backscattering intensity, $F_s$, as a function of depth, $z$, and the backscattering intensity as a function of wavenumber, $K$ (where $K = 2\pi\lambda^{-1}$), are Fourier conjugate pairs [95]:

$$F_s(z) \propto FT\{a_s(K)e^{i\phi_s(K)}\}, \quad \text{Equation 4}$$

where $F_s(z)$ is the OCT signal as a function of depth, with $a_s(K)$ and $\phi_s(K)$ representing the amplitude and phase respectively of the complex backscattered signal as a function of wavenumber. Fourier domain OCT has been shown to confer an increase in signal to noise over time domain OCT as given by [96]:

$$\frac{(SNR)_{FD}}{SNR_{TD}} = \frac{N_s}{2} (SNR)_{FD}, \quad \text{Equation 5}$$

where $SNR_{FD}$ and $SNR_{TD}$ are the signal to noise ratios for frequency and time domain systems respectively, and $N_s$ is the number of sample points taken in an axial scan line. This increase in signal to noise allows for faster imaging speeds.

Two different approaches for achieving Fourier domain systems have been implemented. One spectrally resolves the interference signal using a broad band light source, as in time domain OCT, and a spectrometer. The other approach uses a narrow spectral band width, wavelength swept laser source. Due to its wider availability at 1310 nm, and its simpler detector implementation, the latter approach was adopted for this work.
One additional property of Fourier domain OCT is that, the depth range of the system is determined by optical properties of the laser and the ability to sample the signal, rather than a time delay in the reference arm of the interferometer (as in time domain OCT) which is determined by the range of travel of the reference mirror. The depth range, $\Delta z$, is the field of view in the axial direction and is given by [96]:

$$\Delta z = \frac{\lambda_o^2}{4n\delta \lambda}$$

with

$$\delta \lambda = \frac{\Delta \lambda}{N_s},$$

Equation 6

where $n$ is the index of refraction of the tissue, $\lambda_o$ is the central wavelength of the light source, $\Delta \lambda$ is the full width at half maximum of the wavelength sweeping range, $\delta \lambda$ is the resolution of the spectrometer or the instantaneous linewidth of the wavelength swept source, and $N_s$ is the number of samples taken per axial scan. In addition to imaging the amplitude of the backscattered signal, important information can be obtained through measuring the phase of this signal. Phase sensitive OCT techniques have been widely employed in OCT as a means of measuring flow. In practice, this is performed by comparing the phase of adjacent pixels at similar depths. In time domain techniques the phase difference between adjacent pixels is obtained by demodulating the in-phase and quadrature signals from the OCT signal [97]. Similarly, in frequency domain OCT imaging techniques, the phase difference is also obtained through comparison of adjacent pixels at similar depths. In this case however, the in-phase and quadrature values are obtained from the real and imaginary components of the OCT signal after the inverse Fourier transform has been performed on the re-sorted reflected signal[98]. The steps for signal processing for a time domain and swept source Fourier domain system are shown in Figure 1.9a and b. The signal processing steps are adapted from [97] (for 1.9a) and [98] (for 1.9b).
Figure 1.9 Illustrates the steps to obtain structural and phase difference images in OCT in both time domain a) and Fourier domain OCT b). In time domain OCT the two signals are obtained by multiplying the signal by sin and cos to obtain two perpendicular components. In Fourier domain OCT the two perpendicular components are obtained from the real and complex components following a complex inverse Fourier transform.

OCT’s high axial resolution (10-30 μm) well suited to assess the thickness of the fibrous tissue overlying an arterial plaque. It is commonly believed that a thickness of 65 μm or less correlates to a high probability of plaque rupture [11]; thus, determining this cap thickness with high accuracy is important. OCT has also been able to clearly identify the different arterial layers with the intimal layer appearing as bright, the media appearing darker and the outer layer appearing bright due to the presence of collagen. Characterization of different types of plaque morphologies such as fibrous, calcific and lipid rich components has also been performed[99]. OCT has also been used to measure macrophage density in atherosclerotic plaques[100], giving a measure of the inflammation associated with a plaque. Polarization sensitive OCT has also been used to evaluate the
collagen content of atherosclerotic plaques *ex vivo*, to aid in differentiating between lipid and fibrotic lesions[101]. Fiber optic delivery of the imaging beam is highly amenable to miniaturization, allowing side-viewing probes on the order of 300 μm in diameter to be produced [102].

A traditional drawback to intravascular OCT has been the limited imaging depth due to the opacity of the blood field at optical wavelengths. This difficulty required clearing the blood field by an occluding balloon[103], saline flush[104] or transparent blood replacement[105]. The high speeds of Fourier domain OCT has largely obviated this limitation for patent arteries by allowing the imaging of long coronary artery segments by non-occlusive saline purging and rapid helical pullback of the catheter[106].

1.14 Motivation for forward looking OCT for CTOs

As a tool for imaging CTOs and for providing treatment guidance, OCT potentially offers several attractive features. The lateral resolution associated with optical imaging (2-30 μm) is well suited for identifying small channels within the occlusion that may provide a path through the lesion. The high axial resolution is also important as it should aid in identifying the border between the occlusion and the arterial wall. The high frame rate of Fourier domain OCT systems (between 15-300 fps)[107] is also attractive given the need to image within a moving organ. OCT has also been shown to be able to distinguish and identify the different tissue components in diseased arteries and is highly sensitive to the scattering properties of the tissue. This sensitivity is useful for imaging CTOs as the occlusion is typically composed of collagen which is highly scattering and therefore bright, while the wall of the artery is typically composed of smooth muscle cells, thus appearing dark[108]. OCT is also highly amenable to miniaturization as fiber optic components can be used for transmitting and focusing the light.

Additionally, OCT has been used in combination with laser ablation methods [109]. The combination of laser ablation methods with forward-looking OCT would offer a powerful but safe way to both cross and dilate blocked arteries and is likely the easiest way to incorporate a therapeutic element with a forward looking catheter. This possibility is further explored in the Future Work section of this thesis on page 108.
Potential drawbacks commonly associated with OCT, such as the need to clarify the blood field, are less clinically problematic with a CTO. During a CTO intervention, a balloon is often expanded proximal to the occlusion to prevent a coronary bleed should the wire accidentally perforate the artery. This situation could easily be adapted for use with a suitable OCT imaging catheter to allow for fluid injection to provide a clear optical field. An additional concern with OCT is the limited penetration depth (typically 1-2 mm) in tissue. While it would be clinically effective to visualize the entire length of the occlusion (anywhere from less than 5 mm to longer than 20 mm long in the coronary arteries[18]), microchannels within the CTO offer the possibility of utilizing the entire depth ranging of the OCT system (2-7 mm)[110], provided they can be clarified through a saline injection. OCT also offers the possibility of performing phase sensitive imaging to measure flow through these small channels[111]. This measurement provides an additional contrast mechanism and may indicate whether or not the channel is a safe path to follow.

1.15 Thesis Aims and Overview

A safe and efficient method to open chronic total occlusions in a minimally invasive fashion would allow for a greater number of patients with coronary heart disease to be treated for chest pain without open heart surgery. While there are many devices that provide assistance to the interventionalist using an intravascular guidewire to cross an occlusion, none of these possess effective guidance means. Due to its high resolution, frame rate, and capacity for miniaturization, OCT is an attractive modality for imaging CTOs. We thus sought to evaluate OCT as an intravascular imaging method for guiding interventions in CTOs.

1.16 Thesis Organization

The present chapter (chapter 1) includes this discussion and introduction to CTOs, the clinical motivation for treating them with intravascular techniques, and the need for a forward-viewing intravascular imaging catheter.
Chapter 2 presents \textit{ex vivo} characterization of CTOs with OCT in the form of a published manuscript entitled: \textit{Ex vivo imaging of chronic total occlusions using forward-looking optical coherence tomography}. This paper was published in \textit{Lasers and Surgery and Medicine} in January of 2007 on pages 28 -35. \textit{Ex Vivo} volume rendered OCT images of CTOs are also presented as an addendum.

Chapter 3 evaluates the continuity and morphology of microchannels within a CTO rabbit model using micro-computed X-ray tomography to discern whether or not OCT could be used to follow microchannels continuously. It also examines the evolution of these channels as a function of the occlusion’s age. This work is presented as a manuscript entitled: \textit{Micro Computed Tomography of Chronic Total Occlusions: Patterns of Vascular Formation}. This manuscript is currently in preparation for submission. Application of the \(\mu\)CT techniques used here was also applied to monitor an experimental therapy; this is briefly presented as an addendum.

Chapter 4 provides a review of previous approaches for forward looking OCT. We present a new mechanism for a compact forward–viewing OCT probe in the form of a manuscript entitled \textit{Electrostatic forward-viewing scanning probe for Doppler optical coherence tomography using a dissipative polymer catheter}. This paper was published in \textit{Optics Letters} in April of 2008 on pages 657-659.

Chapter 5 briefly reviews basics of Doppler OCT and defines “Forward-Viewing Doppler” as a Doppler imaging technique in which the probe and the direction of flow are either parallel or anti-parallel. Preliminary forward-viewing Doppler images of flow in phantoms which mimic nearly-occluded arteries are shown. We also present an \textit{in vivo} surgical “cut down” model that allows for isolation and OCT imaging of the occluded artery using scanning optics external to the artery. Flow within an occluded 2 week old artery is observed.
Chapter 6 summarizes the context of this thesis with respect to clinical applications.
Future work needed to move this approach into the clinic is discussed. Finally, the impact of this thesis within the evolution of interventional cardiology is explored.
Chapter 2 – *Ex Vivo* Imaging and Characterization of CTOs with OCT

2.1 Chapter Overview

Work in characterizing the various pathologies seen in peripheral artery occlusions with OCT is presented in a journal publication. Volume rendered images illustrating an ability to differentiate between microchannels within the CTO that remain within the lumen of the occlusion and those that exit into the vessel wall are presented.

2.2 *Ex Vivo* Imaging of Chronic Total Occlusions with Optical Coherence Tomography

While a forward-looking imaging device has long been recognized as useful for characterizing arterial occlusions and guiding interventions, when this work started no forward looking OCT or ultrasound images of a CTO had been demonstrated. Thus, preliminary work was needed to evaluate whether OCT was a viable option for characterizing occlusions and guiding interventions. In order to evaluate OCT imaging of occlusions, we examined peripheral arterial occlusions from patients who had undergone amputation due to severe peripheral limb ischemia. While the ultimate goal of this work is to image within the coronary rather than peripheral arteries, occluded arteries below the knee are similar to the coronary arteries in terms of arterial diameter and type (peripheral and coronary arteries are both muscular as opposed to elastic). Peripheral arteries obtained from amputations are also much less contentious to obtain, as the patient may be consented prior to the procedure to donate the tissue to science. Coronary arteries, however, are typically obtained from a deceased patient and, therefore, require obtaining consent from a family member who has just experienced a great loss. The aims of this work were to (1) determine how well OCT could image the boundary between the vessel wall and the occlusion and (2) to identify how well OCT could characterize different lesions seen in pathology.

The resulting paper was published in the January 2007 issue of *Lasers in Surgery and Medicine*:
Munce NR, Yang VX, Standish BA, Qiang B, Butany J, Courtney BK, Graham JJ, Dick AJ, Strauss BH, Wright GA, Vitkin IA.
Ex vivo imaging of chronic total occlusions using forward-looking optical coherence tomography.

My role as first author in this paper included coordinating with the pathology department to obtain the samples, scanning the samples, directing the processing of the samples with the special histology department at Mt. Sinai Hospital, interpreting and developing the tissue classification schemes, and writing the paper.

This article was “Reprinted with permission of Wiley-Liss, Inc. a subsidiary of John Wiley &Sons, Inc.”
Ex Vivo Imaging of Chronic Total Occlusions Using Forward-Looking Optical Coherence Tomography

Nigel R. Munce, MSc,1,5 Victor X.D. Yang, MD, PhD,1,2,3 Beau A. Standish,1 Beiping Qiang, MD, PhD,1 Jagdish Butany, MD,6 Brian K. Courtney, MD,1 John J. Graham, MD,6,5 Alexander J. Dick, MD, FACC,6,5 Bradley H. Strauss, MD, PhD, FACC,5 Graham A. Wright, PhD,1,6 and I. Alex Vitkin, PhD1,2,7
1Department of Medical Biophysics, University of Toronto, Toronto, Canada
2Division of Biophysics and Bioimaging, Ontario Cancer Institute, University of Toronto, Toronto, Canada
3Roy and Ann Foss Interventional Cardiology Research Program, Terrence Donnelly Heart Centre, St Michael’s Hospital, University of Toronto, Toronto, Canada
4Division of Cardiology, Sunnybrook Health Science Centre, University of Toronto, Toronto, Canada
5Imaging Research Program, Sunnybrook Health Science Centre, University of Toronto, Toronto, Canada
6Department of Laboratory Medicine and Pathobiology; and Division of Pathology, University Health Network, University of Toronto, Toronto, Canada
7Department of Radiation Oncology, University of Toronto, Toronto, Canada

Background and Objectives: Percutaneous coronary interventions (PCI) of chronic total occlusions (CTOs) of arteries are more challenging lesions to treat with angioplasty and stenting than stenotic vessels due primarily to the difficulty in guiding the wire across the lesion. Angiography alone is unable to differentiate between the occluded lumen and the vessel wall and to characterize the content of the occlusion. New technologies to aid in interventional guidance are therefore highly desirable. We sought to evaluate tissue characterization in arterial (CTOs) by imaging ex vivo peripheral arterial samples with optical coherence tomography (OCT).

Study Design/Materials and Methods: Ex vivo arterial samples were obtained from patients undergoing peripheral limb amputation. Samples were imaged in an en face orientation using an OCT system, enabling sequential acquisition of longitudinal images and volumetric reconstruction of cross-sectional views of the occluded arteries. Histology was performed for comparison.

Results: OCT imaging reliably differentiated between the occluded lumen and the underlying arterial wall in peripheral CTOs. OCT correctly identified tissue composition within the CTO, such as the presence of collagen and calcium and was also able to identify intraluminal microchannels.


© 2006 Wiley-Liss, Inc.

Key words: intravascular imaging; arterial disease; interventional cardiology; optical coherence tomography; chronic total occlusions

INTRODUCTION

Chronic total occlusions (CTO) of coronary and peripheral arteries are generally defined as occluded arteries of 3 months duration or longer [1]. Totally occluded arteries are observed in as many as one-third of all X-ray angiograms [2]. The observation of a CTO under X-ray angiography is the most common reason for referral to bypass surgery as opposed to minimally invasive percutaneous approaches [3]. Percutaneous interventions of coronary and peripheral artery CTOs have only limited success rates (approximately 75% in coronary lesions) due to the inability of the operator to easily direct a guidewire through the occluded lumen without dissecting the adjacent arterial wall. A limited number of histological studies have shown that the occluded lumen of a CTO is a complex lesion containing variable amounts of collagen, lipids, calcification, and intraluminal microchannels [5,6]. The border between lumen and the underlying arterial wall cannot be visualized by contrast angiography, leading to the frequent occurrence of directing guidewires into a subintimal location and procedural failure, even possibly vessel perforation. Recently, we have suggested that intraluminal microchannels, 100–200 μm in diameter, which cannot be identified by current imaging techniques, may play a role in predicting the ease with which the occlusion can be crossed with a guidewire [7]. There is thus a need for investigating new imaging modalities to aid in characterization and guidance of intervention in CTOs.

The authors have no competing interests.

*Correspondence to: Nigel R. Munce, MSc, Department of Medical Biophysics, University of Toronto, Toronto, Canada.
Accepted 25 September 2006
Published online 9 November 2006 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20449

© 2006 Wiley-Liss, Inc.
Optical coherence tomography (OCT) is an imaging modality that uses the interference of light reflected back from the tissue with light from a reference arm to form an image based on the depth-resolved reflectivity of the sample, to a depth of approximately 2 mm in tissue [8]. OCT's axial resolution, on the order of 3–15 μm, has been shown to enable differentiation of arterial layers [9] and identification of various arterial pathologies [10,11]. Contrast in OCT is provided by both the intrinsic backscattering properties of the different tissue types, as well as reflections from the different layers. In the intravascular forward-looking geometry, more amenable to interventional guidance, tissue interfaces are likely to be at an acute angle to the imaging direction. Thus contrast is mostly derived from the reflective properties of the tissue itself.

Recently, an optical coherence reflectometry system, which utilized only single-line (one-dimensional) depth profiles of reflections from interfaces, was approved for use in CTOs. Initial studies suggested that this system was able to differentiate between deeper layers of the vessel wall and the occluded lumen [12–15]. However, this system does not provide images of vessel layer boundaries or identify tissue composition within each layer.

We have thus been examining the potential utility of a forward-looking OCT imaging geometry to provide detailed cross-sectional images of the arterial wall and reliably differentiate tissue layers and identify specific tissue composition. We now report on our first ex-vivo experience with OCT imaging of human peripheral CTOs.

MATERIALS AND METHODS

Sample Preparation

Twenty-two samples of peripheral CTOs were obtained from below knee amputated limbs from 14 patients with peripheral artery disease, with informed consent under protocols approved by the hospital research ethics committee. Angiograms were obtained prior to amputation to identify the area of occlusion. The diseased arteries were dissected out of the limbs following amputation and stored in phosphate-buffered saline at 4°C. The arteries were cut into 5-mm sections. The maximum time between amputation and imaging was 1 week.

OCT Imaging

Arterial samples were imaged using a previously described time-domain OCT system [16]. The geometry of the scanning is illustrated in Figure 1. OCT images are presented on a log-based gray scale with scale bars of 1 mm appearing in red in the figures. This system employs a broadband low coherence source with a polarized output of 18 mW at a center wavelength of 1.3 μm with a bandwidth of Δλ = 63 nm, yielding a coherence length (axial resolution) of ~10 μm in tissue. The reference arm consists of a rapid scanning optical delay line and a phase modulator. The sample arm consists of a single-mode fiber capped with a ball-lens, mounted on a three-dimensional computer-controlled micro-positioning stage, yielding a spot size (lateral resolution) of ~20 μm.

Samples were attached to a piece of Styrofoam in order to preserve their orientation for later histological analysis. The specimen was oriented with the vessel axis parallel to the imaging fiber in order to approximate a forward-viewing intravascular imaging geometry. Lateral scanning combined with coherence gate depth scanning yielded a two-dimensional subsurface longitudinally-oriented OCT image, acquired at a rate of one frame per second. The probe was then translated across the sample in the orthogonal lateral direction in 10 μm increments, to yield a series of approximately 300 adjacent longitudinal slices acquired in 5 minutes that were then used for three-dimensional reconstruction and visualization.

For off-line three-dimensional visualization and reconstruction, OCT images were downloaded into Amira Visualization software (Mercury Computer Systems, Berlin, Germany). This software was used to reconstruct perpendicular (cross-sectional) views of the arterial sample, by converting the set of adjacent two-dimensional longitudinal images into a three dimensional volume which
could then be viewed in arbitrary orientations/projections. Reconstruction time was approximately 10 seconds. Brown lines are shown through each OCT image in the figures to indicate the location of the corresponding orthogonal slice.

**Histology Processing**

Immediately following OCT imaging, arterial samples were placed in formalin and sent for histology. A cross-sectional slice was first obtained and then the sample was re-embedded and sectioned longitudinally. Longitudinal sections were taken every 100 µm to ensure similar views in both histology and OCT imaging. Sections were stained with elastin trichrome to identify elastic tissue (black), muscle and blood (red), and collagen (blue). Calcification was identified with Von Kossa’s stain as black. Lipid was identified with Oil Red O in frozen sections (showing lipid as red). Histology slides were scanned under a white light slide scanner (Aperio Technologies, Vista, CA) to allow for high-resolution (20×) large field-of-view histology images.

**RESULTS**

CTOs obtained and scanned could be generalized into several principal types based on histological appearance:

1. Extensively calcified wall, dense collagen occupying the lumen \((n = 6)\).
2. Microcalcifications within the lumen, embedded in collagen \((n = 6)\).
3. Extensive smooth muscle cell infiltration with collagen in lumen \((n = 4)\).
4. High lipid content in lumen \((n = 2)\).
5. Dense collagen within lumen \((n = 4)\).

In the OCT images, dense fibrotic tissue appeared bright, while highly cellular areas and looser connective tissue appeared darker. Such an occlusion is shown in Figure 2 where smooth muscle cells infiltrating the occluded lumen (OL) are seen as a dark region within the occlusion on the OCT images (Fig. 2a,b) and as a red stain within occluded lumen in the elastic trichrome histology (Fig. 2c,d). The muscular media in this case also appears as dark region (M) in both longitudinal (a) and reconstructed axial (b) OCT slices.

Figure 3 shows an occluded artery with an occluded lumen composed of mostly collagen that show up as uniformly scattering region under OCT (Fig. 3a,b). Moderate calcium deposits (C) within the media layers are seen under histology (Fig. 3c,d).

Figure 4 presents a very chronic, heavily calcified occlusion in which intramural calcification appeared as superficially reflective, signal poor regions within the arterial wall as shown in the region labeled C in the longitudinal OCT image in Figure 4a. Representative histology is shown in Figures 4c,d that illustrate large areas of "fallout" indicative of large pieces of calcium.

An example of an occluded artery with extensive intraluminal microcalcifications embedded within the microchannel (MC) is identified within the lumen and is seen on both longitudinal and axial OCT images as well as histology. Medial and Adventitial layers are labeled M and Ad respectively. Histology is elastin trichrome. Bars = 1 mm. [Figure can be viewed in color online via www.interscience.wiley.com.]

Fig. 2. An angiographically-occluded artery is shown in both longitudinal (a) and reconstructed axial OCT images (b). Darker regions seen on OCT slices (a) and (b) within the occluded lumen (OL) represent infiltration of smooth muscle cells; while the brighter regions are seen as regions of fibrosis as evidenced by histology. A long, thin (100 by 1,000 µm)
collagen matrix is shown in Figure 5. These microcalcifications were seen as highly reflective spots under OCT within the CTO that greatly attenuate the OCT signal with depth as shown in Figures 5a, d in the region labeled C.

A CTO with large lipid deposits is presented in Figure 6. Regions of lipids (L) were observed as signal poor spaces within the CTO as shown in OCT images in Figure 6a,b and confirmed using Oil Red stain shown in Figure 6c.

Intraluminal microchannels within the occlusion were identified in most OCT images as small crevices on the longitudinal slices and holes on the cross sectional slices; that were confirmed by histology. The appearance of the different potential components of CTOs under OCT is summarized in Table 1.

**DISCUSSION**

This is the first study to report on ex vivo imaging and characterization of CTOs using OCT. We have been able to use multiple longitudinal OCT slices to generate cross sectional images of occluded arteries. In all cases, these reconstructed axial views exhibited significantly more information than the original longitudinal slices. The calculated reconstructed cross sectional slices were crucial for reliably differentiating the arterial wall layers (occluded lumen, media, and adventitia) and identifying specific tissue composition within the occluded lumen and the media.

**Identification of the Vessel Wall**

Due to its high collagen content, the occluded lumen of the vessels typically had a higher back-scattering signal than the surrounding medial layers allowing for identification of the occlusion. Cases in which the media appeared fibrotic and hence bright on OCT (as in Fig. 5a) still displayed small muscular regions that maintained a dark OCT signal. This variation in intensity from the media illustrates the necessity of imaging in order to identify the different arterial layers. Additionally, the adventitial layer could be identified in most cases, except those in which severe intramural calcification obscured the boundary between the media and adventitial layers as is seen in Figure 4.

**Identification of Specific Tissue Characteristics**

This is the first demonstration of OCT's capacity to visualize microchannels in CTOs. In all cases, endoluminal microchannels, greater than 50 μm in size, could be accurately identified within the occluded lumen by OCT on reconstructed axial slices. Initial imaging studies using NaCT have shown the ability to perfuse these channels with
radio-opaque casting polymers [7] suggesting that clearing these channels of blood with a saline flush, as would be required for in vivo imaging, is possible. In combination with clearing of the blood field in a CTO, microchannels may provide greater OCT imaging depth than one would normally expect if the occlusion were completely solid. This increase in depth range is an ideal application for new frequency-domain OCT systems that could provide up to 4 mm imaging through a clarified straight microchannel [17].

In this study, intramural calcification appeared as highly reflective regions within the wall of the artery with a signal poor region below it. This appearance is different to that reported in previous OCT arterial studies [10,11] using side-viewing OCT geometries that reported a clearly defined signal poor region. We attribute this difference to the segmental nature of the samples scanned in an en face geometry in this study as opposed to a side-viewing probe. In the side-viewing case, calcification is viewed through the intimal layer of the artery and therefore the reflection is not as pronounced as it is when viewing an air-calciun interface as presented here.

The OCT appearance of lipid, smooth muscle cell, fibrotic regions, and microcalcifications reported in this study agrees with previous reports on these components studied in vessel wall imaging [10,11,18]. The identification of lipid by OCT was, however, often obscured due to its colocalization with microcalcifications in the CTO.

Thrombus within or around the occlusion was occasionally seen as a bright signal lining the microchannel(s) of the CTO. Studies by others [19] suggest OCT's ability to identify and differentiate between red and white thrombus within the coronary arteries. Whether OCT can be used to determine the extent of organization within thrombus remains, however, a subject of future work.

**Significance of Imaging CTOs for Tissue Boundaries and Specific Tissue Composition**

Advanced CTO imaging techniques that are additive to contrast angiography remain a highly desired but unrealized goal. Success rates in CTOs, both peripheral and coronary, are much lower than stenotic but non-fully occluded lesions, in part due to inadequate visualization of the occluded segment. Additionally, the lack of imaging modalities capable of discerning the composition of total occlusions has resulted in a poor understanding of the role of each specific tissue component in procedural success or failure. For example, several studies have offered differing views on the role of calcification as an adverse predictive factor for successful CTO recanalization [20,21]. However,
Fig. 5. OCT images of a CTO with lumenal micro-calculcations, labeled C, are shown in (a) and (d). The lumen appears histologically occluded save for the presence of several small (~50 μm) holes that are not seen under OCT (b). Van Kossa’s staining (c) and (f) indicates calcium deposits within the collagen matrix of the occluded lumen and suggesting that these holes are mostly calcium. These deposits are seen as highly reflective dots to a depth of 300 μm under OCT (d). Collagen-rich areas appear as bright, regions to a depth of 1 mm as before. In this case, it is difficult to distinguish the media from the adventitia due to fibrosis of the medial layer resulting in a high backscattered signal from inner regions of the media. Outer portions of the media (M), however, maintain their dense muscular structure and are seen as a thin dark region identified in (a). Loose connective (LC) tissue surrounding the artery is seen as a dark band surrounding the artery. Histology is elastin trichrome (b) and (e) and Van Kossa’s Stain (c) and (f). Bars = 1 mm. [Figure can be viewed in color online via www.interscience.wiley.com.]

contrast angiography is limited in identifying intramural calcifications and their precise location within the wall. It is likely that calcification restricted to deep arterial wall layers will have a different effect than intraluminal calcifications. Other components of CTOs, particularly collagen, smooth muscle cells and lipid, which can currently only be assessed through histological means, may also portend different success rates. New therapies, such as enzyme-mediated collagen degradation [22], may be better-suited for specific lesion pathologies that cannot be currently identified nor differentiated by contrast angiography but are within the diagnostic ability of OCT. Recently, we have also suggested that intraluminal microchannels are an important predictor of lesion crossing and a potential target for angiogenic CTO therapies [7]. The identification of these microchannels by imaging modalities such as OCT could help clinicians select favorable CTOs for treatment, guide therapy during percutaneous interventions, and also assess the effects of angiogenic therapeutic approaches. Conversely, large lipid deposits may identify more complex CTOs with high likelihood of distal embolization. Thus, the identification of these lipid deposits within the CTO could alert the decision to attempt therapy on a CTO to avoid this risk or potentially allow the interventionalist to try to avoid the large lipid core. While more work is required to identify OCT’s sensitivity and specificity in identifying these pathological features, this study suggests that OCT’s ability to provide a detailed subsurface image of an occluded artery may identify an optimal interventional path and assess the variation in the arterial wall integrity across a large region of the artery.

In Vivo Implementation

Practical in vivo intravascular forward-viewing OCT imaging will require several improvements to the probe design used here. The probe must be flexible and self-contained (no exposed moving parts) with a diameter of 2 mm or less in order to facilitate access to the coronary arteries. Forward-viewing OCT probes [23–26], typically have long rigid segments (> 2 cm) at the distal end that would be unsuitable for use within tortuous vasculature. The probe must also image a wide viewing area in order to visualize the entire occlusion. Designs involving scanning a fiber optic across a GRIN lens would be amenable to such requirements; however miniaturization of such probes remains an ongoing research area. In order to obtain three dimensional images in vivo, similar to the ones presented here, in vivo, such scanning probes could be simultaneously rotated using a torque cable. Frequency domain OCT systems comparable to those reported in the literature [27], would be required to ensure clinically acceptable volumetric imaging times on the order of a second or less. Such high frame-rate systems may permit imaging within the time window created by a saline flush alone, without the
Fig. 6. OCT images of an occluded anterior tibial artery demonstrating high lipid content are shown in (a) and (b). A small central microchannel (MC) is seen in both reconstructed cross-sectional OCT slices and histology. Lipid deposition, labeled L, is seen both within the collagen matrix of the CTO as well as accumulation around the central microchannel on the Oil Red O histology shown in (c). These regions are seen as weakly scattering regions in the OCT images. Lipid deposition around the central microchannel appears as small segmental deposits seen in the longitudinal OCT image shown in (a). Areas of the lumen containing a high collagen content once again appear as bright under the OCT images. Histology is Oil Red O in (c) and Elastin Trichrome (d). Bars = 1 mm. [Figure can be viewed in color online via www.interscience.wiley.com.]

need for an occluding balloon. Such a saline flush would be necessary in vivo as blood would severely degrade the image quality.

The intravascular image created in such a system would likely only visualize the media and adventitia layers in the larger scan angles of the image due to the limited imaging depth of OCT. Despite this limitation, the ability to resolve the different arterial layers as well identify constituents of the occlusion and identify microchannels would add a valuable tool to the interventionist’s arsenal.

TABLE 1. OCT Signal Characteristics of CTO Constituents

<table>
<thead>
<tr>
<th>CTO component</th>
<th>OCT appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perivascular tissue (loose connective tissue surrounding the artery)</td>
<td>Dark border surrounding the artery</td>
</tr>
<tr>
<td>Adventitia</td>
<td>Signal rich peripheral border of the vessel</td>
</tr>
<tr>
<td>Media</td>
<td>Signal rich in significant fibrosis or signal poor when it maintains its muscular nature</td>
</tr>
<tr>
<td>Collagen within the lumen</td>
<td>Uniformly back-scattering region. Denser collagen has a higher back-scattering signal (Figs. 3 and 4)</td>
</tr>
<tr>
<td>Smooth muscle cells within the lumen</td>
<td>Dark regions within the collagen matrix of the CTO. Not very reflective (Fig. 2)</td>
</tr>
<tr>
<td>Intraluminal microchannels</td>
<td>Fine cracks within the CTO. Residual blood shows as a bright reflective lining</td>
</tr>
<tr>
<td>Lipid</td>
<td>Lightly scattering in large pools; Can be identified also as small “segments” (Fig. 5)</td>
</tr>
<tr>
<td>Microcalcifications within the CTO</td>
<td>Highly reflective dots. When abundant, they create shadows (Fig. 5)</td>
</tr>
<tr>
<td>Intramural calcium</td>
<td>Highly reflective on the surface; otherwise signal poor (Fig. 4)</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS
The authors acknowledge the work of the special histiolytic lab at Mt. Sinai Hospital, as well as the help of Peter Faure in the department of pathology at Toronto General Hospital. Support for this project was provided by the Canadian Foundation for Innovation, the Ontario Centers of Excellence, Photonics Research Ontario, as well as the Canadian Institutes of Health Research.

REFERENCES
2.3 Addendum to this work

While the work presented in the journal above described 3-D characterization using a time-domain OCT system, since publication our lab has acquired several frequency domain OCT systems. The signal to noise of Fourier domain systems is $N/2$ times larger than a time domain system, where $N$ is the number of samples taken in an axial scan (as shown in Eq. 5 in chapter 1). This increase allows for faster scanning times, and thus we were able to perform 3 dimensional volumetric imaging of occluded arteries approximately ten times faster than with the time domain system.

We were also able to demonstrate segmentation of selected components of the occlusion for three-dimensional visualization. This work used the Amira software program (Mercury Computer Systems) to manually label different regions from a three dimensional OCT imaging stack. The different regions were identified based on their OCT image appearance, knowing that the media is typically signal poor and collagen within the occluded lumen is signal rich. Example images are shown in Figure 2.1a and 2.2a.

Figure 2.1a) illustrates a segmented OCT volume of an occluded human anterior tibial artery. The red volume represents the segmented medial wall (labeled M), while the yellow volume illustrates a channel passing through the centre of the occlusion (labeled MC). The space occupied by the occluded lumen is labeled OL. b) shows a representative Movat pentachrome stained section from the same tissue segment as shown in a). In Movat tissue staining red represents muscular tissue, yellow represents collagen, blue represents proteoglycan rich tissue and black represents elastic tissue. Scale bar in b) is 1 mm.
Figure 2.2 a) illustrates a segmented OCT image volume stack of an occluded human anterior tibial artery in which we identify a channel crossing the medial wall of the lesion. As in the previous figure, the red volume in a) represents the medial wall (labeled M) while the yellow volume is segmented from areas identified as microchannels. (labeled MC) Representative Movat stained histology is shown in b) obtained from the same tissue segment. Scale bar in b) is 1 mm.

2.4 Conclusions

This work demonstrates that OCT is able to identify various vascular pathologies seen in occlusions. We were able to identify the border between the occluded lumen and the media of the artery in most cases. We were also able to identify microchannels within the occlusion using OCT. In all cases, it was found that the reconstructed “face-on slices” were easier to interpret and to identify features than the longitudinal slices. This observation emphasizes the importance of having a probe capable of three dimensional forward-viewing imaging.

One potential drawback of this work is that samples were obtained from cases that likely failed intravascular interventions. Therefore, we may have omitted from characterization samples upon which intravascular therapy was easy to perform. Such arteries may be composed of less dense collagen and possess a greater number of microchannels than the very fibrous and often calcific occlusions that we typically saw. An additional drawback was the absence of cardiac CTOs from the characterization due to the difficulties in obtaining this tissue. Finally, expansion of this study to include blinded readers to ascertain how well different interpreters can identify the boundary between occlusion and vessel wall would also add to this work. These follow-ups remain to be pursued in future investigation.
From the published article and the additional work, we saw that microchannels within the lesion play a large role in our capacity to image through the occlusion. Provided that the microchannels are free of blood, OCT is able to suitably image these channels for several millimeters. Thus, provided that these channels were continuous throughout the lesion, OCT could be employed in a step-wise fashion to safely traverse the occlusion. OCT was also able to identify instances where the microchannels appear to traverse into the arterial wall. Such locations may be very problematic when using a guidewire as they may lead the guidewire to exit and perforate the vessel wall. Yet little is known regarding the global architecture of microchannels within CTOs. While it has been suggested that these channels may provide a path through the lesion[19], little is known about their global architecture within an occlusion. It is also not known how often they traverse through the vessel wall and exit the vessel. These questions motivated us to study the morphology of microchannels in CTOs using micro computed x-ray tomography.
Chapter 3 – Three Dimensional Microvascular Morphology of Chronic Total Occlusions

3.1 Chapter Overview

The aim of the work presented in this chapter was to ascertain the three dimensional morphology of CTO microvasculature and how it evolves over time. The importance of understanding the 3 dimensional structure of CTO microvasculature is discussed within the context of developing an intravascular forward-viewing imaging probe. A manuscript, to be submitted to Circulation Research, is presented that, shows that, although CTOs possess long continuous channels, these channels can also exit to the arterial wall.

3.2 Motivation for 3-D Vascular Morphology Study of Chronic Total Occlusions

Important unanswered questions regarding microvessels in CTOs remain, including to what degree intraluminal microchannels are continuous in a CTO and how frequently they exit from the lumen into the vessel wall. Such information is important in evaluating the role microchannels may provide for a forward looking intravascular device. For example, if the channels are straight and continuous, it should be possible to clarify these channels through saline injection and visualize a long track through the occlusion. If the channels are somewhat tortuous, then clarifying them is still possible but visualization is limited by the curvature of the channels. If, however, the channels are discontinuous, then clarification is difficult and visualization will be limited by the ability of the imaging signal to penetrate through blood and tissue. The shape of these channels and frequency that they exit the lesion is also important. If these channels rarely exit the lesion then the need for imaging guidance of guidewire crossing following these channels is reduced. Furthermore if they exit the lesion at a shallow angle as opposed to a sharper angle, it may be more difficult to identify them as channels leading to the arterial wall.

Previous work by Katsurgawa et al. has suggested, in a limited histological study of 10 coronary CTOs, that CTOs with tapered entrances (5 out of the 10 studied) typically have channels that traverse the lesion (4 out of the 5), while blunt occlusions have channels that are seen to exit into the arterial wall (5 of the 5 blunt CTOs)[18]. Dvir et al.,
report on visualizing fine recanalization channels in 43 of 61 patients with coronary artery occlusions by reconstructing vascular volumes using several angiograms taken at different angles[72]. However, the authors were not able to observe any exiting channels.

Initial histological work, shown below, examining serial slices taken every 2 mm in a rabbit occlusion model suggested that the microchannels in these occlusions were also continuous as the microchannels in each section appeared similar locations in each axial slice.

Figure 3.1 Masson’s Trichrome stained histology sections of a 6 week old occlusion in a rabbit thrombus model. Sections were taken approximately every 2 mm from proximal (a) to distal (d). Microchannels within the occluded lumen are identified with red arrows appear to be continuous along the length of the lesion. Inside of these channels microfilm can be seen as a dark material.

Histology sections alone, however, cannot easily determine the three dimensional structure of these channels. Although 3-D reconstruction from multiple histological slices
has been performed by others in small animals [112, 113], such techniques require hundreds of slices to be taken and can be challenging to ensure that each slice is correctly aligned. For the occlusions in this study, over 500 slices would be required if short axis sections every 30 \( \mu \text{m} \) were used to reconstruct the entire occlusion. Pursuing such a method would have allowed for precisely determining whether a microvessel was located in the adventitia, the media or the occluded lumen. However, given that we were investigating 4 time points with at least 8 samples per time point the amount of data created would have been difficult to handle and the software tools for reconstructing 3-d volumes from multiple histology sections are not well developed.

Micro computed tomography is an imaging technique in which the sample is rotated on a stage while being irradiated by a micro-spot x-ray source to form a three dimensional volumetric image. To obtain contrast in the vasculature, a lead based contrast agent is injected immediately after sacrifice. This imaging technique naturally results in aligned slices as the sample is not cut. It also allows for the use of segmentation software that has been developed for conventional x-ray CT imaging. Therefore to investigate the morphology of the microchannels in CTOs, micro-computed tomography was used.

For this work, a rabbit femoral artery occlusion model was used. This model involved the injection of thrombin into the lumen of a surgically exposed artery and waiting to allow for a thrombus to form. The natural history of this model has been characterized in terms of the physical remodeling that the artery undergoes, such as the cross-sectional area of the intraluminal microchannels, the amount of collagen within the artery, and the inflammation seen in the occluded artery [114]. While it is difficult to say precisely how this evolution correlates with what is seen in human pathology, many similarities exist. These include the breakdown of the internal elastic lamina, the conversion of thrombus to collagen and the presence of microchannels. Important to note however, is the absence of calcification in both arterial wall and the occlusion itself in the rabbit model. This lack of calcification may result in greater negative remodeling (shrinking) of the occluded rabbit artery as compared to the pathology seen with human CTOs.
3.3 Micro Computed Tomography of Chronic Total Occlusions: Patterns of Vascular Formation

The following paper is in preparation and will be submitted to Circulation Research. Micro Computed Tomography of Chronic Total Occlusions: Patterns of Vascular Formation

Abstract

Objectives: The objective of this study was to characterize the intraluminal and extramural microvasculature evolution of a chronic total occlusion (CTO) rabbit model.

Background: Despite their name, chronic total occlusions are often incomplete occlusions, possessing small vessels that are seen on pathology. However, neither the three dimensional anatomy nor the origin and evolution of these vessels over the time course of the lesion is well defined. Knowledge of the morphology of microvessels would be of great interest for developing new interventional approaches.

Methods and Materials: 47 thrombotic occlusions were created in a rabbit femoral artery model. Animals were sacrificed at 2, 6, 12, and 24 week time points to give >8 occlusions per time point. The arteries were filled with a low-viscosity radio-opaque polymer compound at 150mm Hg pressure. Samples were scanned in a micro computed tomography (μCT) system to obtain high resolution volumetric images. Analysis was performed in an image processing package that allowed for labeling of multiple materials.

Conclusions: Two classes of microvasculature were consistently observed in the experimental occlusions: a circumferential neo-vasculature appearing just outside the vessel labeled as extramural as well as intravascular vessels inside the artery. We observed a dramatic rise in the extramural vasculature at the 2 week time point. This rise
in extramural vasculature was followed by an increase in the intravascular vessels within the central region of the occlusion at the 6 week time period as measured by μCT. The intravascular channels within the occlusion appear highly continuous at all time points later than 2 weeks. While these channels do typically traverse the lesion, they also exit the vessel frequently at sharp angles. As the lesion ages the channels become finer and more tortuous, potentially making the occlusion more difficult to cross with a guidewire.
Introduction

Complete occlusions of coronary arteries are seen on approximately one third to one half of all angiograms [22, 23]. Furthermore, approximately 1% of patients over the age of sixty have symptomatic occlusive peripheral arteries[115] with this prevalence being dramatically higher (~30%) in the diabetic population[116]. A chronic total occlusion (CTO) is defined as an angiographic occlusion with TIMI= 0 or 1 which is older than 3 months[15]. Within the coronary arteries, several studies have shown that successful revascularization of a CTO corresponds to a reduction in symptoms [41], and improved left ventricular function[117]. Recanalization of peripheral arteries is important for limb salvage and decreased incidence of infection. The primary challenge in minimally invasive treatment of CTOs is the difficulty in crossing the lesion with a guidewire prior to balloon angioplasty [54, 55].

While local composition of CTOs has been previously reported, information on the global architecture of an occlusion, as well as it evolution, remains elusive. Pathologically, a CTO is primarily composed of organized collagen formed from an aging thrombus. While a CTO is, by definition, occluded under x-ray angiography, histologically approximately half are less than 99% occluded[16].

Neovascularure within an occluded artery is seen arising from three distinct processes. Firstly vessel proliferation arises in the vasa vasorum (Vv) as a response to injury. The Vv is the network of fine vessels located in the adventitia and the more peripheral layers of the tunica media of the artery. Previous work by others in patent arteries have used micro computed tomography (μCT) to identify two classes of vessels within the Vv: 1st order vessels which run longitudinal and parallel to the artery as well as 2nd order vessels which are circumferential to the vessel[20]. These authors were able to show that in a balloon-injured artery, the number and density of adventitial Vv vessels increases.

Secondly, intimal vascularization arising from a plaque has also been observed. This vasculature is thought to originate from the Vv as a response to inflammatory and hypoxic factors[118] and is believed to play a major role in destabilizing the plaque, potentially leading to plaque rupture[9].
Thirdly, recanalization channels resulting from reorganization of thrombus within the occlusion have been noted. These channels have been seen within both coronary[16, 18] and peripheral arterial CTOs [119] as well as in animal models[19, 68, 120]. Previously we have suggested that these channels may provide a path for successful guidewire crossing as it is likely that the channel would be the path of least resistance through the dense collagen of the occlusion[19]. Furthermore, recent work in our group using Doppler ultrasound of a CTO induced by depositing a polymer plug into the superficial femoral artery of a pig illustrated that these channels do possess flowing blood[21]. This work was not, however, able to visualize the entire three-dimensional architecture of these channels or track their evolution as a function of time.

Thus important questions remain about the three dimensional vascular morphology of chronic total occlusions and the evolution of the microvasculature as the lesion ages. To address these questions, we examined CTOs created in a rabbit model using μCT at multiple time points.

**Methods**

**The Occlusion Model**

Approval for experiments was obtained from St Michael’s and Sunnybrook Hospital Animal Care Committees. Bilateral arterial occlusions were initiated in 28 Male New Zealand white rabbits (Charles River Canada, St Constant, Quebec) weighing 3.0 to 3.5 kg, as previously described[68]. Briefly, a femoral artery segment was isolated and ligated at each end. The segment was then injected with ~0.1 ml of 100 IU/ml of bovine thrombin solution and then the proximal ligature was removed in order to allow blood to mix with the thrombin to create a thrombus. The distal ligature was maintained up to 60 minutes to ensure a persistent occlusion. Animals were then returned to their cage and fed a regular diet. Animals were sacrificed at 2, 6, 12, and 24 weeks [n ≥ 8 occlusions per time point] following creation of the CTO.
Contrast Perfusion

An x-ray angiogram (as shown in Figure 1) was performed in order to verify the occlusion prior to sacrifice using a catheter in the abdominal artery introduced via the carotid artery. After the angiogram, the catheter was withdrawn and intravenous heparin (1000 units) was administered approximately ten minutes prior to sacrifice to prevent blood coagulation in the microvasculature. Immediately following sacrifice, a syringe was inserted into the abdominal aorta and the tip was tied in place with a ligature to prevent back flow. Following an injection of 30 ml normal saline to flush clotted blood from the main arterial system, Microfil (FlowTech, USA) was injected at a pressure of 150 mmHg as measured by a handheld manometer. The Microfil was given an hour to set, then the femoral arteries were surgically removed and left in formalin for 48 hours. Specimens were then embedded in 2% w/w agarose gel. The samples were imaged in a μCT system (MS-8, GE Medical Systems, London, Ontario), [121]. Three-dimensional cone beam CT data sets were acquired in 2.5 hours with 905 views at 28-μm resolution. An x-ray source of voltage 80 kvp and a beam current 90 mA was used. A 3-D data volume was reconstructed at 14-μm resolution using the Feldkamp algorithm for cone beam CT geometry.

Image Analysis

The single 3-D data volume file generated by the μCT software (GE MicroView) was split into multiple axial slices using software (MRIcro) so that it could be imported into a volumetric image analysis software (Amira, Mercury Computer Systems). Amira was used to label the two distinct types of vasculature seen within the CTO: intravascular vessels associated with the recanalization channels in the lumen; and an extra-arterial vessels representing vessels in the arterial wall and immediately adjacent to the artery. Labeling of the intravascular channels was done by first identifying the patent vessel just prior to the occlusion; this vascular area was labeled with a tool that acted as a seed growing algorithm, propagating the intraluminal region through connected regions in successive axial slices. A similar step was performed at the distal end. This seed growing algorithm typically labeled all connected vasculature associated with the occluded artery segment. In order to identify vessels that were extramural, we applied two methods. Firstly extramural vasculature could be directly identified at the proximal and distal ends.
as the small vessels surrounding the patent artery prior to the occlusion. These vessels were typically arranged in a circumferential ring around the patent vessel (as shown in Figure 1a) and were labeled as a different material in Amira using a tool that filled in homogenous areas and stopped when the pixel value changed abruptly. These circumferential vessels were labeled for each slice as they progressed into the occlusion. The circumferential nature of these vessels was used to define a border between the extramural and intravascular vessels. This border was marked on the screen as a reference as shown in Figure 1a-h by the dotted yellow line. As we progressed through the axial slices of the occlusion, vessels that were arranged in a semi-circular shape that fit on or outside of this border were labeled as extramural. Secondly, at time points greater than 2 weeks, sufficient contrast was consistently obtained between the tissue surrounding the artery and the artery itself. This contrast was enhanced at these later time points as the artery negatively remodeled thus increasing the relative amount of loose connective tissue surrounding the artery. In these slices, vessels that appeared on the border of loose connective tissue and the artery were labeled as extramural. Intravascular vessels which crossed the arterial border were relabeled as extramural. Labeling was performed by two independent readers (NM and MW). The software package was used to color code and display the intravascular vessel category as red and the extramural vessels as blue in an isosurface.

The Amira software package also calculates the number of voxels in each axial slice that were identified as intravascular and extramural. The start and end of the occlusion were defined as the axial slices in which the vessel lumen diameter, as measured by μCT, decreases by 90% as compared to the patent artery. The endo- and extra-luminal vascularity as a function of the lesion length was normalized to a length of 500 arbitrary units using software (MATLAB, Mathworks) so that occlusions of different lengths could be compared. We then obtained an averaged vascular distribution at each time point by averaging these normalized vascular distributions.

Communicating channels were defined as points in which the intravascular vessels crossed the defined border between the intravascular and extramural vessels. These events were counted by scrolling through all of the axial slices of each occlusion and counting the number of such incidences. The number of these events was averaged for each time point.
Results

Forty-seven occlusions were successfully created in 28 rabbits. Three of these occlusions were not analyzable due to poor filling with Microfil and an additional three possessed occlusions that were less than 1 mm in length. Of the occlusions analyzed, we obtained an average inter-observer variability in intravascular vessel volume of ~10% by two trained readers.

Typical three-dimensional microvascular volumes at 2, 6, 12 and 24 weeks are displayed in Figure 2a-d. Intravascular channels are labeled in red while vessels labeled in blue are extramural vasculature associated with the occluded artery. Typically the occlusion was formed between a branching collateral and the distal bifurcation of the rabbit femoral artery. We defined the start and end of the occlusion as the axial slices in which the intravascular vessel area decreased by ninety percent relative to the fully patent artery. The average length of the occlusion was 18.1 ± 4.1 mm at 2 weeks, 10.7 ± 0.8 mm at 6 weeks, 14.1 ± 3.7 mm at 12 weeks and 15.0 ± 2.7 mm at 24 weeks. Noticeable in the volumetric images is the progression from an amorphic entrance at the early two-week time point to a tapered (sometimes meandering) structure at both the proximal and distal ends of the occlusion at later time points. Also of note is the large increase in intravascular vascular volume seen between the two- and six-week time points. The number of separate intravascular channels appears to decrease at later time points. We were also able to observe extramural vasculature appearing as circumferential vessels surrounding the occluded artery. These vessels were most predominant at the early time points and appear to diminish over time.

Figure 3a displays the average vascular distribution of the intravascular microchannels as a function of length along the occlusion for all time points. We observed a sudden increase in the vascularity of the central region of the occlusion at the six week time point. As the lesion aged, this central region decreased but greater recanalization was seen from both proximal and distal ends of the occlusion. Figure 3b displays the averaged vascular distribution of the extramural vessels along the length of the occlusion. At the early 2-week time point we see an initial centralized distribution of extramural vasculature.
consisting of two broad peaks. At the 6-week time point we see a shift of this distribution towards the proximal end. At the 12-week time point these vessels appear evenly distributed across the occlusion. Similarly at the 24-week time point, the extramural vascularity is also evenly distributed but substantially less prevalent than previous time points.

The total average vascularity of the lesions was also investigated by evaluating the average number of voxels labeled either as intravascular or extramural across all axial imaging slices. It should be noted that a typical number of voxels per slice in a patent vessel is approximately 3000. Figure 4 illustrates a peak in the total intravascular vessel volume at the 6-week time point. This vascular volume gradually falls off as the occlusion ages. The average extramural vascularity, however is seen to peak at the very early time points and falls off rapidly as the occlusion ages.

To investigate continuity of the intravascular vessels within an occlusion we evaluated the percent continuity by identifying a continuous segment as one that was longer than 1 mm, and defining the percent “continuity” of the channels in a CTO as the sum of the lengths of all of these segments divided by the total length of the occlusion. Although a useful first quantification metric, this concept as defined above is not without its problems. Note that parallel intravascular microchannels that were observed to join together were counted as single segment in the context of this continuity measurement. However, this measurement does allow for the possibility of unconnected channels larger than 1 mm creating an artificially high value for the continuity metric (including possibly exceeding the 100% level). In practice, typical occlusions possessed between one and three disconnected (separate) segments as detailed in Table

We observed very little continuity of the intravascular microchannels at the two-week time point but saw average continuity of 85% at time points six weeks or greater (figure 4c).
The properties of the intravascular vascular segments at different time points are summarized in the Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Age of Occlusion</th>
<th>Average Number of Segments</th>
<th>Average Segment Length (mm)</th>
<th>Location of Vascular Segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.6</td>
<td>3.5</td>
<td>50% - only 1 segment from proximal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30% - several segments</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20% - only 1 segment from the distal end</td>
</tr>
<tr>
<td>6</td>
<td>1.5</td>
<td>6.1</td>
<td>25% - only 1 segment proximally</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>62.5% - segments from both proximal and distal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.5% - only 1 segment from the distal end</td>
</tr>
<tr>
<td>12</td>
<td>1.5</td>
<td>8.5</td>
<td>12.5% - only 1 segment proximally</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50% - several segments</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>37.5% - only 1 segment from the distal end</td>
</tr>
<tr>
<td>24</td>
<td>1.9</td>
<td>5.2</td>
<td>89% - segments from both proximal and distal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11% single microchannel in the mid-occlusion</td>
</tr>
</tbody>
</table>

Communicating channels were also consistently observed at all time points as points in which the intravascular vessels appeared to exit the artery. An example of such channels as seen on μCT slices is shown in Figure 5a. A graph of the number of occurrences of these communicating channels as a function of time is shown in Figure 5b.

**Discussion**

In this work we identify regional vascular changes and their evolution in a rabbit femoral artery thrombus occlusion model.
The early stage (2 week old) occlusions are characterized by rounded blunt proximal and distal ends with minimal penetration of channels into the body of the occlusion. The proximal entrance of the occlusion is typically located just after a branching artery, as has been observed by others[18]. We identify a dramatic increase in the extramural vascularity at the two-week time point. These channels appear as a fine diffuse, ring-like vasculature surrounding the occlusion similar to the form of 2nd order Vv structures as reported by Kwon et al. Intravascular channels are typically minimal at this early time point. Those that are present are highly discontinuous and appear to originate from the extramural vasculature.

The 6-week time point corresponds to a dramatic increase in intravascular volume. The proximal and distal ends of the occlusion begin to take on a tapered appearance. In some instances the intravascular vessels appears as multiple vessels while in other occlusions it appears as a single large vessel. In both instances these channels are traceable over long distances of the occlusion. In examining the distribution of the intravascular vasculature we see that there is a peak in the central region of the occlusion. The extramural vasculature is still present but no longer appears as a mesh-like neovasculature, but rather is consolidated into several more distinct vessels, similar to the 1st order Vv described by Kwon et al. The distribution of the extramural vessels appears to be shifted slightly proximal relative to the intravascular vasculature.

At the 12-week time-point we see a decrease in the intravascular vascular volume as the channels begin to narrow. Typically these channels appear as single narrow microvessels which are continuous over the majority of the CTO. The overall distribution of the intravascular volume still has a central peak within the body of the lumen but it has regressed relative to the earlier time point. The extramural vasculature at this time-point has also decreased relative to the 2- and 6-week time points and typically appears as distinct regions across the artery.

At the 24-week timepoint the intravascular channels appear more tortuous and finer still. The intravascular vascular volume appears evenly distributed and the channels are still continuous across the lesion. The extramural vasculature is less prevalent than at other time points. Both proximal and distal ends exhibit fine gradual tapers.
There has been a great deal of debate in the field as to the nature and evolution of vascularity within occluded arteries. Particularly, questions pertaining to the role of the Vv in revascularizing an occluded artery remain unclear. Angiogenesis within the arterial medial wall has been widely noted as a response to hypoxia caused by growth of the intima in injured arteries[122]. In this work, we identify a peripheral “extramural” vasculature that appears very prominently at the early time points and falls off sharply as the occlusion ages. Most likely these vessels represent the Vv; however, as μCT lacks sufficient contrast to reliably differentiate between the occluded lumen and the vessel wall, it is difficult to definitively identify them as such. Multiple communications between this extramural network and the body of the occlusion are seen between the 2 and 6 week time periods. Corresponding to this extramural vasculature we see a rise in the intravascular vascular volume at the 6 week time period in the central region of the occlusion suggesting that in-growth of extramural vessels are responsible for an increased vascular volume in the central portion of the occlusion. The larger amount of neovasculature observed within the center of the lesion may be due to greater hypoxia in this region. One illustrative example of this ingrowth is shown in figure 6 of a two week old occlusion, where the extramural vasculature appears to feed intravascular vessels. This new intravascular vasculature appears as fine, highly discontinuous fragments within the CTO.

As the lesion ages, we observe revascularization driven from both proximal and distal ends of the occlusion. This process results in the development of the tapered entrances similar to those seen in angiography[54]. Whether this recanalization is due to pressure driven mechanisms analogous to those seen in river formation[123] or to the action of specialized circulating cells, as has been observed in venous thrombi[124], remains unclear. What is observed in this work, however, is that the initial vascular volume peak within the central portion of the occlusion seems to merge with the distal and proximal ends as the lesion progresses. This results in a continuous, albeit tortuous, path through the lesion. The central intravascular vascular volume peak observed at the six week time point decreases suggesting that many of the vessels in this region, either become non-functional or lead the lesion to becoming patent.

Thus, we suggest that recanalization channels originate first from the vasa vasorum forming a large number of tortuous vessels within the center of the occlusion at early time
points. As the occlusion ages, the distal and proximal ends of the occlusion also begin to recanalize into the lesion. Eventually the central channels join up with these peripheral inroads.

Numbers of fenestrations directly connecting the intravascular vasculature with the Vv peak at a 6 week time point and gradually decrease as the lesion ages. This decrease may be caused by negative remodeling of the artery at late time points as well as a decrease in the extramural vasculature that was associated with late stage occlusions. The fenestrations appear to exit the vessel at very sharp angles as is shown in the volume rendered image in figure 6c. Whether these locations are indeed the typical sites that a wire is likely to either dissect the artery or create a sub-intimal passage is a subject of future investigations.

**Study limitations**

The CTO model differs from the clinical case primarily due to its lack of atherosclerosis and calcification. The occurrence of calcification within the vessel walls either prior to or post thrombotic occlusion likely has significant implications for the architecture of the occlusion.

Additionally, due to the nature of the μCT studies used here, animals could not be followed serially. This limitation results in the need for us to compare different animals at different time points; thus, increasing variability. Newer μCT systems coupled with blood-pool contrast agents may be able to address this challenge in the future[125].

**Clinical Implications**

Intravascular microchannels represent an important therapeutic target for either endovascular or pharmacological revascularization strategies. This work illustrates that they are continuous over large segments of the occlusion and do provide a path through the lesion. They may also however exit the lesion frequently making blindly following their path potentially very risky. This observation is important given that a number of devices have been introduced that may follow the “path of least resistance” through a CTO [62, 64].
Recent work has suggested that conventional fluoroscopy can be used to build up three dimensional images of microchannels within CTOs[72]. These authors also reported highly continuous microchannels in CTOs. While such images may be important for guiding procedures it remains to be seen whether such techniques can identify fenestrations within the CTO and whether conventional guidewires exhibit sufficient control to avoid exiting the lesion at these points should these holes be identified. Forward-looking intravascular imaging catheters using either ultrasound[87] or optical coherence tomography[126] have been proposed to enable such an imaging solution. However, effective miniaturization remains an unmet challenge.

This work puts forth the concept that neovasculature arising from the vessel wall does play an important role in recanalizing the lesion. This observation may explain why lesions in which the wall is severely calcified are more difficult to cross. It also raises the possibility of targeting a viable arterial wall with angiogenic factors or stem cells to aid increase vessel in-growth, and potentially recanalization of the occluded artery.
Figure 1: Micro-CT slices shown in a) (proximal) to h) (distal) identify tracking through the lesion of both the intravascular channels (shown in red) and extra-vascular vessels (shown in blue). The lumen is marked with an “L” in the patent sections a) and h). The dotted yellow line represents the outer border of the artery and was drawn based on the difference in average intensity of the tissue in the artery as compared to the loose tissue surrounding the artery. In i) we show the resulting volume generated from the labeled materials and the location of the axial slices shown. An angiogram of a 12 week old CTO is shown in j) with red arrows identifying both the proximal and distal ends of the occlusion.
Figure 2: Progression of microvasculature in a rabbit femoral CTO model at 2 weeks a), 6 weeks b), 12 weeks c), and 24 weeks d). White arrows label the proximal end of the occlusion while black arrows show the distal end.
Figure 3: The average intravascular vessel volume of microvessels in an occlusion as a function of distance in the occlusion for several time points is shown in a). The average extramural vascular distribution across occlusions at several time points is shown in b).
Figure 4- The total intravascular a) and extramural b) vascularity is shown as a function of the age of the occlusion. The average continuity of the lesion as a function of time is shown in the graph shown in c).
Figure 5: An axial micro-CT slice shown in a) illustrates a point in which intravascular channels (red) appear to communicate with extramural vessels in the arterial wall (shown in blue). A longitudinal slice through the occlusion shown in b) illustrates several fenestrations identified with yellow arrow heads. The number of fenestrations observed as a function of the occlusion age is shown in c). A volume rendered image of a 12 week occlusion in d) shows several extramural vessels (blue) communicating with intraluminal vessels. The inset image in d) illustrates the widefield view of the occluded artery with the proximal entrance being located in the upper left hand corner and the yellow box illustrating the magnified region shown in the main image.
Figure 6: illustrates a 2 week old occlusion that appears to show extramural vessels (blue) feeding intraluminal vasculature found at the center of the occlusion. White and black arrows indicate the proximal and distal ends of the occlusion, respectively. Of importance to note is the appearance of a central region of intravascular vessels (red) that appears to be fed from the extramural vessels (blue).
3.3 Addendum on this Work—Using μCT to Monitor Intravascular Therapy

I have also been able to use some of the methods described in this work in collaboration with Dr. Bradley Strauss as a method for monitoring response to therapy in a rabbit CTO. Dr. Strauss is working on the development of vascular endothelial growth factor coated microspheres as a means of stimulating angiogenesis within the vessel wall.

Below shows an image of a VEGF treated 12 week old occlusion.

Figure 3.2: Micro-CT volumetric images of the proximal entrance of a 12 week old occlusion treated with VEGF coated microspheres. Efforts to determine whether there is a significant increase in the vascularity as compared to untreated occlusions are ongoing. Image A shows the intravascular vessels at the proximal end of the CTO in yellow. Image B uses a low volumetric threshold to identify small extramural vessels (volume rendered in red here). C, and D show cross sectional images with, C, and without, D, volume rendered vessels. Arrows show vasculature believed to be induced by angiogenic stimulation.
3.4 Addendum to this Work – Preliminary Investigation into Tissue Hypoxia in Chronic Total Occlusions

The μCT work in this chapter suggests that there is a dramatic growth of microvasculature both within the vessel wall and the occluded lumen. One potent factor that may drive such neovascular growth is low oxygen concentration in the artery and the surrounding tissue. Hypoxia has been associated with angiogenesis in vascular disease [118, 127]. In order to examine hypoxia in the rabbit model of CTOs, we performed pimonidazole (PIMO) staining. PIMO is commonly used for staining for hypoxia. PIMO is given intravenously prior to biopsy or sacrifice. The compound binds to thiol-containing proteins in cells[128] where the oxygen tension is less than 10mmHg[129]. The tissue is then resected, stored in formalin for 24 hours and sectioned. An antibody to PIMO is then used to stain the tissue section.

As a preliminary investigation into tissue hypoxia in CTOs, we injected 4 rabbits with PIMO intravenously at 60 mg/kg one hour prior to sacrifice. Two of the rabbits had lesions that were 2 weeks old while the other rabbits had occlusions that were 12 weeks old. Slides were also stained with Movat to highlight structural details. In Figure 3.3, we present an arterial section that is proximal to the occlusion from one of the rabbits with a 2 week old lesion. The artery is patent and subsequently the PIMO stain shown in Figure 3.3b appears to be negative as seen by the absence of the brown stain in and around the artery.

![Figure 3.3 Illustrates a patent artery proximal to an occlusion shown stained with Movat pentachrome in a) and PIMO in b). The adventitia of the artery is labeled “Ad” and the media is labeled with an “M”. The PIMO stain, as one would expect in a patent artery with adequate blood supply is negative for hypoxia. Scale bars represent 500 μm.](image)
In figure 3.4, we show a section from the same artery, at a location just inside the occlusion. Figure 3.4a shows the Movat stained slide for structural information and Figure 3.4b shows the Movat stained image. Note the darker appearance of the cells within the occluded lumen in the region that is outlined with a white dotted line. Also note the absence of staining around the small channel (labeled MC) within the occluded lumen suggesting that the region around this channel has an oxygen tension greater than 10mmHg.

In figure 3.5, we show an arterial section taken from a slice that is located within the center of one of the 2 week old occlusions. Figure 3.5a shows the Movat stained section for structural detail, while 3.5b is the PIMO stained section. Again, a positive stain is seen within a region of the occluded lumen. This area is outlined with a white dotted line in Figure 3.5b. Additionally, positively stained areas are also seen within the medial layer of the artery; these regions are identified with red arrows on Figure 3.5b.
Figure 3.5 Illustrates a section taken from the centre of a 2 week old occlusion. Image a) is a Movat stained section and image b) is a section stained with PIMO for hypoxia. In both slides, the adventitia of the artery is labeled “Ad” and the media is labeled with an “M”. A region of cells within the occluded lumen that appear to stain positive for hypoxia is outlined with a white dotted line. Regions in the media that appear to be positive for hypoxia are identified with red arrows. Scale bars represent 500 μm.

In figure 3.6, we show an arterial section taken from a 12 week old artery. Figure 3.6a shows the Movat stain. Noticeable on this section is the shrinkage of the artery. The medial wall has atrophied to such an extent that is difficult to distinguish it from the adventitia. Only a small portion of the internal elastic lamina is visible in Figure 3.6a. The occluded lumen shows evidence of fat as seen by the honeycomb-like segments within the lumen labeled with an “F”. There is a single small microchannel within the occluded lumen that is labeled with an “MC” on both images. The PIMO stained slide shown in Figure 3.6b appears to be negatively stained. This lack of staining is consistent with the near total fibrotic appearance of the artery.

Figure 3.6 Illustrates sections taken from a 12 week old arterial occlusion. Image a) illustrates a Movat stained section in which the medial wall has severely hypertrophied to the extent that it cannot easily be identified. Image b) illustrates the corresponding PIMO stained slide which appears negative for hypoxia. Scale bars represent 500 μm.
This preliminary staining work suggests that earlier time points experience a greater hypoxic response while at later time points the tissue has become fibrotic and not as responsive.

3.5 Conclusions

This Micro-computed tomography study convincingly demonstrates two points relevant to the development of a forward-viewing OCT probe. Firstly, microchannels within the lumen of a CTO are continuous over long segments and hence a forward-viewing OCT probe could image several millimeters down these channels provided that they could be clarified. Secondly, these channels also exit the lesion frequently; thus, having a means to image their direction in vivo, rather than simply following them blindly, is vital to successful interventions.
Chapter 4 – Forward-Viewing Intravascular OCT Probe Development

4.1 Chapter Overview

A motivation for the development of a forward looking intravascular optical coherence tomography catheter is presented. The technical requirements of a forward-looking OCT probe suitable for operation in the cardiovascular system is reviewed in light of these requirements. Efforts at engineering some of these designs are discussed. Difficulties in doing so, led to the design of a new probe based on electrostatic actuation using a dissipative polymer catheter. The paper, published in Optics Letters, is presented here. Finally, a follow up discussion on difficulties and potential improvements associated with this probe design is included.

4.2 Motivation and Technical Requirements for an Intravascular Forward-Viewing Catheter

Given the aim of visualizing both intravascular microchannels (50 -200 μm in diameter) as well as the interface between the occlusion and the medial wall, a high resolution imaging technique is required. Additionally, in order to image within a moving organ such as the heart in a manner suitable for providing interventional guidance, high frame rate imaging is needed. Three-dimensional imaging ability would also be highly desirable as it avoids confusion that can arise from off axis slices. These requirements limit the use of non-invasive imaging techniques such as computed tomography and magnetic resonance imaging which, in best case scenarios, have resolutions on the order of 250 μm and 700 μm respectively[130].

Thus we are led towards an intravascular approach to provide real-time high resolution imaging of the occluded artery. As previously mentioned, a forward-looking, intravascular imaging catheter for procedural guidance remains a highly sought after but challenging endeavor. Principal difficulties are size constraints – the catheter must be on the order of 1 mm to fit within the coronary arteries, as well as possess rigid sections no
longer than ~4 mm in length. These requirements have hindered a large number of attempts at building practical imaging catheters. While a forward-looking phased array ultrasound catheter should allow for imaging through several millimeters of tissue, miniaturizing the electronics and transducers while maintaining image quality is complex [87, 88, 131]. Thus we have focused on developing forward-looking intravascular optical coherence tomography as a means for providing imaging guidance.

4.3 Review of Forward-Looking Optical Coherence Tomography Probes

Optical coherence tomography (OCT) has shown tremendous potential in diagnosing lesions in the cardiovascular system [99, 100, 132]. While no forward-looking intravascular devices have been reported, there have been many different embodiments of forward looking OCT probes. There have been three principle approaches to developing a forward-looking imaging probe.

The first is a simple cantilever approach inside a catheter, in which the fiber optic is actuated from side-to-side to form an image. The fiber may be scanned in front of a lens in order to achieve an amplified angular field of view, or a lens may be incorporated into the fiber itself. Boppart et al. were the first to report on a cantilever style OCT imaging probe[133]. In this work, the authors describe two embodiments of a long piezo-electric driven shaft used to oscillate a fiber both with the lens affixed to the fiber (Figure 4.1a) and with the fiber scanning in front of a system of lenses (Figure 4.1b). Liu et al. also describe the use of a cylindrical piezo-electric crystal to drive a fiber optic in resonance in front of a graded index of refraction (GRIN) lens [134] as shown in Figure 4.1c). This technique resonantly scans the fiber with a frequency on the order of kilohertz as is shown in Figure 4.1 d). As this scanning speed is significantly faster than is used in most OCT systems, the authors compensated for this by decreasing the axial scanning speed of the reference arm to scan on the order of several hertz. This scanning scheme is in contrast to conventional implementations of both time and Fourier-domain OCT which scan axially on the order of kilohertz and laterally on the order of tens of hertz. The authors termed this technique lateral priority scanning. This probe was 2.4 mm in outer diameter but was hindered by the long rigid length (32 mm) necessary for the cantilever and the piezo-
electric crystal. Wang et al. also described a cantilever technique in which an electroactive polymer was used to bend a fiber optic inside of a 5 x 5 x 10 mm square tube to form an image[135] (as shown in Figures 4.1 e and f). This technique has the advantage of using only low voltages to scan the fiber but is hindered by slow scanning speeds (several Hz).

Figure 4.1 Displays examples of forward-viewing probe designs published by other groups. Images a) and b) illustrate designs published by Boppart et al. that use a piezo-electric actuated cantilever. Image a) shows a variation in which the fiber and the lens are scanned together while image b) shows the fiber being scanned behind the lens. Images c) and d) illustrate a design by Liu et al. which employs a piezo-electric tube to drive a fiber in resonant frequency. Image c) shows the design while d) shows the fiber being oscillated. Images e) and f) illustrate the use of an electroactive polymer to scan a fiber with a GRIN lens affixed to its end. Image e) shows the design by Wang et al. and f) illustrates the bending of the electroactive polymer that was used in their work. Images a) and b) are from Optics Letters, 1997, Vol 22, No. 21, p. 1618-1621; c) and d) are from Optics Letters, 2004, Vol. 29, No. 15, pp. 1763-1765; and e) and f) are from Optics Letters, 2005, Vol. 30, No.1 p. 53-55. Reproduced with permission.

The second approach to forward viewing that several groups have proposed is using micro electro-mechanical systems (MEMS). Zara et al. used an integrated force actuator, consisting of hundreds of thousands of deformable micromachined capacitors to bend a mirror that scans the OCT beam in a forward direction[136] as shown in Figure 4.2 a and b. Electrostatic actuation of micromirrors has also been applied in a forward-looking probe[137]. Pan et al. were able to apply this probe to image dysplastic tissue in the bladder with a 4.3 mm diameter probe[138] as shown in Figure 4.2 c and d. While these designs are encouraging, the difficulty with this approach within the context of
intravascular devices has been the ability to package the MEMS element within a catheter less than 1.5 mm in diameter.

Figure 4.2 illustrates two different examples of MEMS based forward-viewing probes. Images a) and b) illustrate a design published by Zara et al. In this design, an integrated force actuator (labeled IFA) is used to bend a mirror which scans the OCT beam in a forward direction. Images c) and d) show the design by Xie et al. This forward-viewing design uses an electrostatic MEMS based mirror to scan the OCT beam across the sample. Images a) and b) are reproduced from Optics Letters, 2003, Vol. 28, No. 8, p 628-630; Images c) and d) are from Applied Optics, 2003, Vol. 42, No. 31, p.6422-6426. Reproduced with permission.

The third approach is the use of optical fiber bundles. The advantage of bundle-based approaches is the absence of motion at the distal end of the probe. As there are no moving parts, there is no energy expended inside the probe, thus making these probes safe for use in vivo. Conventional fiber bundles are made through either extrusion of plastic or glass or by fusing many small fibers together. Typical bundles used in microendoscopy consist of over 10,000 fused multimode optical fibers spaced in a “honey comb” arrangement as shown in Figure 4.3. The individual fibers have an 8 µm diameter core with a 12 µm cladding diameter. Imaging fiber bundles are most often described as spatially coherent. This property means that a point on one end of the fiber bundle will be transmitted to a point in the same location on the other end of the fiber.
The use of multimode fibers is, however, problematic for OCT, as they allow for the propagation of multiple optical beam paths within the fiber. Each of these beam paths has the potential to act as the “sample beam” within the OCT system. This property thus significantly decreases the signal to noise ratio of a fiber bundle OCT system. Additionally, in order to minimize cross talk between adjacent fibers, manufacturers typically use large differences in index of refraction between the core and cladding of the individual fibers. This large difference in index of refraction, results in the numerical aperture (NA) of the individual fibers being high (0.55). Numerical aperture is a measure of both the acceptance angle of an optical element as well as the divergence of light propagating out of it. As the NA of the fibers in these fiber bundles is approximately twice the NA of conventional single mode fibers, a greater divergence in the light exiting a fiber bundle is seen. This characteristic results in significantly higher losses when the light is coupled into a long working distance, low NA lens used for focusing the light. Furthermore, conventional glass fiber bundles are typically very rigid. For example, the minimum bending radius of a 1 mm diameter glass fiber bundle is 50 mm, thus making access to the coronary arteries difficult. One potential solution to this problem is to use polymer fiber bundles which are significantly more flexible with a minimum bending radius of 25 mm for a 1 mm diameter bundle. These fiber bundles however, introduce an additional difficulty in that polymers typically used in polymer optical fibers are opaque to wavelengths above 1 micron – such wavelengths are typically used in OCT. One possible approach to address this problem is, therefore, to use an OCT system with a centre
wavelength at 840 nm to allow for transmission through the polymer bundle. I was able to set up such a system using a novel interferometer design and a polymer fiber bundle (from Nanoptics of Gainesville, Fl). The unique aspects of this interferometer were the use of a variable length sample arm, as opposed to a variable length reference arm, and the use of a novel 800nm broadband circulator (Oplink of Fremont, CA). In this set-up (shown in Figure 4.4), light from the laser is split such that ninety percent is sent to the sample arm and ten percent is retained in a “reference arm” path. The reference arm in this interferometer is unique, in that it does not have any free space element within it. In the sample arm, we use a circulator to direct the light towards a collimator on a sliding rail. The rail and collimator are aligned with a scanning mirror. Moving the collimator on the sliding rail allows us to vary the length of the sample arm to match the length of the reference arm. The scanning mirror scans the collimated beam through a 10x objective lens which focuses the light to a spot onto the end of the fiber bundle. This scanning spot could visually be observed at the distal end of the fiber bundle as a blurred red line across the bundle’s end face. The distance between the objective and the proximal end of the bundle was adjusted until this line appeared sharpest. The bundle was composed of 7,400 individual polymer multimode fibers composed of polymethylmethacrylate (PMMA) with an overall diameter of 1 mm.
Figure 4.4: Schematic of the interferometer used for OCT imaging with an 840nm swept source laser and a polymer fiber bundle. The sample arm of the interferometer contains a collimator on a sliding rail. This element allows for the length of the sample arm to be variable. The galvanometer mirror scans the beam across a 10x objective lens which serves to focus the light into the polymer fiber bundle. The reference arm in this interferometer consisted only of a length of fiber and a polarization controller.

We were able to obtain images of an IR card using the interferometer design without the fiber bundle, by replacing the 10x objective shown in Figure 4.4 with a lens (focal length of 3 cm) and adjusting the sample arm length. An example image is shown in Figure 4.5a. We were also able to obtain images from the reflection of the distal surface of the bundle, as shown in Figure 4.5b; we were not able to image an object outside of the bundle. This deficit was most likely due to the low power (1.5 mW) of the laser used in this system.
While others were able to report ex vivo application of fiber bundle OCT to form two dimensional OCT images[139], clinical in vivo imaging with fiber bundle OCT has not been demonstrated due to significant problems in the flexibility and implementation of a fiber bundle probe. While the ultimate solution to this problem would be a high density single mode coherent fiber bundle with high transmission at near-IR wavelengths, such a bundle is not yet available.

A second approach to fiber bundle imaging has been to produce single mode coherent fiber arrays. In contrast to a bundle which consists of thousands of fibers arranged in a honeycomb pattern, a typical fiber array consists of either 8 or 16 fibers arranged in a single line. This method, advanced by Barton et al., uses single mode fibers, etched to 15 μm, placed adjacent to each other in an array [140, 141]. The array is placed into contact with a GRIN lens in order to focus the light. In this approach, the reference arm is also a corresponding array of optical fibers. A scanning galvonometer mirror in the reference arm allows for each channel to be accessed individually. While this technique has
demonstrated preliminary images 120 μm wide (composed of 8 fibers, spaced 15 μm apart), it is labor intensive to expand the field of view to take full advantage of the 1mm diameter of the GRIN lens. This challenge is due to both aligning all of these etched fibers properly and packaging them effectively in a catheter less than 2 mm in diameter. Traditional methods for aligning fibers consist of bulky substrates with multiple V-grooves etched into them. Conventional V-groove arrays are designed for either 12 or 16 fibers. Expanding these to suit 60 fibers would result in a prohibitively large substrate.

Careful thought must also be given to matching each individual fiber’s length in multichannel fiber array designs. Most approaches to multi-channel OCT have addressed this issue by treating each channel as an individual interferometer [140, 142]. An alternative approach would be to try to match the lengths of the fibers such that the variation in their lengths was within the ranging depth of the OCT system and to scan the OCT beam across the distal end of each fiber as we did with the polymer fiber bundle with the 840 nm OCT system. New OCT systems that implement frequency domain mode-locking techniques to achieve ranging depths on the order of 7 mm or more [143, 144] would be desirable for such systems as they would permit greater variation in the individual channel lengths.

Other efforts in the development of fiber bundle based micro-endoscopy have been the use of incoherent fiber bundles[145]. In an incoherent fiber bundle, the arrangement of the fibers at the proximal end of the bundle does not necessarily have any relation to the arrangement at the distal end. The light beam is scanned through an objective lens and illuminates each fiber at the proximal end of the bundle. While this approach has not been applied to OCT, the random arrangement of the fibers may reduce cross-talk between the fibers. Difficulties in manufacturing such a bundle include etching ~ 1200 fibers to 40 micron diameters, and packaging these fragile fibers within a 1 mm diameter tube.
4.4 Electrostatic Forward-Looking Probe

In order to address the requirements mentioned above - flexibility, fast scanning, small diameter, short rigid length and ease of packaging - an electrostatic approach was undertaken. It was observed that by indirectly coupling a conductive cantilever to ground through a dissipative polymer, an oscillatory motion could be induced. By using a metallic coil, a fiber optic could be placed within the cantilever to enable scanning of an optical beam. As this technique only requires two electrodes and a cantilever, it is highly amenable to miniaturization. The following paper, published in Optics Letters on April 1, 2008, details the theoretical and experimental characterization of the probe:


As the lead author on this paper, I built the prototypes for this probe, developed a theoretical model to test the motion of the cantilever, tested the motion using a high speed camera, and wrote the paper.
Electrostatic forward-viewing scanning probe for Doppler optical coherence tomography using a dissipative polymer catheter

Nigel R. Munce,1,2 Adrian Mariampillai,1 Beau A. Standidge,1 Mihaela Pop,1,2 Kevan J. Anderson,1,2 George Y. Liu,1 Tim Luk,1 Brian K. Courtney,2 Graham A. Wright,1,2 I. Alex Vitkin,1,3,4 and Victor X. D. Yang2,5,6

1Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada
2Imaging Research, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Toronto, Ontario M4N 3M5, Canada
3Ontario Cancer Institute/University Health Network, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada
4Department of Radiation Oncology, University of Toronto, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada
5Department of Electrical and Computer Engineering, Ryerson University, 350 Victoria Street, Toronto, Ontario M5B 2K3, Canada
6Corresponding author: yvong@ece.ryerson.ca

Received December 14, 2007; accepted January 31, 2008; posted February 13, 2008 [Doc. ID 90794]; published March 21, 2008

A novel flexible scanning optical probe is constructed with a finely etched optical fiber strung through a platinum coil in the lumen of a dissipative polymer. The packaged probe is 2.2 mm in diameter with a rigid length of 6 mm when using a ball lens or 12 mm when scanning the fiber proximal to a gradient-index (GRIN) lens. Driven by constant high voltage (1–3 kV) at low current (<5 μA), the probe oscillates to provide wide forward-viewing angle (13° and 33° with ball and GRIN lens designs, respectively) and high-frame-rate (10–140 fps) operation. Motion of the probe tip is observed with a high-speed camera and compared with theory. Optical coherence tomography (OCT) imaging with the probe is demonstrated with a wavelength-swept source laser. Images of an IR card as well as in vivo Doppler OCT images of a tadpole heart are presented. This optomechanical design offers a simple, inexpensive method to obtain a high-frame-rate forward-viewing scanning probe.

Catheter-based optical coherence tomography (OCT) has shown promise in diagnosing lesions in both the gastrointestinal [1] and cardiovascular systems [2]. With the advent of frequency domain OCT systems with frame rates exceeding 100 frames/s [3], new optical probes for in vivo applications are required to fully capitalize on the benefit of these imaging speed capabilities. Side-viewing rotary probes are based on fiber-optic rotary joint devices and have been the workhorse of endoscopic and intravascular OCT systems owing to their high frame rate, minimal sensitivity to errors along the probe's length, and relatively simple implementation. While this imaging geometry is ideal for pulsatible-vessel surveys, forward-looking devices may be more amenable to providing interventional guidance. Several groups have proposed different forward-looking strategies for OCT probes based on electroactive polymers [4], fiber bundles [5], piezoelectric scanning [6] and piezoelectric-induced resonance [7], microelectromechanical systems [8,9], and counter-rotating graded-index (GRIN) lenses [10]. The dimensions of the rigid proximal end combined with packaging issues and overall probe complexity are the main difficulties in achieving a low-cost, single-use probe design for use in catheter-based forward-viewing systems in vivo. Electrostatic actuation of optical fibers has previously been implemented for optical switches [11]. The inherent rigidity, however, of standard 9/125 single-mode fiber typically necessitates long (~3 cm) cantilever lengths and/or high field strengths in order to obtain relatively small (~200 μm) lateral displacements at the tip.

In this Letter, we implement an electrostatically driven cantilever within a catheter to create a compact, wide-angle, rapid-scanning forward-viewing probe. The novel approach is the use of an electrostatic dissipative polymer to allow the cantilever to oscillate under constant voltage. A schematic of the probe is shown in Fig. 1(a). To increase flexibility, the cladding of a single-mode fiber is etched to 60 μm diameter using a wet-etching technique previously described [12]. The fiber is placed within a 250 μm diameter platinum alloy coil, which is then placed within the 400 μm diameter central lumen of a triple lumen catheter shown in Fig. 1(b). The custom-made catheter is extruded using a dissipative polymer (polyether block amide, Pebax). Dissipative polymers are conductive on the surface only; thus the charge deposited within the material bulk is held for several hundred milliseconds and then migrates to the conductive surface. The two peripheral lumens (diameter 270 μm) contain insulated wires with insulation coatings removed at the cantilever end to provide electrical contact. One of these wires serves as an electrode while the other acts as ground, which are connected to the high voltage (HV) and ground leads of a dc high-voltage power supply, respectively. The current limit of the power supply is set at 20 μA; however, typical currents needed are less than 5 μA.
Fine solder wire is wrapped around the catheter surface and is connected to ground. Initially, the cantilever is neutral and attracted to the HV electrode; however, as it is not directly connected to ground there is no significant electrostatic discharge. When the platinum coil touches the HV electrode, it acquires the same potential. The acquired charge takes a finite time to dissipate through the catheter; during this time the cantilever repels away from the electrode toward the ground wire. When the cantilever touches the ground wire, it becomes neutral again, creating a discharge measurable as a pulse in a parallel circuit located outside of the catheter and serves as a trigger signal for imaging acquisition. As the cantilever returns to ground it is once again attracted to the HV wire, thus creating an oscillating motion.

In one implementation, a 1 mm focal length ball lens is fusion spliced to the distal end of the etched fiber. Alternatively, a 1.8 mm diameter GRIN lens (focal length, 2 mm; pitch, 0.3) is placed in front of the etched fiber to focus light and amplify the angular displacement of the beam. The small tube can be filled with mineral oil for index matching. The advantage of the GRIN lens is the amplification of the angular displacement of the fiber through the lens; however, this increases the required rigid length from 6 to 12 mm to accommodate the length of the GRIN lens, and it introduces coma aberrations into the image. A photograph of the probe in motion in air outside of the tube is shown in (1c).

The probe behaves similar to a damped driven oscillator in that there is a certain voltage required to initiate motion. Once the probe begins to oscillate, the voltage may be decreased producing a corresponding decrease in frequency response of the cantilever. The frequency of oscillation as a function of the driving voltage was measured for motion in both air and oil using the frame trigger signal, as shown in Fig. 2(a). For oil immersion, cavitation bubbles were observed at driving high voltages >3.5 kV, 9 μA. The cantilever motion in air exhibited a lower threshold voltage to initiate motion, and a steeper frequency response as compared with the motion in oil. However, slower speeds were possible in oil, which may be advantageous for some imaging applications. In contrast to the frequency response, the amplitude of oscillation is the same in air as in oil, since the cantilever always oscillates between the two electrodes.

We separate the oscillatory motion into an attractive and a repulsive phase and assume that the charge on the cantilever remains constant during one cycle. The attractive phase starts when the cantilever is neutral. We define r = 0 as the middle of the tube (radius = R). The arrangement is modeled as a spring of constant k_s with a velocity-dependent resistance (proportionality constant α), attracted to a mirror charge q_m a distance 2(r + R) away from the cantilever with Coulomb's electrostatic constant k_e as represented by the third term in the following:

$$\frac{\partial^2 r}{\partial t^2} = -k_s r - \alpha \frac{\partial r}{\partial t} + \frac{k_e q_m^2}{4(r + R)^2}. \tag{1}$$

The repelling phase is when the cantilever has briefly touched the electrode, obtaining some amount of charge (q_e), repelling it from the electrode, as represented by the third term in the following equation; it is also drawn toward the grounding wire owing to the mirror charge effect as represented by the fourth term:

$$\frac{\partial^2 r}{\partial t^2} = -k_s r - \alpha \frac{\partial r}{\partial t} + \frac{k_e q_e}{(r + R)} + \frac{k_e q_m^2}{4(r - R)^2}. \tag{2}$$

In Eq. (2), k_e q_m^2 represents the charge on the cantilever.
Fig. 3. (Color online) (a) Sector image of an IR card taken using the electrostatic ball-lens probe, resulting in 10° scan angle. (b) Sector image of an IR card taken with an electrostatic probe in which the fiber was scanned behind a GRIN lens, demonstrating 33° scan angle. (c) Structural image of the heart of Xenopus laevis taken with the GRIN lens design identifies left (L) and right (R) aortic arches and (d) corresponding Doppler image showing the aortic branches. A small gill vessel is identified with a V. Horizontal scale bars, 1 mm. Scale corresponds to phase shift owing to Doppler effect.

ver and the mirror charge above the ground wire, while \( q_{\text{elec}} \) represents the electrode charge during repulsion. These two equations can be solved for \( r(t) \) to generate a family of triangular waveforms for comparison with experimental measurements. A high-speed CCD camera (MICAM02, BrainVision Inc.) was used to capture images with a temporal resolution of 2.2 ms to analyze in detail the motion of the cantilever. Driving voltage was slowly increased to 2100 V from such that the probe began to oscillate continuously in mineral oil. The center of the cantilever tip was tracked by software. The results of this motion tracking are shown for three cycles in Fig. 2(b), illustrating a triangular-like waveform at 11 Hz (or 22 fps when both slopes of the triangle are used for imaging). The small difference between theoretical and experimental results is likely caused by the cantilever not being exactly equidistant from the electrode and ground wires at its equilibrium position.

OCT imaging was performed with the prototype probes in the sample arm of a Mach–Zehnder interferometer using a wavelength swept laser centered at 1300 nm with a tuning range of 110 nm and sweeping rate of 43 kHz. In Fig. 3(a) an OCT image of an IR card is shown, acquired with the ball-lens design oscillating at 30 Hz (60 fps) in air with a viewing angle of 13°, while in Fig. 3(b) an OCT image is taken using the GRIN lens probe oscillating at 5 Hz (10 fps) in oil with 33° viewing angle. For Doppler OCT in vivo demonstration, we used the GRIN lens electrostatic probe to image the heart of an anesthetized Stage 45 Xenopus laevis embryo as shown in Figs. 3(c) and 3(d). These images demonstrate the ability of the probe to generate Doppler images comparable with those obtained with bulk scanning optics.

In conclusion, we have demonstrated a method for rapidly actuating an optical fiber inside a compact catheter in order to obtain forward-viewing OCT images. While high voltage was used to actuate the cantilever, typical currents were \(~ 2 \mu A\). Furthermore, the presence of a ground wire within the catheter and the Teflon tube minimize the chance that a discharge would be delivered to the tissue under examination. The small outer diameter (2.2 mm) and the short rigid length (6 mm) of the probe using the ball-lens design can be further miniaturized to allow for navigating the proximal coronary arteries. While the GRIN lens design has a longer rigid tip section (12 mm) and may not be suitable for the more tortuous sites of the coronary vasculature, the increased angular field of view of this design will likely be useful in other more accessible lumens and orifices of the body. The potential combination of this probe with laser ablation strategies [13] may allow for the application of OCT as an interventional tool in addition to its current diagnostic role, potentially facilitating procedures and expanding the boundaries of minimally invasive interventions.

Support from the Natural Sciences and Engineering Research Council of Canada, Canadian Foundation for Innovation, Ontario Centers of Excellence through the Photonics Research Ontario program, and the Canadian Institutes of Health Research is gratefully acknowledged.

References
4.5 Manuscript Addendum

4.5.1 Misprint in the paper

The third term in the right hand side of Eqn. 2 in the paper is missing a square term in the denominator. It should read:

\[
\frac{\delta r^2}{\delta^2 t} = -k_r r - \alpha \frac{\delta r}{\delta t} + k_s q_b q_{elec} + \frac{k_s q_b^2}{(2(r - R))^2}.
\]

4.5.2 Automated Image Segmentation with Trigger Pulses

OCT images taken using the probe for the *Optics Letters* publication were segmented manually by looking for an inflection point in the curvature of a front surface of a series of repeating images as shown in Figure 4.6 below.

Figure 4.6: Individual scans from the electrostatic probe were segmented from the original OCT image by identifying inflection points in the image. Dotted red lines indicate where the images were segmented. These segmented images were subsequently converted to a sector display in software.
As mentioned in the article, an electrical pulse is observed in the ground channel each time that the cantilever touches it. This pulse was observed to be periodic with a millisecond rise and fall time. An example of such pulses is shown in Figure 4.7, which displays a series of pulses that were produced when the cantilever was driven with 3000 Volts.

We, therefore, sought to use this pulse to automate the segmentation of the images acquired with the electrostatic OCT probe. A system was set up that acquired both the OCT and the trigger signal that occurred when the cantilever made contact with the ground electrode using 2-channel software written by Thorlabs (Newton, New Jersey). In the typical swept-source OCT signal acquisition, both the OCT signal (the combined sample and reference signal) and the Mach Zender Interferometer (MZI) clock are simultaneously acquired for each axial scan. The MZI clock is used to recalibrate the signal so that it is evenly spaced in wavenumber. In the two channel set-up, a single reference MZI clock is acquired prior to use for recalibration, as opposed to real-time calibration. This stored clock signal allows the input channel on the data acquisition card to be used to simultaneously acquire both the OCT signal and another input. A comparison of the typical data acquisition setup and the modified 2-channel setup used for trigger signal acquisition is shown in Figure 4.8 below.
We connected the ground electrode of the probe to a Tektronix 100X high voltage probe to lower the voltage input to the data acquisition card. A program was then written by a summer student, Louis Tan, which identified the peaks and segmented the images based on the location of these peaks relative to the OCT signal obtained. This program was able
to segment the images based on this trigger signal; however, several important issues were identified in the probe’s operation.

First, the probe occasionally missed the trigger signal, leading to multiple images being grouped together. One possible explanation for these missing pulses signals is due to charring that occurred on the surface of the cantilever such that an the electrical pulse is not received when the cantilever touches the ground electrode. Second, in addition to a signal obtained when the probe touched the ground wire, a signal was also occasionally obtained when the cantilever touched the electrode. This variability in the trigger signals can be illustrated in a plot of the number of segmented frames as a function of the trigger signals obtained, as shown in Figure 4.9. If the frequency of the cantilevers oscillation was constant and the probe produced a pulse each time it touched the ground electrode, a straight line would be expected. Missing trigger pulses would however would result in a step pattern as the number of A-scans remains constant. This pattern is seen in Figure 4.9 below in the area outlined by the red box as a rounded step pattern suggesting that there are two phases on in which every trigger is captured and one phase in which every other signal is captured.

Figure 4.9: Shows a graph of the number of A-Scans as a function of the number of trigger signals received by the data acquisition card. Since the A-scan rate is constant, this graph should be a straight line if the frequency of the trigger signals is constant. While a) shows an acquisition over several seconds b) shows a zoom in of the trend inside of the red square shown in a).
4.6 Conclusions

Principal difficulties associated with the probe described here were the inability to effectively trigger the scanning. While this limitation is not prohibitive to clinical operation, it does make interpreting images in the context of real-time guidance more challenging.

In addition to difficulties associated with image segmentation, several other limitations of the developed probe warrant further discussion. First, as with any cantilever based design, the field of view is coupled to the diameter of the probe. In this electrostatic probe, the cantilever oscillates between the two electrodes. While the 200 $\mu$m diameter wires used as electrodes for this probe may seem small by macroscopic standards, in designing a probe on the order of 1 mm diameter, these wires occupy a significant amount of space. Thus, a small diameter probe with this actuation method is somewhat limited by the size of the wires used. While reducing the wire diameter is possible, it also introduces difficulties in making sure that the wires are not crooked. This difficulty arises because as the diameter of the wires is reduced they become less rigid and more susceptible to becoming kinked.

An additional concern regarding this design is the use of oil immersion as a means to both dampen the oscillation rate and to provide greater coupling between the fiber and the GRIN lens. The concern is that, should the probe rupture and the oil leak into the blood stream, it may create oil emboli which can lead to serious consequences[146].

Although these issues would complicate clinical implementation in human patients, we were able to apply the probe in phantoms mimicking severely narrowed arteries to obtain preliminary forward looking images as presented in the following chapter.
Chapter 5 – Doppler Optical Coherence Tomography Imaging in Chronic Total Occlusions

5.1 Chapter Overview

The motivation for forward-looking Doppler imaging is reviewed in the context of providing guidance for CTOs. Preliminary forward-looking Doppler OCT images are presented in a flow phantom model. We also describe work in a surgical cut-down rabbit femoral artery occlusion model to allow for in vivo demonstration of Doppler OCT imaging of microchannels.

5.2 Doppler Optical Coherence Tomography

Doppler Optical Coherence Tomography (DOCT) is the flow sensitive, functional extension to OCT. Unlike a true Doppler technique that examines the frequency shift of the reflected light, DOCT typically compares the phase of the signal of two pixels that are adjacent in time to estimate a velocity. In time domain OCT, this phase shift is typically measured in the carrier wave of the interference signal using quadrature demodulation methods. In swept source systems, the interference signal is resorted so that it is evenly spaced in wavenumber, and then undergoes a complex inverse Fourier transform (concept shown in Figure 5 in Chapter 1). The phase of each pixel (from negative to positive $\pi$) is obtained from the real and imaginary components of the interference signal after this transform, and displayed in a color map image.

5.3 Motivation for Forward Viewing Intravascular Doppler OCT.

The results from the $\mu$CT studies presented in Chapter 3 indicate that recanalization channels are continuous over long distances in a CTO; further, when channels do exit into the arterial wall, they do so at sharp angles. Previous work by Thind et al. supports these observations and also demonstrates that microchannels in CTOs do have flowing blood that can be identified by power Doppler ultrasound[21]. Thind et al. measured the peak velocity of blood flow in these microchannels at approximately 2 cm sec$^{-1}$. Thus, we
hypothesize that DOCT can be used to detect flow in microchannels in CTOs. The equation for the Doppler frequency shift is:

\[ <v> 2n_t \cos \theta = \lambda_o f_d \], \quad \text{Equation 5.1}

where \(<v>\) is the mean velocity of the pixel being imaged, \(n_t\) is the index of refraction of the object being imaged, \(\theta\) is the Doppler angle (the angle between the imaging beam and the direction of the moving object), \(\lambda_o\) is the centre wavelength of the energy being used to image and \(f_d\) is the measured Doppler frequency shift. This “Doppler” shift is calculated from the difference in phase between pixels adjacent in time, as described by:

\[ f_d = \frac{\Delta \phi}{2\pi T} \], \quad \text{Equation 5.2}

where \(\Delta \phi\) is the difference in phase, and \(T\) is the time measurement interval between the two pixels. For intravascular forward-looking DOCT, the direction of flow would be close to parallel or anti-parallel to the imaging direction. In this situation, the “\(\cos \theta\)” term in Eqn. 5.1 is close to its maximum absolute value of 1 and therefore sensitivity to flow should be optimal. Should one of the channels exit into the wall (and thus change direction), a dramatic change in the Doppler image should be seen.

5.4 Forward-Viewing DOCT: Experimental Set-up

To test the ability of the forward viewing probe to generate Doppler images similar to what may be seen in vivo, a suitable phantom needed to be developed. In order to create a phantom mimicking a blunt occlusion with a microchannel, a 400-\(\mu\)m inner diameter (ID), 600-\(\mu\)m outer diameter (OD), Teflon tube was placed inside a larger 2.8 mm ID, 3.8 mm OD polycarbonate tube such that the smaller tube extended significantly out from both ends of the larger tube. The 3.8-mm tube was then filled with a mixture of TiO\(_2\) powder and polydimethylsiloxane (PDMS) at a ratio of 0.2 mg/ml and allowed to set. After the PDMS had cured, one end of the tube assembly was cut with a scalpel to create a clean face as shown in the Figure 5.1a. This assembly was then inserted into a stretchable tygon tube such that the tygon tube fit snugly over the tube assembly to create a layered structure that allows for fluid to be injected at one end, as pictured in Figure 5.1c.
Figure 5.1: Image a) illustrates a flow phantom in which a 400 μm ID tube is embedded in a PDMS filled tube. In image b) the entire assembly is shown inside a stretchable tygon tube that serves to simulate the vessel wall. Image c) shows a drawing of the assembled flow phantom.

Other types of structures that were also tested included an oval shaped channel (Fig 5.2a), a tapered entrance created using a pipet tip (Fig 5.2b) and a channel which entered from the side of the polycarbonate tube (Fig 5.2c).
The electrostatic forward-viewing probe described in Chapter 4 was used for imaging in these experiments. The design utilizing GRIN lens as opposed to a ball lens was selected for this work due to the increased angular field of view. In order to be able to effectively visualize the entire cross section of the occlusion, the 5mm working distance GRIN lens was substituted with a 20 mm working distance GRIN lens. The probe was connected to a custom made swept source OCT system with a center wavelength of 1320 nm that employed a Fourier domain mode locking technique to achieve an axial scanning frequency of approximately 43 kHz and a ranging depth of 6 mm[144].

As a first step in the experimental set-up, the entire flow phantom and the distal tube were filled with saline to create an optically clear field of view. A mixture of intralipid and saline was then added to a 10 ml syringe and this syringe was attached to the distal end of the small Teflon tube. The syringe was placed in an infusion pump to simulate flow coming through the microchannel. The probe was then placed inside the phantom and the high voltage power supply that was connected to the probe was activated so that the probe began to oscillate. The distance from the probe to the cleaved face of the flow
phantom was adjusted such that the entire proximal end of the “occlusion” could be visualized. The set-up for this work is detailed in Figure 5.3.

Once a clear view of the proximal end of the flow phantom was obtained, the infusion pump was activated at rate of 1 ml/min. The data acquisition was initiated once the Intralipid was seen in the microchannel tube, several centimeters away from the imaging area. The purpose of this timing sequence was to capture the arriving bolus of Intralipid. Images were segmented manually and converted to a sector display in a similar method as shown in Figure 4.6.

5.5 Forward-Viewing DOCT: Imaging Results

A time sequence of images displaying the arrival of a bolus of Intralipid in the phantom is shown in Figure 5.4 using the side entering design (Figure 5.2c), as this assembly allowed for the channel to be more centered within the “occlusion”. This design used a 600-μm ID, 900 μm OD tube, wedged in through the side of a Teflon tube to mimic a microchannel that exits from the lumen through the wall.
Images in Figure 5.4 illustrate an ability to perform Doppler imaging when the flow is anti-parallel to the imaging direction. The flow profile, as seen on the DOCT images Fig 5.4d-f, appears parabolic through the channel. The Doppler images display aliased flow as seen by the yellow and blue colors (representing negative and positive $\pi$ phase shifts respectively) being close to each other. In this situation, the phase difference between the adjacent lines is greater than $2\pi$. However, the measurement range for phase is limited from $-\pi$ to $\pi$. Thus, phase differences that are greater than this range will cycle between $-\pi$ and $\pi$.

We were also able to examine several other geometries of the CTO phantoms, including imaging flow through a tapered entrance as shown in Figure 5.5 and the oval shaped microchannel as shown in Figure 5.6.
Figure 5.5: A schematic drawing of imaging flow directed towards the forward viewing electrostatic probe is shown in a). The red arrow indicates the direction of flow. Structural b) and Doppler c) images of Intralipid flow arriving in a tapered phantom (as shown in figure 5.2c) are displayed. Scale bars represent 1 mm.

Figure 5.6: Imaging flow through an oval channel using a forward-viewing Doppler OCT probe. Image a) once again shows the orientation of the probe with respect to the flow phantom. Image b) shows the face-on view of the flow phantom and the orientation of the imaging slice through it. Image c) illustrates the structural image taken of Intralipid arriving through the channel, while d) shows the corresponding Doppler image.
The Doppler images presented in Figs. 5.5 and 5.6 again display aliasing artifacts associated with fast fluid flow. In both cases, the Doppler images serve to provide additional contrast over the structural images. This contrast will be important in situations where the surrounding occlusive material and the fluid have similar scattering properties, such as with collagen and blood.

5.6 Experiment Limitations and Conclusions

Principal challenges associated with this work were that the probe’s limited field of view resulted in the need for an increased working distance of the probe in order to view the entire width of the flow phantom. Due to the limited imaging depth of the OCT beam in Intralipid, this long working distance only allowed for imaging the arrival of the Intralipid, rather than steady state flow at the proximal end of the channel. Furthermore, this large working distance resulted in an artifact of the unresolved complex conjugate referred to as “image wrapping”. This effect is illustrated in Figure 5.7 which shows a bolus of Intralipid arriving and propagating out of a microchannel opening. As the leading edge of the fluid approaches and then exits the field of view defined by the coherence gate of the interferometer, it appears as a mirrored image superimposed on the rest of the image.

![Figure 5.7: Illustrates a sequence of structural images (a-c) taken 60 milliseconds apart and their corresponding Doppler images (d-f) that show the “wrap around” artifact due to the complex ambiguity in Fourier domain OCT. The red dotted line in the structural images (a-c) represents the leading wave front of the bolus of Intralipid. In image b) this wave front is seen to “wrap around” or head in the opposite direction. This effect is due to the inability to resolve the complex conjugate ambiguity in Fourier domain OCT. Scale bars represent 1mm.](image-url)
Despite the limitation of image wrapping, these preliminary forward-viewing Doppler imaging results, suggest that we can use Doppler OCT imaging in a forward-viewing probe to obtain additional contrast as compared to structural OCT alone. Flow speeds used in this work correspond to average velocities on the order of 5 cm/sec. This velocity is of the same order of magnitude as the peak pulsatile flow of 2 cm/sec observed in microchannels within a pig occluded artery model that Thind et al. reported on[21]. In order to minimize the image wrapping, probes that could be placed closer to the proximal end of the phantom while maintaining a large field of view are needed. A larger imaging angle would allow the imaging catheter to be placed closer to the lesion of interest while still imaging both sides of the arterial wall.

5.7 Design of Experiments to Test \textit{in vivo} DOCT Imaging of Occluded Arteries

In order to test OCT’s ability to detect \textit{in vivo} flow within a CTO, we modified the experimental protocol described in Chapter 3 for the creation of a CTO in the rabbit femoral artery, to allow for surgical exposure of the lesion at several weeks following its creation. Briefly, the original model involves surgically exposing and isolating a rabbit femoral artery, ligating the artery both distally and proximally, injecting approximately 0.1 ml thrombin solution (200 international units/ml) at the proximal end of the artery and then removing the proximal ligature to allow blood to flow into the lesion and create a thrombus. For animals that were to be imaged at 6 or more weeks, we also wrapped the artery with a sterile surgical biocompatible plastic sheet that could be left in the closed surgical site for up to 12 weeks. This plastic wrap facilitated subsequent isolation of the artery from the surrounding connective tissue at time points of six or more weeks. Shown in Figure 5.8a is a picture of an isolated artery just after thrombin injection. The distal ligature was maintained for one hour to prevent the thrombus from lodging into more distal arteries while the proximal ligature is loosened to allow for the inflow of blood. Once the thrombus had formed within the isolated artery, it was imaged using a Thorlabs OCT system (centre wavelength ($\lambda_o$) 1325 nm, bandwidth ($\Delta\lambda$) 110nm and axial scan speed of 8 kHz) equipped with a handheld scanner attached to a surgical arm. The scanner
consisted of a pair of scanning mirrors and a lens which serve to scan and focus the “sample arm” beam of the OCT system. This scanner was positioned above the artery as shown in Figure 5.8b such that it scanned axially across the artery.

Figure 5.8: Illustrates the creation of a rabbit thrombus occlusion in a) which shows an isolated rabbit femoral artery with a closed ligature at the distal end. A biocompatible plastic (not shown) was used to protect the artery after thrombus formation for animals that were to be imaged at 6 weeks or more. Image b) illustrates the scanning set-up used for in vivo imaging of the exposed artery.

5.8 Doppler OCT in vivo Imaging Results

Initial imaging was performed prior to thrombus injection on a surgically isolated patent artery. Images were acquired over a time course of several seconds. Figure 5.9 shows a structural OCT image and a Doppler image sequence of an isolated femoral artery after being topically treated with lidocaine. The lidocaine served primarily as an analgesic; however, it also acts to dilate the artery.
The images in figure 5.9 illustrate rapid flow through the patent artery. Images in Figure 5.9 b and c) display aliased flow near the wall of the artery. Deeper imaging of the flow near the centre of the artery is not possible due to the high attenuation of the blood. In the images shown in Fig. 5.9d we see that the flow appears as a speckle-like mosaic of colors as opposed to arcs as in images 5.9 b, c and f. This appearance is due to an increase in the flow rate in the artery caused by the pulsatile nature of the flow in the artery. At these times of high flow rate, there is insufficient time between adjacent axial scans to see a continuous variation in the phase differences. One potential solution to address this imaging artifact is to use an OCT system with a faster axial scan rate such that the time between adjacent axial scans is less.
We also imaged the artery after approximately 30 minutes after a thrombus had formed within the lumen. Interestingly, we were able to observe Doppler signals from parts of the thrombus. A Doppler OCT image sequence from a proximal segment of an artery nearly filled with thrombus is shown in Figure 5.10.

![Image](image.png)

**Figure 5.10:** Illustrates an isolated rabbit femoral artery that appears to be almost entirely filled with thrombus. The structural image a) shows very fresh thrombus as a signal rich region in the arterial lumen. The interface between this thrombus and the arterial wall is identified using a red dotted border. The Doppler image sequence in images b-f) illustrates a pulsatile portion of the thrombus (identified with an arrow in image b). Scale bars represent 1 mm.

Pulsatile flow within the proximal portion of the thrombus was seen 30 minutes after the thrombus had formed, suggesting that the thrombus develops softer regions very early on. It is difficult to tell in the images in Figure 5.10 whether or not the Doppler signal is a result of flowing blood in the thrombus or the result of deformation of the soft clot being pushed by upstream blood.
At two weeks, we were able to see Doppler signals within the proximal portions of the occlusion as shown in Figure 5.11.

![Figure 5.11](image)

Figure 5.11: Illustrates a surgically exposed 2 week old occlusion with both structural a) and Doppler b-f) images. The Doppler image identifies a central microchannel. A red dotted curve is used to identify the outer border of the artery on the structural image in a). A light blue arrow is used to note the location of the channel within the artery. A representative Movat stained histology slide is shown in g). The Adventitia is labeled “Ad” and the media is labeled with an “M”. A light blue arrow again identifies the central microchannel in both the structural OCT a) and histology images g). Scale bars in a-f are 1mm. The scale bar in image g) is 0.5mm.

The structural OCT image in Figure 5.11 shows that the artery has shrunk in diameter as compared to images taken of the artery just after thrombus formation. The Doppler OCT images show that the central microchannel does possess pulsatile flow. The Doppler signal is, however, significantly less than that seen in the patent artery in Figure 5.9, as evidenced by the absence of aliased blood flow in the microchannel in the 2 week old occlusion. Using Eqns. 5.1 and 5.2, an estimated Doppler angle of 80 degrees and the known properties of the swept source laser that was used for imaging, we can estimate the peak velocity seen in this channel as approximately 8 mm per second. This is similar to the 1 cm per second peak blood flow velocity that Thind et al. observed with ultrasound in microchannels in a pig model of a CTO[21]. The Movat stained histology slide also shows a central microchannel within the occluded lumen.
At 6 weeks, however, the density of the collagen prohibited imaging to less than 200 μm and no microchannels could be located. Interestingly, process of wrapping the artery with sterile surgical plastic at the time of inducing the lesion and allowing the occlusion to age while still surrounded by the surgically-implanted plastic wrap appeared to prevent negative remodeling (shrinking) of the artery at the 6 week time point. This step was necessary, however, for isolating the artery at these later time points from the surrounding connective tissue. In cases where the wrapping process was not performed, the artery could not be visually identified from the surrounding collagen and fat matrix.

Figure 5.12: 6 week occlusion showing limited structural OCT imaging depth in a) and an absence of Doppler signal in b). Scale bar represents 1mm.

One goal of this work was also to be able to identify similar vascular patterns as were observed in the μCT work presented in Chapter 3. While we were able to clearly identify intraluminal microchannels at the 2 week time point (Figure 5.10), definitive detection of extramural vessels within the arterial wall proved difficult. This challenge was a combination of the small size of these vessels (10-50 μm) and vibrations induced through the surgical arm and animal respiration and heartbeat. One promising image, however, potentially showing flow in small vessels is presented in Figure 5.13. This DOCT image was taken in the mid portion of the artery after a thrombus had formed within the lumen. The thrombus appears as bright but highly attenuating within the artery in the structural OCT image below. Evident in the Doppler image in Figure 5.13 b-d at approximately the
10 o’clock position are small signals that may represent flow within vessels located in the media of the artery.

Figure 5.13: Illustrates a thrombus filled artery approximately 1 hour after thrombus creation. The arterial wall is outlined with dashed red lines in the structural OCT image in a). A time sequence of Doppler OCT images are shown 80 ms apart in b-d). Small Doppler signals seen at the 10 o’clock position (identified with an arrow) may represent small vessels within the arterial wall. White scale bars represent 1 mm.

It should be noted, that within the context of intravascular imaging for CTO guidance, resolving the small vessels in the arterial wall would likely not be needed in order to successfully guide therapy. However, in the context of detecting areas of an artery that are susceptible to develop a plaque that is likely to rupture, imaging these vessels may be important. Other authors have noted an increase in vasa vasorum prior to onset of plaque
formation[147], and thus the development of methods to identify this vasculature is of importance.

5.9 Limitations of These Experiments

The principal limitation of this work was the shallow imaging depth associated with OCT in highly scattering media such as blood and dense collagen. This limitation allowed for imaging microvasculature within the occlusion only to depths of ~300 μm or less in tissue.

Three-dimensional imaging of the occlusion was also limited due to vibration isolation difficulties. Primary sources of vibration were both animal motion due to breathing and heart rate, as well as vibration induced in the long surgical arm holding the OCT scanner. These periodic vibrations were seen in the longitudinal reconstructed slices from the 3-D image set, as seen in Figure 5.14c identified with the red arrows. 3-D image sets were acquired using the two axis beam scanner shown in Figure 5.7b. 1024 axial scans were acquired 20 μms apart to create a 3-D image stack. These axial slices were imported into the Amira™ software program, which was used to visualize corresponding longitudinal slices.
Figure 5.14: A 3-D data set of a proximal segment of a 2 week old occlusion. An axial slice is shown in a) with a dotted border in red identifying the outer layers of the artery and the letters OL identifying the location of the occluded lumen. A reconstructed longitudinal slice is shown in part b). A dotted red line is again used to label the outer border of the artery. Image c) shows an enlarged version of the longitudinal slice shown in b). Note the periodic jagged vibrations seen along this border due to motion artifacts that are identified with arrows in image c).

5.10 Future in vivo Imaging of Arterial Occlusions in Animal Models

A possible solution to the issue of limited penetration depth in the occluded artery is to develop a suitable arterial occlusion model in smaller rodent model such as a mouse or a rat. This model could employ a window chamber or could simply be a surgical exposure model similar to that presented here. This should address the difficulties associated with a limited penetration depth. It would also allow for the imaging system to be placed over the lesion site without the use of a long surgical arm, thus limiting vibration. A mouse model would allow the use of animals such as Apolipoprotein E knockout [148] that cause
the mice to have a higher lipid level in the blood stream, resulting in the development of plaque. Other interesting mouse models may include the use of a diabetic [149, 150] or uraemic [151] mice to try to induce calcification and to identify differences in the lesion’s evolution as compared to a normal mouse. Occlusions may be induced through ligating a femoral artery microsurgically. Other possible avenues of investigation include the use of high frequency ultrasound in combination with the surgical cut-down and arterial wrapping technique presented here. This concept may still be limited by vibrations and the ability to scan across the length of the occlusion due to the anatomy of the vessel.

An additional possibility is the use of large animal arterial occlusion model to image potential microvessels at the proximal entrance of the occlusion. Thind et al. have demonstrated microvessels in a pig femoral artery model[21] based on a model developed by Bailey et al.[152] . This model is advantageous for experimental intravascular imaging catheters because the peripheral arteries are less tortuous than those found in the heart.
Chapter 6 – Concluding Remarks

6.1 Accomplishments of this Work

The research presented in this thesis has accomplished several goals. It demonstrates that, as an imaging technology, OCT can characterize in an *ex vivo* setting various pathologies that occur in occluded arteries. Prior to this work, it was unclear what an intravascular forward-looking OCT catheter would visualize when imaging an occlusion. It demonstrates, *ex vivo*, that OCT can identify channels that exit from the occluded lumen to the vessel wall, provided that these exiting points are within the imaging depth of the system.

This work also puts forth new ideas regarding the origin and evolution of microchannels within an occluded artery. It suggests a new model for microvessel formation in an arterial occlusion: one in which microchannels within the centre of the occlusion originate from vessels in the arterial wall, while channels forming at the proximal and distal ends appear later and may be the result of recanalization pressure. We show, in all time points except the earliest 2-week time point, that these intravascular channels are continuous over long lengths of the occlusion.

We also present three dimensional μCT images that display for the first time to our knowledge openings within the occluded artery’s wall in which an intravascular channel communicates with the vessel wall. These occur frequently across all time points greater than 2 weeks. Within the context of a forward-viewing imaging modality, this work demonstrates that microchannels within the occlusion do often provide continuous paths through the lesion yet also exit frequently; thus, necessitating image guidance in order to avoid.

This work also introduces a novel approach using a dissipative polymer to couple a cantilever to a ground charge so that it may be employed to create a compact forward-viewing scanning catheter. We were able to use this catheter to demonstrate, for the first time to our knowledge, forward-viewing Doppler OCT.
6.2 Relevance to Interventional Cardiology

This work presents several important points that are relevant to interventional cardiology.

The ex vivo characterization demonstrates the potential of a forward–viewing high resolution imaging catheter to differentiate between the occluded lumen and the arterial wall. This work also illustrates the need for three-dimensional forward viewing as the reconstructed axial images were easier to interpret than the acquired longitudinal images. It also demonstrates that forward-viewing OCT can identify channels that exit into the vessel wall. Furthermore, with the addition of Doppler imaging, there is a possible contrast mechanism for identifying whether a path is safe to follow or not. A forward-viewing OCT probe also raises the possibility of identifying pathologies that may have a greater chance of creating embolic debris. Thus, future clinical decisions to treat a patient with a CTO may rely on identifying the pathology in vivo to decide whether or not to use methods to protect against embolization.

This work also reports on microchannel morphology within CTOs. Two clinically relevant results are discussed. Firstly the three dimensional structure of microchannels observed in the micro computed tomography (μCT) studies suggests that, although they are highly continuous within the lumen, they also exit the lesion frequently into the vessel wall. Thus, blindly following a channel with a guidewire may lead to the wire being placed within the vessel wall. Furthermore, a number of intravascular devices appear to follow the “path of least resistance” through the lesion. The frequent observance of these exiting points calls into question such approaches. Secondly, the μCT imaging work here also suggests that microchannels within the vessel wall have an important role to play in recanalizing a lesion. Thus, strategies for promoting angiogenesis within the vessel wall may be a promising therapeutic avenue in occluded lesions.
6.3 Future Work: Combining Imaging and Intervention

The ultimate clinical utility of a forward looking intravascular device will likely depend on its capacity to be integrated with therapy. Laser-based revascularization is one potential modality that could be used in combination with a forward looking probe such as the one described here. Incorporating fiber optics into the outer sheath of the catheter could enable simultaneous intravascular imaging and ablation of the plaque. Laser ablation with either a femtosecond or an excimer laser is advantageous, as it creates sub-micron debris as it ablates the tissue [153, 154]. This small particle size should minimize blockage of downstream capillaries. One possible design showing the incorporation of multiple fibers into the outer sheath of the catheter is shown below.

![Figure 6.1: Illustrates the incorporation of fiber optics into the outer sheath of the electrostatic scanning catheter discussed in chapter 4. The fiber optics may potentially use either femtosecond or ultraviolet ablation methods.](image)

In terms of device delivery, an important consideration is that the imaging/ablation probe must be either small enough to fit into a 6 French catheter (I.D. 1.6 mm) in order to access the coronary arteries or possess a guidewire port to allow the device to reach the intended site. Effective replacement of the blood with saline in the imaging field will also be an important part of in vivo intravascular imaging that must be addressed for forward-viewing OCT. This task may be performed through the same “over-the-wire” catheter that contains the imaging catheter, provided that this catheter has a small enough profile to access the lesion. Alternatively a custom balloon may be incorporated into the imaging catheter along with suction and flushing ports.
6.4 Future Work: Modifying Medical Therapy toward Natural Recanalization of Arterial Occlusions

One interesting observation in the μCT study was the ~50% patency rate observed in arteries 6 weeks or more after an occlusion had been initiated. The hypothesis that intravascular channels in an occlusion arise from vessels in the arterial wall and may help drive recanalization prompts the strategy of driving arterial wall angiogenesis as part of optimal medical therapy of occlusions. While such ideas are currently being investigated by Dr. Strauss for chronic total occlusions, it is likely that these strategies may be highly effective in the sub-acute phase of the disease (48 hrs – 6 weeks). As the occlusion ages, fibrosis and calcification of the arterial wall may limit the effectiveness of these strategies. Thus, an in-depth investigation to determine if there is an optimal time window for angiogenic therapy of arterial occlusions is warranted.

6.5 Conclusions

While this thesis is written at a time of controversy around the efficacy of angioplasty in occluded arteries, several important points must be noted. Conventional belief is that the benefits of angioplasty are compromised by the increased likelihood of reinfarction and in-stent thrombosis. While next generation of angioplasty stents, such as bioabsorbable stents[155, 156] or stents that actively attract endothelial cells[157] may address some of these challenges, such results remain unproven in clinical trials. At the same time, however, advances in minimally invasive bypass surgery have not produced a significant reduction in side effects associated with bypass. Additionally, the prevalence of heart disease and conditions such as diabetes in the general population mean that a greater number of people will require cardiac intervention. Thus safely opening chronic total occlusions may have significantly more impact on patient’s outcomes if current difficulties involved with angioplasty are effectively addressed.

Secondly, among those with stable angina, the issue of effectively identifying patients who will benefit from therapy is unresolved. This issue was examined for patients with greater than 70% stenosis in the COURAGE trial subset reporting on quality-of-life[36] in
which intravascular balloon angioplasty and stenting is recommended in patients who are unresponsive to maximal medical therapy.

Thirdly, this work should also be interpreted within the larger context of rebuilding the heart in a minimally invasive fashion. Stem cell injection to cardiac tissue via catheter-based means or through surgical delivery has captured a tremendous amount of attention recently as a potential approach for repairing damaged heart muscle. Little attention, however, has been given to repairing the diseased and often occluded arteries that are needed to supply this new muscle. Angiogenic approaches have been suggested that target the myocardium with factors such as VEGF. This neovasculature, however, is typically small and tortuous. Thus, myocardial repair must either explore methods of growing large coronary arteries [158] or effectively repairing the existing ones. Performing either in a minimally invasive fashion will likely require advances in imaging technology.

Ultimately, the work presented in this thesis increases our understanding of the morphology and evolution of arterial occlusions and represents preliminary steps toward being able to safely intervene on an occluded artery in a controlled manner.
References

38. Soares, P.R., et al., *Coronary revascularization (surgical or percutaneous) decreases mortality after the first year in diabetic subjects but not in nondiabetic subjects with multivessel disease: an analysis from the Medicine, Angioplasty, or Surgery Study (MASS II)*. Circulation, 2006. 114(1 Suppl): p. 1420-4.
42. Dzavik V Fau - Buller, C.E., et al., *Randomized trial of percutaneous coronary intervention for subacute infarct-related coronary artery occlusion to achieve long-term patency and improve ventricular function: the Total Occlusion Study of Canada (TOSCA)-2 trial*. (1524-4539 (Electronic)).
44. Erne P Fau - Schoenenberger, A.W., et al., *Effects of percutaneous coronary interventions in silent ischemia after myocardial infarction: the SWISSI II randomized controlled trial*. (1538-3598 (Electronic)).


