Cellular-resolution endoscopic optical coherence tomography and image analytics

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Cellular-Resolution Endoscopic Optical Coherence Tomography and Image Analytics

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in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

2018

Statement of Originality

I hereby certify that the intellectual content of this thesis is the product of my original research work and has not been submitted for a higher degree to any other University or Institution.

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Date

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Yuemei Luo

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Abstract

Coronary artery disease (CAD) and gastrointestinal (GI) cancers are among the top killers worldwide and in Singapore. Acute myocardial infarction (AMI, = heart attack), the clinical manifestation of CAD, is one of the leading causes of global morbidity and mortality. Due to the high prevalence and high risk of AMI, identification of vulnerable plaque associated with AMI is critical for early detection and subsequently interventional treatment. Esophageal, gastric and colorectal cancers are among the commonly diagnosed GI cancers worldwide and early diagnosis of these cancers is vital for the effective therapeutics. Intraepithelial neoplasia (IEN) may develop rapidly into invasive cancers without noticeable symptoms. Therefore, early detection of IEN when they are confined in epithelium is the key to achieving a good prognosis. However, we are limited in methods to obtain histology images of coronary arteries because it is hardly practical to take biopsy from coronary arteries. Even though biopsies can be routinely taken from GI tissues, sampling error may also lead to diagnostic errors. Therefore, a nondestructive imaging tool that can provide cellular-resolution images is critical for improving diagnostic accuracy.

We have developed a desktop micro-optical coherence tomography (μ OCT) imaging system and a novel endoscopic μ OCT fiber-optic probe towards cellular-resolution imaging in coronary arteries and GI tracts. The desktop μ OCT imaging system is an improved version of previously reported μ OCT system in that it has a fiber-optic flexible handheld probe, which enables *in vivo* imaging of living animals. The first main technical contribution of this thesis is the development of the endoscopic μ OCT fiber-optic probe. The μ OCT fiber-optic probe achieves an axial resolution of 2.48 μm in air and transverse resolution of 4.8 μm , which are 4 times and 4.17 times better than the current endoscopic OCT devices, respectively. In particular, we used a novel beamsplitter design at the distal end of the fiber-optic probe so that we can realize the common-path design, annular focusing, and an all-glass optical path. The common-path design eliminates the dispersion difference between the sample and reference arms so that optimal axial resolution of 2.48 μm in air

can be achieved, as well as eliminates the polarization mismatch between the two arms during the probe rotation for circumferential scanning. The annular focusing enables a 1.3 times extended depth-of-focus (DOF) which mitigates the problem of DOF limitation. The all-glass optical path makes it easier to fabricate the probe. Besides, we also employed a rigid outer sheath surrounding the probe so that areas of interest were properly maintained around the relatively small focal region to alleviate the issue of limited DOF.

In order to investigate the capability of μ OCT for visualizing cellular structures, we firstly imaged rat colon *in vivo* using the desktop μ OCT imaging system. Imaging results show that the detailed microstructures, such as the crypt lumens and the goblet cells, could be clearly identified which was supported by corresponding histology images. Secondly, we conducted *ex vivo* imaging of fresh intact swine colon, swine coronary arteries, and human atherosclerotic coronary arteries by the endoscopic μ OCT fiber probe. In normal swine colon we were able to visualize cellular-level microstructures such as goblet cells; we also clearly visualized smooth muscle cells and foam cells in atherosclerotic plaques. These results demonstrate that this endoscopic μ OCT fiber-optic probe is capable of visualizing cellular-level morphological features of both GI tracts and coronary arteries.

The second main technical contribution of this thesis is the design and simulation of the second fiber probe, which may improve the DOF by 2 times. The rationale for this effort is that the DOF of the above-mentioned first probe is still not enough for *in vivo* use. This second probe design follows the principle of multiple aperture synthesis (MAS) and digital refocusing. It uses a novel calcite-based polarization beamsplitter to create two apertures at the pupil plane of the objective lens and to form three apertures in the detection path. The phases of interferometric signals collected through these three sub-apertures can be digitally manipulated so that the three beams can be "refocused" at an out-of-focus point. I have completed the optical design and simulation of the optical performances, and fabricated the second fiber probe.

In addition to the above-mentioned two technical contributions, I have conducted mechanical simulations to model the plaque stability in coronary atherosclerosis. As firstly pointed out, rupture of vulnerable plaque is critically associated with cardiovascular thrombosis and even AMI, whereas its detailed mechanisms are not

fully understood. Recent studies have found abundant cholesterol crystals in ruptured plaques, and it has been proposed that the rapid expansion of cholesterol crystals in a limited space during crystallization may contribute to plaque rupture. However, the potential effect of cholesterol crystals on plaque rupture remains elusive due to the lack of the geometry of cholesterol crystals for analysis. In previous studies, μ OCT can clearly visualize cholesterol crystals within arterial tissues *ex vivo*, and opens the possibility for evaluating the relationship between cholesterol crystallization and plaque rupture. Based on the measured geometric information of cholesterol crystals in human atherosclerotic aorta tissues, we developed a two-dimensional finite element method model of atherosclerotic plaques containing expanding cholesterol crystals and investigated the effect of the magnitude and distribution of crystallization on the peak circumferential stress.

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Chapter 1

Introduction

1.1 Background and Motivation

1.1.1 Clinical Needs

1.1.1.1 Early Detection of Neoplastic Changes in Gastrointestinal (GI) Tracts

Colorectal, gastric and esophageal cancers are among the prevalently diagnosed cancers worldwide, claiming about 1.8 million lives and more than 2.5 million new diagnosed cases annually [1]. These cancers are also the most diagnosed cancers in Singapore: e.g., colorectal cancer is the most common cancer among men and the second most cancer among women. In most cases, if detected and treated early, these cancers may be curable [2]. Therefore, screening of gastrointestinal (GI) tracts at an early stage is an effective method for early detection of these cancers.

Intraepithelial neoplasia (IEN) of the GI tracts, which is the precursor of GI cancers, generally derives from the epithelium of the mucosa. At the stage of IEN, neoplastic cells are restricted to epithelial layer. Once neoplastic cells occupy the whole thickness of epithelium, they begin to infiltrate subepithelial layer, and consequently, the malignancy of cancer increases dramatically [3]. Hence, it is of vital importance to detect lesions as early as possible especially at the stage of IEN, and successful detection of IEN is a significant task for cancer prevention.

Currently, random biopsy under white-light endoscopy together with subsequent histopathological analysis of biopsy tissues is still the gold standard for a definitive diagnosis of cancer-related lesions. However, the standard wide light endoscopy (WLE) and random biopsy may not provide an accurate diagnosis, since neoplastic changes are often within the lower half of the epithelium and may not be visible under WLE. This situation is even worse in Barrett’s esophagus and ulcerative colitis where the lesions have a patchy distribution throughout the entire organ. Moreover, biopsy-based histopathological analysis is invasive and time-consuming, and often carries the risk of missed diagnosis. Meanwhile, artifacts introduced by tissue-processing in histopathological analysis may hinder our understanding of micro-anatomic features of biological tissues in their native state.

Therefore, a non-invasive endoscopic imaging tool with the ability to capture real-time and histology-grade information of epithelium is critical screening the intraepithelial tumors invisible under WLE and consequently enhancing the diagnosis accuracy of early lesions.

1.1.1.2 Evaluation of Vulnerable Plaques in Coronary Artery Disease (CAD)

Coronary artery disease (CAD) is the most common form of heart disease in all over the world, claiming approximately 7.3 million lives worldwide and more than 5 thousand lives in Singapore per year [4, 5]. In normal conditions, the coronary arteries supply blood to the heart on constant flow and the arterial walls are comprised of regular layered structures including intima, media and adventitia. Acute myocardial infarction (AMI, = heart attack), the clinical manifestation of CAD, is a serious condition and often lethal if not treated immediately. It is commonly caused by the rupture or erosion of atherosclerotic plaques and usually characterized by the buildup of plaques within the coronary wall [6, 7]. The rupture of vulnerable plaques could induce the formation of blood clot or thrombus, subsequently narrowing the artery lumen and restricting the blood flow to an area of heart muscle, and thereby, starving the muscle of blood and eventually causing an acute coronary event [8]. If vulnerable sites can be detected before rupture, effective intervention and medical treatments can be adopted to prevent clinical events. These evidences suggest that the identification of vulnerable plaques before rupture is crucial to preventing the worst situation.

Thin-cap fibroatheroma (TCFA), one of the atherosclerotic lesions in morphology, is frequently observed in patients dying with AMI and considered as a precursor of plaque rupture [9]. The major characteristics of TCFA contain a very thin fibrous cap (less than 65 μm in thickness) together with macrophage infiltration and a very large lipid pool [7–11]. Besides, cellular and extracellular compositions of the fibrous cap, including accumulation of macrophages, cholesterol crystals and T-lymphocytes, smooth muscle cells, collagen and elastic fibers and so on, are the key information for determining the plaque vulnerability [6, 12, 13]. Hence, quantification of these cellular and extracellular compositions can assist the assessment of the plaque.

Histopathological analysis of excised biopsy samples is the clinical gold standard for accurate diagnosis of many important diseases. However, it is technically challenging or prohibiting, if possible, to conduct the biopsy of coronary arteries in patients. Most of our knowledge about the CAD comes from the histopathologic study of cadaver tissues, which may not reflect the pathophysiological processes *in vivo*. An investigating tool, which can quantitatively image cellular and extracellular components as well as provide real-time imaging information, is needed for identifying vulnerable plaques and adopting effective therapeutic strategies to save lives before AMI.

1.1.2 Optical Coherence Tomography (OCT)

For imaging GI tracts, there is an urgent need by using a non-invasive diagnostic tool like μOCT , which can screen through a large luminal area. This is because neoplastic or malignant changes in GI tracts tend to spread over a very large mucosal area in a patchy pattern such as Barrett's esophagus, which is very different from lesions in other tubular organs. For imaging coronary arteries, the clinical gold standard, histopathological analysis of excised biopsy sample, is technically challenging or prohibiting because it is hardly practical to take biopsy from coronary arteries. So a non-invasive investigating tool such as μOCT is needed for identifying vulnerable plaques. This also agrees with the fact that main established applications of OCT endoscopy are diagnosis of Barrett's esophagus and coronary artery disease [14–18].

Optical coherence tomography (OCT) firstly proposed in [19] is a non-invasive and high-resolution interferometric laser scanning based imaging technique. Additionally, owing to its ability to provide real-time cross-sectional images, it becomes promising to be an alternative of traditional invasive random biopsy for diagnosis of various diseases [20–23].

Figure 1.1 reveals the working principle of OCT based on Michelson interferometer [24]. A low coherence (broad bandwidth) [21] light source is split into two paths: one goes to a reference mirror (reference arm) and the other one is directed to sample (sample arm). The reflected light from the reference mirror and the backscattered light from the sample are recombined at the same fiber coupler and interfere with each other.

FIGURE 1.1: Schematic of the generic OCT system. L: lens. This figure refers to [21].

The interferometric signals are detected and processed to delineate axial profiles retrieved by Fourier transform. A two-dimensional (2-D) or cross-sectional image can be obtained by transversely scanning the sample light radiation using a beam scanner while continuously acquiring axial-line (A-line) profiles. A three-dimensional (3-D) image can be obtained by transversely scanning the sample light using 2-axis (X and Y) scanners and constructed by combining multiple cross-sections, shown as Figure 1.2 [24].

Technically, OCT can be viewed as a combination of the low coherence interferometric technique and reflectance confocal microscopy. In the axial/depth direction, the working principle of OCT is based upon low coherence interferometry, wherein the profiles of the refractive index changes of the sample along the depth direction are recorded by the interferometric signals. In the transverse direction, OCT optics is identical to reflectance confocal microscopy, in which confocal detection

FIGURE 1.2: OCT image formation principles [24].

pinhole acquires a small volumetric signal of the sample by rejecting the out-of-focus backscattered light.

Resolution and scanning range are two of significant behaviors for OCT imaging described in axial, lateral and transverse directions, respectively [24]. Due to the symmetry of the utilized Gaussian beam in transverse-lateral section, both two characteristics in transverse and lateral directions almost keep the same. Thus, only the resolution and scanning range in axial and transverse directions are analyzed in our study, depicted in Figure 1.3 [24].

The axial resolution, the depth-resolving ability, is equal to the coherence length of the light source and thus predominated by the laser source as [24]:

$$\Delta z = l_c = 2 \ln(2) \lambda_c^2 / \pi \Delta \lambda^2 \quad (1.1)$$

where λ_c and $\Delta \lambda$ are the central wavelength and the full width at half maximum (FWHM) bandwidth of the light source, respectively.

The transverse resolution is regarded as the diffractive limited spot size of a focused beam, and is defined as the beam waist diameter with an assumed Gaussian input beam. It is generally determined by the wavelength of the light source, the focal length of the objective lens, and the diameter of the beam passing through the objective lens. It can be expressed as [24]:

$$\Delta x = 2 \lambda / \pi N A \quad (1.2)$$

FIGURE 1.3: Illustration of the resolution and scanning range in the axial and lateral directions [24]. DOF: depth-of-focus.

where λ is the wavelength of the light source, NA is the numerical aperture (NA) of the objective lens $NA = d/2f$, d is the diameter of the beam passing through the objective lens, f is the focal length of an objective lens.

The DOF, the distance about the plane of focus where objects appear acceptably sharp in an image, is defined as an axial distance that is twice the Rayleigh range, can be expressed as [24]:

$$z = 4\lambda/\pi NA^2 \quad (1.3)$$

A new generation of OCT, termed micro-optical coherence tomography (μ OCT), is capable of providing an ultrahigh spatial resolution of 1 - 3 μ m and herein capturing cellular and subcellular structures in intact arterial tissues *ex vivo* [25, 26]. It opens the possibility to discern real-time cytological information in GI tracts and intracoronary arteries at the cellular level equal to histology.

Even though μ OCT offers high resolution to visualize tissues at the cellular and subcellular level, μ OCT is still an *ex vivo* imaging technology for GI tracts and intracoronary arteries and cannot be used for clinical diagnosis as of now, due

to the inability to access the internal lumens by a miniaturized probe to deliver the sample beam to the imaging sample. It is essential to design and fabricate a miniature and flexible probe for accessing internal lumens for real-time imaging.

1.1.3 Trade-off between Transverse Resolution and Depth-of-focus (DOF)

One of the major issues with μ OCT to image tissues *in vivo* is the limited depth-of-focus (DOF). From the Eq. (1.2), the transverse resolution is mainly dependent on the NA of the objective lens in the sample arm. From the Eq. (1.3), the DOF is proportional to the square of NA. In other words, the DOF is inversely proportional to the square of transverse resolution. Consequently, there exists a trade-off between the transverse resolution and the DOF: a higher transverse resolution results in a shorter DOF, and vice versa. In μ OCT system, the high NA of the objective lens provides a high transverse resolution (small spot size) but shortens the DOF. As a result, the limitation in DOF easily results in the defocus problem when imaging through a relatively thick tissue depth *in vivo*.

1.2 Objective

The objective of this thesis is to develop and validate endoscopic μ OCT systems for imaging cellular microstructures of GI mucosa and coronary arterial wall. Firstly, in order to test if μ OCT is capable of acquiring cellular-resolution structures of GI mucosa, we constructed a desktop μ OCT imaging system with a flexible hand-held probe and imaged rat colon *ex vivo*. The desktop μ OCT system has the best possible spatial resolution and sensitivity with regard to the fiber-optic endoscopic system, so that results provided by the desktop μ OCT tell us what μ OCT can do and what it cannot. Secondly, we designed and fabricated a fiber-optic μ OCT probe that achieves an axial resolution of 2.48 μm in air and a transverse resolution of 4.8 μm . We prioritized the design requirement of spatial resolution for this first probe, and only moderately mitigated the limitation of DOF by use of annular apodization and a rigid sheath. In addition, to achieve a circumferential scanning, we constructed a rotary joint and then combined it with the probe during imaging.

Thirdly, we verified the feasibility of this first μ OCT endoscopic probe for *ex vivo* visualization of the cellular-level morphological information in GI tracts and coronary arteries. Fourthly, after we confirmed the feasibility of cellular-resolution imaging by μ OCT endoscopic probe, we designed and fabricated the second version, which provides further extended DOF without compromising spatial resolution. Finally, we aim to establish an analytical method for the evaluation of the vulnerability of plaques based on mechanical instability brought by cholesterol crystal accumulation recorded by the fabricated μ OCT probe. Based on the geometric information of cholesterol crystals from the high-resolution images acquired by μ OCT in human arteries, we attempted to model and simulate the cholesterol crystal growth in atherosclerotic plaques, which provides a possibility to evaluate the mechanical effect of cholesterol crystallization on the plaque rupture.

1.3 Significance of the Study

The ability to image and identify cellular-level histological information of GI cancers and coronary atherosclerosis is essential for their diagnosis and management. My study fills the technical gap between the clinical need and current standard-of-care tools by enabling non-invasive stain-free cellular-level endoscopic imaging and intravascular imaging for the first time. The technologies developed through this study may ultimately enable visualization of the cellular structures critical for diagnosis of disease without excisional biopsy.

First of all, my study improved the spatial resolution of endoscopic OCT by 4-6 times and provided preliminary evidences that support cellular-resolution imaging. Although endoscopic OCT systems have been reported and commercialized [14–18, 27], the resolution is about 10 - 30 μ m, which is too coarse to visualize cellular scale structures. This limitation in spatial resolution is one of major reason why OCT is not widely embraced by medical community except for ophthalmology. The typical impression about OCT for a large number of clinicians is a technology that can resolve mucosal layers. Our effort in this study will change this stereotype by demonstrating the capability of visualizing a whole new level of micro-anatomical information — cellular-level information.

Secondly, my study also provided solutions to the major technical problems including DOF limitation, unbalanced dispersion, and signal coupling efficiency. These solutions may ultimately overcome these technical roadblocks towards clinical utility of this cellular-level imaging tool. We provided two distal end optical designs. The first probe design solved the problem of unbalanced dispersion and moderately mitigated the problem of DOF and signal coupling efficiency. The second probe design aims to solve all the above-mentioned problems.

1.4 Novel Contributions of the Study

First of all, I designed and fabricated the first circumferential scanning μ OCT endoscopic system with a fiber-optic probe. The endoscopic μ OCT system achieves an axial resolution of 2.48 μm in air and a transverse resolution of 4.8 μm . In particular, we adopted an apodizing beamsplitter design at the distal end of the probe to realize moderate DOF extension and utilized a rigid sheath to surround the probe during the scanning to mitigate the limited DOF.

Secondly, I demonstrated for the first time the capability of endoscopic μ OCT to visualize cellular-level details in freshly acquired GI tracts and human atherosclerotic coronary arteries. We have visualized for the first time the mucus granules of goblet cells in normal swine colon *ex vivo* and smooth muscle cells, macrophages, collagen fibers in human atherosclerotic plaques *ex vivo*.

Thirdly, I designed and fabricated the first μ OCT fiber probe with multiple aperture synthesis (MAS) function. The MAS technique has been proven to be an effective method to extend the DOF without coupling loss of optical signal in desktop free-space imaging systems. My research miniaturized and integrated the MAS optics in the distal end of μ OCT fiber probe, so that the DOF limitation can be further mitigated compared to the first μ OCT probe.

Lastly, I proposed and demonstrated a new imaging based method to evaluate the mechanical instability caused by the cholesterol crystals in atherosclerotic plaques. This new method provides complementary diagnostic information about the vulnerability of the plaques and may help to improve the diagnostic accuracy of coronary artery disease.

1.5 Outline of the Thesis

This dissertation consists of 8 chapters.

Chapter 1 starts with the clinical needs of OCT system, especially high-resolution OCT termed μ OCT, for imaging GI tracts and intracoronary arteries over other current techniques. Then the basic theory of OCT approach is briefly illustrated, followed by the introduction of the two main limitations for high-resolution OCT application: the lack of miniaturized probe and the limited DOF. After that, the objective and significance of this study is stated.

Chapter 2 reviews related literatures including the state-of-the-art imaging techniques as well as DOF extension techniques applied in OCT.

Chapter 3 demonstrates the capability of μ OCT to image GI tracts at the cellular level by utilizing a desktop μ OCT systems for visualizing rat colon *ex vivo* and *in vivo*, respectively.

Chapter 4 constructs an endoscopic μ OCT system with a flexible miniature probe, which allows inserting the sample arm into the imaged lumens. Subsequently, its capability for GI tracts imaging is testified by conducting *ex vivo* imaging on swine colon. Specifically, I elaborate the design and fabrication methodology for the probe. In order to extend DOF moderately, the probe adopts apodization together with common-path design.

Chapter 5 presents a combination of this probe-based μ OCT system and spectroscopic OCT to improve the color contrast. Then a demonstration about the ability for visualizing coronary arteries is provided by acquiring cellular and extracellular compositions of atherosclerotic plaques.

Chapter 6 provides a miniaturized optical design of the MAS optics and the corresponding μ OCT fiber-optic probe design. The principles of MAS and digital refocusing are elaborated, and the performances of this advanced μ OCT probe are also predicted through numerical simulation.

Chapter 7 reports a novel mechanical model and analysis method I developed for evaluating the effect of cholesterol crystallization on plaque vulnerability, which may assist in preventively evaluating the mechanism of plaque rupture.

Chapter 8 concludes this thesis and puts forward potential ideas for future works.

Chapter 2

Literature Review

2.1 Pathological evolution of GI diseases and atherosclerotic lesions

2.1.1 Pathological process of epithelial cancer

Epithelial cancer is the predominant type of cancers accounting for 80 – 90% of all cancers in adults [28]. It is a fatal disease originated from epithelium and is characterized by uncontrollable division and proliferation of abnormal neoplastic cells [3]. Among all epithelial cancers, squamous cell cancer is the most common type and remains as the major cause of cancer-related death [3, 29].

Epithelial cancers have the similar pathophysiological nature and share a same pattern of multistage development: normal epithelium, intraepithelial neoplasia, superficial infiltrating cancer and advanced infiltrating cancer [30]. An example of the development of squamous cell epithelial cancer in esophagus is illustrated in Figure 2.1 [3, 30]. At the stage of intraepithelial neoplasia which could be further divided into mild, moderate and severe stages (Figure 2.1(B)), neoplastic cells are restricted to epithelial layer and have no or very low risk of metastases; however, once neoplastic cells occupied the whole thickness of epithelium, they begin to infiltrate to subepithelial layer where nutritional supply is sufficient, and consequently, the risk of metastases and the malignancy of cancer increase dramatically [30]. Clinical practice also indicates that better health outcomes are closely associated

with successfully detecting cancer at an early stage especially at the stage of intraepithelial neoplasia. Therefore, successful detection of intraepithelial neoplasia is an important task for cancer prevention.

FIGURE 2.1: Multi-stage development of esophageal squamous cell cancer. (A) Progression from a benign lesion to invasive cancer [3]. IN: intraepithelial neoplasia. (B) Stages of intraepithelial neoplasia [30]. HSIL: LSIL: low-grade squamous intraepithelial lesion; LSIL: high-grade squamous intraepithelial lesion. CIN1, CIN2, and CIN3 account for low-, moderate-, and high-grade dysplasia, respectively. HPV: human papillomaviruse. E6 and E7, are the 2 viral oncoproteins.

2.1.2 Pathological process of atherosclerosis

Atherosclerosis is a multi-step process ranging from endothelial dysfunction to plaque development, plaque progression, and plaque rupture, shown as Figure 2.2 [31]. Once plaques rupture, it usually leads to thrombus, narrow the blood flow and eventually causes cardiovascular events [31].

In the process of plaque formation, the early stages consist of infiltration of the affected site by T-lymphocytes and monocytes, which then transform into macrophages (foam cells), followed by proliferation of fibrous tissue [31–33]. Initially the endothelium attempts to repair itself by attracting T-lymphocytes, monocytes and platelets to the injured site. When failed to repair, the endothelium becomes permeable and the lymphocytes and monocytes migrate into the deep layer of the intima [33]. In this stage, low-density lipoprotein (LDL) particles are engulfed by monocytes and then are transformed into macrophages (foam cells), smooth muscle cells begin migrating from the media, and the fatty streak is formed [33]. The

FIGURE 2.2: Progression of atherosclerosis [31]. EPA has been reported to have beneficial effects on many of these steps. RLP, remnant-like particle; LDL, low-density lipoprotein; ox-LDL, oxidized low-density lipoprotein; CRP, C-reactive protein.

process is reversible at this stage, and if this stage can be detected, interventional treatment could be adopted to prevent worse situations.

2.2 State-of-the-art Imaging Techniques

2.2.1 Histopathology

Clinical diagnosis of many diseases of internal organs relies on histological examination of biopsy samples randomly excised under the standard white-light endoscopy (WLE), which is the current gold standard for the screening. However, the process of biopsy is invasive to the organ under investigation and destructive to the sampled tissue volume, which may introduce complications for patients such as bleeding and perforation, and places substantial burden to the healthcare system of patients [34, 35].

WLE and random biopsy is also limited in guiding endoscopic interventions, especially for colorectal cancer. Colorectal cancer is generally considered as a progression from benign adenoma, and thus, detection of adenomatous polyps and the following removal before their development into malignant tumors is the key to preventing colorectal cancer. Colonoscopic polypectomy is routinely utilized

for colorectal polyps which are commonly demonstrated during screening colonoscopies. Since colonoscopic polypectomy is associated with a risk of perforation and bleeding with a rate of 0.4-0.7% of cases [36], indications for colonoscopic polypectomy should ideally be limited to an adenomatous polyp that has the potential of developing into an invasive colorectal carcinoma. However, conventional colonoscopic examination so far has yielded no morphological criterion differentiating between neoplastic and non-neoplastic polyps to avoid unnecessary polypectomies with subsequent colonoscopy and attendant costs.

In addition, as histology requires workup and the evaluation is time-consuming, which may limit the ability of the endoscopist to immediately determine the necessity for intervention during ongoing endoscopy, possibly resulting in the need for repeated endoscopies. Furthermore, redundant biopsies have to be taken for many lesions that are subsequently determined to be not malignant. Thus, pathologists must bear a heavy burden of the work, and patients incur increased costs and associated risks (such as bleeding). Moreover, because of local fibrosis after biopsy, endoscopic resection is difficult [37].

To solve these problems, non-invasive screening and surveillance tools need to be investigated to decrease the frequency of biopsy and consequently minimize the risk of the cost and complications.

2.2.2 Non-invasive Imaging Approaches for GI Tracts

Recently, several types of imaging modalities have been proposed and utilized for GI tracts imaging to address these issues, including narrow-band imaging (NBI) [36], chromoscopy [36, 38], confocal laser endomicroscopy (CLE) [39, 40], fluorescence endomicroscopy [41, 42], optical coherence microscopy (OCM) [43–45], and OCT [14–16, 21, 27, 46–48].

NBI and magnifying chromoscopy are capable of clearly visualizing pit patterns and vascular structures, which are very significant for the colorectal lesions diagnosis [36, 38]. Even though NBI and chromoscopy can offer additional information of the imaged areas, neither of them can directly visualize the histological and cytological information.

CLE can provide cellular/subcellular resolution to image the microstructures of colorectal, gastric and esophageal epithelium [39, 49–52]; however, it is subject to sampling errors similar to those of biopsy due to the limited field of view [35, 49–52]. Additionally, comparisons between CLE and standard histopathological examination is difficult, since CLE provides the images in *en face* planes which are parallel to the surface of the lumen, while the histopathological examination routinely displays the cross-sectional images in vertical planes.

Based on a line illumination and detection scheme, a line-scanning OCM has been developed to generate *en face* cellular-resolution images of human specimens, demonstrating its capability for upper and lower GI tracts imaging [43–45]. Owing to the high resolution provided by OCM, both crypt structures and goblet cells were clearly visualized [43–45]. With regards to fluorescence endomicroscopy, Bao et al. showed that two-photon fluorescence endomicroscopy was advantageous for gastric cancer diagnoses over that using one-photon [41]. Zhuo et al. demonstrated the potential of multiphoton microscopy (MPM) for differentiating colonic polyps [42]. Nevertheless, all the above-mentioned methods are associated with certain technical issues, e.g., suffering from limited sensitivity and scanning speed, and providing only *en face* images with limited penetration depth. Such issues finally hinder the clinical use of those imaging tools.

OCT is a promising 3-D imaging tool for obtaining real-time images for its non-invasive and high-resolution properties [19, 53]. Fiber-optic endoscopic OCT techniques with a spatial resolution of 10–30 μm have been developed for imaging gastrointestinal (GI) tracts [16, 27, 47, 48, 54–58], which can provide significant morphology information. But they are still limited to detect the cellular-level structures. Since it is straightforward to improve axial resolution of OCT by use of a light source with boarder spectral bandwidth, advances in ultrahigh axial resolution technique has significantly improved the image quality [59]. However, the transverse resolution is not enough to provide cellular-level information.

2.2.3 Non-invasive Imaging Modalities for Coronary Arteries

Among current screening tools for coronary arteries, non-invasive /minimum-invasive angiography techniques, including X-ray, computed tomography (CT) or multi-detector CT (MDCT) [60], magnetic resonance imaging (MRI) [60], intravascular ultrasound (IVUS) [61, 62], and intravascular OCT (IVOCT) [17, 18, 27, 63–68], have greatly improved the clinical outcome of the diseases such as CAD.

X-ray angiography, CT and MRI are commercially applied for non-invasive atherosclerotic plaque screening with a spatial resolution in the order of hundreds of microns [60], which is 2-3 orders of magnitude lower than that of histology. probe-based intravascular ultrasound (IVUS) with the resolution of 70-100 μm is clinically developed to characterize plaques [61, 62]. IVOCT so far is the highest-resolution *in vivo* imaging modality to visualize the microstructural features of coronary atherosclerotic plaque with approximately 10 μm axial and 20 - 30 μm transverse resolutions [17, 18, 27, 63–68].

To summarize, all above-mentioned techniques are useful for staging when diseases reach a higher stage, but lack the capability to detect early lesions due to the insufficient resolution.

2.2.4 Background and Development of OCT

OCT first proposed for the ophthalmic application by Fujimoto et al. [19] is a non-invasive imaging tool, enabling in situ and real-time visualization of microscopic volumetric morphology without tissue removal. To date, OCT is not only commercially employed in ophthalmic imaging [69, 70], but also increasingly used in dermatological imaging [71, 72], airway function evaluation [73, 74], gastrointestinal tract imaging [48, 75] and cardiovascular disease diagnosis [25, 66, 67].

There have been two generations of OCT technology: time-domain OCT (TDOCT) and frequency-domain OCT (FDOCT) [76, 77]. In TDOCT, which was proposed firstly, the mirror in reference arm can be moved axially, so the light path length is changed as a function of time [19]. Moving the mirror can lead to the change of reference path length, and the interference can be observed only when the path

difference between reference arm and sample arm is within coherence length of light source and peaks when path difference matches the coherence length. Thus, the implementation of the moving reference can realize the sectioning of the sample.

But during the past years, FDOCT which shows increased sensitivity and improved scanning speed has replaced TDOCT to become commonly applied OCT [78–80]. The system of FDOCT is similar to that of TDOCT while the reference mirror in FDOCT is immobilized corresponding to the sample position [76–80]. Particularly, FDOCT can be subdivided into swept source OCT (SSOCT) and spectral domain OCT (SDOCT) [76, 77, 79]. In the case of SSOCT, the light source has narrow instantaneous bandwidth but can be swept rapidly in wavelength, and the spectral interference signals is detected as a function of time by a photoreceiver [21, 79]. In the case of SDOCT, a broadband and continuous-wave source is applied and a spectrometer containing a grating and a charge-coupled device (CCD) is used at the detection part to measure the interferometric signals in which the grating is to disperse the interferential light into CCD to record the relative light intensity [81, 82]. Compared to SSOCT, SDOCT can provide higher resolution. In our study, μ OCT, termed as a case of SDOCT, is chosen to receive ultrahigh resolution of $\sim 1\text{--}4\text{ }\mu\text{m}$.

2.2.5 Principle of spectral domain OCT (SDOCT) system

A basic schematic of SDOCT is illustrated as Figure 2.3 [24]. A broad-band light source containing various frequency components is split to the reference reflector and imaging sample as reference beam and sample beam, respectively. By travelling a certain distance, the backreflected beam from fixed reference reflector and the backscattered beam from difference depths of internal sample structure interfere. There exists a phase delay between reference beam and sample beam depending on their path length difference. For a sample comprising a series of reflectors, different depths of the reflector has different delay to reference beam and produce different modulations. The total interference intensity can be detected by a spectrometer as a function of frequency. And by Fourier transforming the detected spectral intensity to resample it in z -space, the delay related to path length difference z as well as reflectivity of relative reflectors in sample can be measured to reconstruct

the sample depth profile, because the reconstruction of an unknown sample is directly dependent on the sample reflectivity profile.

FIGURE 2.3: Schematic of the typical Michelson interferometer in SDOCT [24]. BS: beam splitter. E : electric field. z : light path length. r : reflectivity. k : spatial frequency. ω : angular frequency. ρ : detector responsivity. Subscript i means the input. Subscripts R and S mean reflector and sample, respectively. I_D is the detected intensity.

Since frequency ν of the source oscillates much faster than any detector's responding time, the influence of angular frequency ($\omega = 2\pi\nu$) on combined intensity can be eliminated. Thus, when assuming sample comprising a series of N discrete reflectors, the detected intensity as a function of spatial frequency k is expressed as [24]:

$$I_D(k) = \frac{\rho}{2} \left\langle \left| \frac{s(k, \omega)}{\sqrt{2}} r_R e^{i2Kz_R} + \frac{s(k, \omega)}{\sqrt{2}} \sum_{n=1}^N r_{Sn} e^{i2Kz_{Sn}} \right|^2 \right\rangle. \quad (2.1)$$

Here, $S(k) = \langle |s(k, \omega)|^2 \rangle$ is the power spectral density of light source which is decided by source.

By using Euler's rule, the combined intensity, also called spectral interferogram, can be recalculated as the following [24]:

$$\begin{aligned}
I_D(k) &= \frac{\rho}{4} [S(k) [r_R^2 + r_{S1}^2 + r_{S2}^2 + \dots]] && \text{DC Term} \\
&+ \frac{\rho}{2} \left[S(k) \sum_{n=1}^N r_R r_{Sn} (\cos [2k(z_R - z_{Sn})]) \right] && \text{CC Term} \\
&+ \frac{\rho}{2} \left[S(k) \sum_{n \neq m=1}^N r_{Sn} r_{Sm} (\cos [2k(z_{Sn} - z_{Sm})]) \right] && \text{AC Term.} \quad (2.2)
\end{aligned}$$

Shown in Eq. (2.2), detected spectral interferogram $I_D(k)$ contains three components: direct-current (DC) term, cross-correlation (CC) term and auto-correlation (AC) term.

By Fourier transforming the detected signals $I_D(k)$, the interferometric intensity $i_D(z)$, can be obtained as [24]:

$$\begin{aligned}
i_D(z) &= \text{FT}[I_D(k)] = \frac{\rho}{4} [\gamma(z) [r_R^2 + r_{S1}^2 + r_{S2}^2 + \dots]] && \text{DC Term} \\
&+ \frac{\rho}{4} \left[\gamma(z) \otimes \sum_{n=1}^N r_R r_{Sn} (\delta [z \pm 2(z_R - z_{Sn})]) \right] && \text{CC Term} \\
&+ \frac{\rho}{4} \left[\gamma(z) \otimes \sum_{n \neq m=1}^N r_{Sn} r_{Sm} (\delta [z \pm 2(z_{Sn} - z_{Sm})]) \right] && \text{AC Term.} \quad (2.3)
\end{aligned}$$

Here, $\delta(z)$ accounts for the impulse function and $\gamma(z)$ is the coherence function which is the Fourier transform of $S(k)$.

The Eqs. (2.1) and Eq. (2.2) include three distinct components [24]:

- (1) DC term is constant and considered as DC component which is just dependent upon the power spectral density, the reflectivity of reference and the sum of sample reflectivities. Since the reflectivity of reference reflector ($\sim 0.9 - 1.0$) is far greater than that of the samples (on the order of $\sim 10^{-2} - 10^{-2.5}$), the DC term is largely dominated by the reference part and shown little relation to the sample part.
- (2) Apart from the constant items $S(k)$ and r_R , the CC term is also depended on the spatial frequency and the path length difference between reference arm

and sample reflectors, which is the significant component for sample imaging in OCT. For multiple reflectors in a sample, the CC term is the superposition of all interference by each reflector and reference, and the amplitude of each interferometric CC term is proportional to the product of reference reflectivity and relative sample reflector reflectivity. Since the reference reflectivity keeps constant for a given OCT system, the sample reflectivities can be estimated by the amplitudes CC term.

- (3) The AC term, seen as artifacts in OCT system, is generated by the interference between the backscattered light from different sample reflectors. Unlike CC term, their amplitudes of AC terms are determined by the reflectivities of different sample reflectors which are far smaller than that of reference reflector, thus the AC term is much smaller than CC term. Additionally, in order to decrease the effect of AC term, a reference with as large as reflectivity should be selected to expand the difference of amplitude between CC and AC term.

2.2.6 Micro-optical Coherence Tomography (μ OCT)

In order to improve resolutions of OCT devices in both axial and transverse directions, μ OCT technology with a desktop μ OCT system was developed for imaging human coronary arteries and respiratory airways [25, 83–86]. The desktop system achieves $2 \times 2 \times 1 \mu\text{m}$ (transverse $\times$ lateral $\times$ axial) resolution in tissue, and enables clear *ex vivo* visualization of many key characteristics and processes associated with pathophysiology of coronary arteries, including macrophages/foam cells, smooth muscle cells and leukocytes adhesion [25, 83–86]. This breakthrough in spatial resolution brings about a quantum leap in acquired information including cellular and extracellular details that have never been seen in intact human tissues (Figure 2.4), demonstrating that μ OCT is promising to capture the histology-grade structural features.

Recently, a linear scanning μ OCT bronchoscope has been successfully demonstrated for imaging mucociliary clearance *in vivo* [73, 74]. However, no progress has been made to develop a circumferential scanning probe-based μ OCT for GI tracts and coronary arteries imaging. The major technical difficulty is the well-known

FIGURE 2.4: Imaging comparison among (a) OCT, (b) μ OCT and (c) histology of a calcium plate (Ca) within the coronary artery wall [25]. Scale bar: 200 μ m.

trade-off between the transverse resolution and DOF, which precludes practical applications of probe-based μ OCT.

2.3 Endoscopic OCT probe

Endoscopic OCT is a procedure that applied to visualize the surfaces of internal organs by inserting a miniature probe into the body, and various endoscopic OCT systems with different designed probe have been published [87, 88]. For application, it has widely used for airway function evaluation [73, 74], gastrointestinal tract imaging [50, 75], cardiovascular disease diagnosis [25, 66, 67], urological application [89, 90], neurological application [91–93], nasal imaging [94] and so on.

Based on the clinical application, the probe can be designed to be flexible and rigid: the flexible probe is more suitable for vascular and GI tracts imaging due to the flexible structures of imaging tissues, while the rigid probe is desired for the application involving incision-based procedures such as image-guided biopsy [87, 95]. According to the imaging direction, the probe can be categorized into side-view imaging (rotational imaging) design and forward-view imaging design:

the side-view imaging provides the circumferential information of tissues around the probe, while the forward-view imaging can provide information of tissues in front of the probe [87, 95]. The side-view imaging is more available for imaging hollow organs such as GI tracts and coronary arteries, and the forward-view imaging is more attractive solid tissues or organs for imaging deep within the tissues [87, 95].

With respect for probe optics, the first OCT probe proposed by Tearney et al. [42] consists of a single mode fiber (SMF), a gradient-index (GRIN) lens to focus the emitted light to a spot and a prism to deflect the light for side-view imaging. Another design of angle-cut ball-lensed fiber to replace the GRIN lens was proposed to improve the imaging performance [96]. In order to improve the lateral resolution and signal intensity, a multi-channel probe with a multifocus array tip was proposed to allow high NA for the fiber, as well as the increased probe fabrication complexity [97]. A new probe design with the use of a diffractive lens after the GRIN lens was fabricated to compensate the chromatic aberration caused by the broadband light source [38]. Another novel probe called needle probe with an ultra-small size and a sharp distal end can be inserted directly into the solid tissues [93, 98].

2.4 DOF Extension Techniques

In order to acquire cellular-resolution images of large volumetric tissue, it is necessary to extend the DOF without compromising the high transverse resolution. In the past decades, various DOF extension techniques have been applied for OCT systems and can be classified into hardware-based extension approaches and digital refocusing solutions by software correction.

Hardware-based extension approaches include phase [99–101] or amplitude [25, 102] apodization, Bessel beams with axicon optics [103–105], and adaptive optics [106, 107].

In phase or amplitude apodization methods, the illumination and collection efficiency of light is low over the extended DOF, easily resulting in signal loss [25, 99–102]. Axicon lens makes a narrow focal line along the optical axis and can be approximated as a zero-order Bessel beam, which maintains uniform transverse

distribution over a DOF more than 10 times larger than the Rayleigh range, however, this method suffers signal loss of 26 dB and pronounced sidelobe artifacts [103–105].

Adaptive optics, using pupil segmentation or active phase modulation of the pupil, can recover near-diffraction-limited performance from a variety of samples exhibiting aberrations [106, 107]. Nevertheless, this approach needs stable phase when acquiring sequential depth profiles, which is practically challenging if imaging tissues *in vivo* where heart pulse would break the phase stability. Besides, its time-consumption property also renders it hard to manifest during *in vivo* OCT imaging.

Several digital refocusing solutions are proposed by digitally correcting for defocus-induced wavefront distortion to extend the DOF, including interferometric synthetic aperture methods (ISAM) [108, 109], depth-encoded synthetic aperture based refocusing [110, 111], and multiple aperture synthesis (MAS) based refocusing [112].

ISAM computationally reconstructs volumes with an identical resolution in all planes compared to that achieved at the focal plane in conventional OCT, by solving the inverse scattering problem for interference microscopy [108, 109]. However, this approach demands high stability of the phase when capturing consecutive images at different depths and suffers from heavy computation cost.

The depth-encoded synthetic aperture based refocusing proposed by Mo et al. extends the DOF by dividing apertures and then digitally correcting the wavefront curvature without the need of stable phase to acquire consecutive OCT images [110, 111]. Besides, it shows a good compatibility with existing OCT hardware. However, this method easily suffers from signal loss originating from the decreased coupling efficiency due to the NA mismatch of the fiber pinhole between illumination and detection optics. The reason is that the different angular (spatial) frequency components are separated into non-Gaussian beams in the illumination path, while coupled into the Gaussian pin-hole detector in the detection path. In addition, this method is not scalable since increasing the number of apertures will dramatically increase the signal loss.

MAS based refocusing technique applied in our group is capable of digitally correcting the wavefront distortion without the signal loss or sidelobe artifacts [112]. This method allows Gaussian beam illumination for each aperture, and in the detection path, the backscattered light field is coupled into the same Gaussian

pin-hole detector with matched NA, herein achieving the optimal illumination and detection coupling efficiency. As a result, MAS method can become an effective method to achieve high transverse resolution along an extended DOF, which shows potentiality for *in vivo* probe-based OCT.

2.5 Spectroscopic OCT

Absorption and scattering are two mechanisms to form the color in the nature. In contrast to those produced by selective absorption, structured color reflected by a non-luminous object is mainly caused by the interaction between light and specific structures. In biology, structural color is commonly observed at the surfaces of animals and plants [113, 114] (skin, feather, epicarp) through light receptors, such as naked eyes, microscope, telescope and so on, but partially understood and less studied in internal organs, what is to say, in three dimensions.

Spectroscopic OCT, a kind of structured color imaging method based OCT which arises from tissue scattering has been employed to evaluate precancerous process [115, 116] or burning severity [117, 118] with non-invasive, three dimensional, high-resolution spectral information, while no specific structured color has been reported. In detail, structured color imaging method based OCT system uses the same data acquired for conventional OCT imaging, and can provide spectral information at each site of the sampled volume with the help of spectral analysis.

Leitgeb et al. first demonstrated the ability to perform SOCT using frequency-domain OCT [119]. Similar to the short time Fourier transform (STFT) for time-domain OCT, the spectral interferogram had been short-frequency Fourier transformed (Figure 2.5) [120]. The basic idea of spectroscopic OCT is to use time-frequency analysis method to get the frequency information along the imaging depth and the most widely used time-frequency analysis method is the STFT. Physically, the STFT can be considered as the result of passing a signal through a bank of band-pass filters with constant bandwidth.

For structure characterization, previous work using this technology has been conducted to investigate the scattering signals mainly from endogenous cells or exogenous spherical particles at the micrometer level [121–123], but few works has been done on the cylindrical structures at micrometer level, such as collagen fibers. In

FIGURE 2.5: Frequency Domain spectroscopic OCT. SOCT: Spectroscopic OCT. SFFT: sparse fast Fourier transform. [120]

this thesis, we applied the spectroscopic OCT on the imaging of coronary artery to distinguish the structure of collagen fibers.

Chapter 3

Imaging Cellular Structures of GI Tracts *Ex Vivo* Using μ OCT

This chapter presents a demonstration about the capability of μ OCT to detect cellular structures of GI tracts, such as crypt structures and goblet cells in normal rat colon. Specifically, Section 3.1 gives a brief background on the motivation to visualize intestinal crypts and goblet cells by μ OCT. After that, in Section 3.2, the study method is described including both experimental setup and procedures. Section 3.3 presents the experiment results, and the following discussion and summary of this chapter are illustrated in Section 3.4 and Section 3.5, respectively.

3.1 Background

The mucosal microanatomy of the large intestine is characterized by the presence of crypts of Lieberkühn, which is associated predominantly with goblet cells. Crypts of Lieberkühn, also named as intestinal crypts, usually are found as the invaginations in the intestinal epithelium lining of the small intestine and large intestine. They are the home for vigorous proliferation of epithelial cells, and hence are vital to fueling the self-renewal process of the epithelium [124]. Goblet cells are the glandular epithelium cells to secreting gel-like mucins for mucous membranes protection as well as homeostasis maintenance [125–127]. During the progression of bowel diseases, such cellular-level intestinal microstructures, intestinal crypts together with goblet cells, undergo morphological changes, and are also critical

indicators of pre-malignant lesions for early detection of intestinal cancers [128]. Particularly, the presence of goblet cells in gastric mucosa may indicate intestinal metaplasia, which is a sign of premalignant stage of gastric cancer [129]. In the colon, the structural alterations of crypts and goblet cells are associated with the assessment of ulcerative colitis, a condition predisposing to the development of colonic cancer [130–132]. Therefore, the visualization of intestinal crypts and goblet cells and their structure changes in cross-sectional view, particularly *in vivo*, would assist early diseases diagnosis and treatment.

μ OCT, a non-invasive imaging tool with the ultrahigh spatial resolution and the capability to generate both cross-sectional and 3-D images *in vivo*, has been demonstrated to visualize cellular and subcellular structures in coronary arteries [25, 26] and pulmonary tracts [73, 74, 85, 133–135]. However, there has been no report on its capability in identifying cellular-level microstructural details in GI tracts such as intestinal crypts and goblet cells.

In this chapter, we demonstrated the development of a desktop μ OCT system, which achieves a resolution of 2.0 μm in both axial and transverse directions for such imaging purpose. *Ex vivo* 3-D images, together with real-time cross-sectional images *in vivo* in rat colon mucosa, were acquired. Such results were further validated as compared to their corresponding histology images. To our knowledge, our study is the first demonstration of μ OCT as a tool for visualizing cellular-level microstructures of intestinal mucosa *ex vivo* and *in vivo*.

3.2 Methodology

3.2.1 Imaging System Construction

In this study, a typical desktop μ OCT system was utilized for both crypts and goblet cells imaging in rat colon, and the system schematic is depicted in Figure 3.1. A supercontinuum light source (SC-5; Yangtze Soton Laser Co., Ltd, Wuhan, China) with a broadband spectrum covering a wavelength range of 480 nm - 2200 nm and a repetition rate of 5 MHz was employed as the light source. To make it suitable for our application, a short pass dichroic filter (DMSP1000; Thorlabs Inc., Newton, New Jersey, USA) with a transmission band of 520 nm - 985 nm

was placed after the laser output. The reason to choose this wavelength range is that it has a central wavelength of about 850 nm and a wide bandwidth which can provide a high axial resolution. The laser power after the filter was measured to be 19.8 mW, and its spectrum received by the CCD camera is shown in Figure 3.1(B) thanks to the spectral responses of both fiber coupler and diffraction grating. Such a spectrum covers all the 2048 pixels of the CCD camera.

FIGURE 3.1: Schematic of the constructed μ OCT system. (a) Diagram of the desktop μ OCT system. (b) Laser output spectrum detected by the line-scan CCD array. DF: dichroic filter; SMF: single mode fiber; PC: polarization controller; L1 - L5: achromatic lens; W: ultraviolet (UV) fused silica window; M1: reference mirror; L6: camera lens; CCD: charge-coupled device; IMAQ: image acquisition.

The laser output was split into two paths by a broadband 75:25 fiber coupler (TW850R3A2; Thorlabs Inc.). The beam output from the 75% port was directed to the sample arm optics, which consists of a collimation lens L3 (AC050-015-B-ML; Thorlabs Inc.), 2-D galvo scanners (GVSM002/M; Thorlabs Inc.) as well as an objective lens L4 (M Plan Apo NIR 20 $\times$; Mitutoyo Inc., Kawasaki, Japan). The power illuminating on the sample was measured to be 10 mW in this study. The reference arm optics followed the same length as the sample arm, and it consists of a collimation lens L1 (identical to lens L3), a ultraviolet (UV) fused silica window (#49643; Edmund Optics Inc., Barrington, New Jersey, USA) that was used for dispersion compensation purpose, as well as a focal lens L2 (identical to lens L4). The dispersion compensation method proposed in [86] was adopted in this paper.

The light backreflected from the reference arm and that backscattered from the sample arm were recombined by the fiber coupler to generate interferometric signals. Such signals were finally collected by a spectrometer, which was comprised of a collimation lens L5 (AC127-030-B-ML; Thorlabs Inc.), a diffraction grating (1200 lines/mm at 830 nm; Wasatch Photonics Inc., Logan, Utah, USA), a camera

lens (Nikon AF Nikkor 85 mm f/1.8D; Tokyo, Japan), and a line-scan CCD camera (E2V, AViiVA EM4-EV71YEM4CL2014-BA9). The detected signals were transferred to a computer through camera link cables and an image acquisition card (KBN-PCECL4-F; Bitflow Inc., Woburn, Massachusetts, USA) at 12-bit digital resolution to achieve a balance between the system scanning speed and imaging performance. In the experiments, both the camera and the galvo scanners were synchronized by a triggering signal generated by the computer.

3.2.2 System Performance

The magnification power of the sample arm optics was ($1.5\times$), and thereby, the system transverse resolution was predicted to be $\sim 1.96\text{ }\mu\text{m}$, while with the broadband light source employed, the theoretical axial resolution was calculated to be $\Delta z = 1.73\text{ }\mu\text{m}$ in air. The nominal DOF is around $90\text{ }\mu\text{m}$ according to our Zemax simulation.

We verified the system transverse resolving power by imaging a 1951 USAF resolution chart with a scanning speed of 20, 480 A-lines/second. Figure 3.2(A) depicts an *en face* image of the resolution chart, which consists of 512×512 pixels, covering an imaging area of $0.26\text{ mm} \times 0.26\text{ mm}$. As can be seen, the line pattern of group 7 element 6 with a line spacing of $2.19\text{ }\mu\text{m}$ were clearly resolved, demonstrating that the system's transverse resolution should be finer than $2.19\text{ }\mu\text{m}$. Such measured value matches well with our predicted value of $\sim 1.96\text{ }\mu\text{m}$. To characterize the system's axial resolution and sensitivity, we placed an actuated iris diaphragm (SM05D5; Thorlabs Inc.) with total attenuation of 30.3 dB into the sample arm optics, and acquired 512 A-line profiles by placing a BK7 glass surface (with an attenuation of 14 dB) at the focal plane. To measure the axial point spread function (PSF), during the post-processing, we plotted an axial profile at a fixed transverse position from the imaging data. The measured axial PSF was shown in Figure 3.2(B), showing an axial resolution of $\sim 2\text{ }\mu\text{m}$ in air. Since the signal-to-noise ratio (SNR) was measured to be 59.1 dB at a length difference of $\sim 80\text{ }\mu\text{m}$, the system sensitivity was estimated to be $\sim 103.4\text{ dB}$ at a path length difference of $\sim 80\text{ }\mu\text{m}$, and it was slightly lower than the theoretical value of 105.6 dB.

FIGURE 3.2: Spatial resolution characterization of the OCT system. (a) *En face* image ($0.26 \text{ mm} \times 0.26 \text{ mm}$) of the 1951 USAF resolution chart. (b) Measured axial PSFs versus path length differences between the sample and reference arm optics of the constructed μ OCT imaging system.

3.2.3 Study Design and Imaging Protocol

In this study, both *ex vivo* and *in vivo* imaging on rats were conducted. The aim of this *in vivo* imaging is to provide the contrast between cytoplasm and cell nuclei when the imaging animals are alive. Although many previous studies [43–45] have provided the imaging results of colon tissue *ex vivo*, the tissue was not supplied with blood and maintained in physiological conditions (such as temperature, humidity, oxygen and nutrition), so that the mucosal structure and function might have been modified considering there are rich enzymes in the colon lumen. In this study, this *in vivo* imaging experiment can tell us what OCT can capture in the living animals with heartbeat and breath. This is important because it provides justifications for continued efforts on the development of endoscopic OCT system for human use.

Imaging was conducted on freshly explanted rat colon samples. The study protocol was approved by institutional animal care and use committee (IACUC) at Nanyang Technological University [ARF-SBS/NIE-A0312]. Three rats were utilized in this study. Specifically, to avoid artifacts caused by μ OCT setup or histology procedure, each experiment was repeated at least three times with the same μ OCT setup.

For *in vivo* imaging, the rats were anaesthetized using Ketamine (80 mg/kg bodyweight) and Xylazine (8 mg/kg bodyweight) in the study. After confirmation of anesthesia, surgical procedure was conducted on the rats. A 2 ~ 3 cm longitudinal

incision was performed along the intestinal tract to fully expose the internal wall of colon (lumen side). Subsequently, the anaesthetized rats were mounted supine on the scanning stage for *in vivo* colon imaging. Imaging was performed within 2 ~ 3 minutes after the surgical procedure, and each image acquisition procedure took less than 2 minutes for a rat.

To obtain tissue samples for *ex vivo* study, we sacrificed those three rats after the completion of *in vivo* experiments and harvested the colon tissues right after cessation of vital signs. When harvesting those tissues, we marked the regions of interest using tissue marking dye. All those marked regions were excised and then fixed using formalin for histological analysis. Those fixed sections were cut in both cross-sectional and *en face* directions relative to the luminal surface to allow comparison with μ OCT images.

3.3 Results

3.3.1 3-D Visualization of Colon Mucosa

Freshly excised specimens were utilized to conduct μ OCT imaging *ex vivo* within 5 mins after being excised. Each specimen was cut open longitudinally and flushed by phosphate buffered saline (PBS) solution three times to remove the food debris and mucus. In the imaging process, a small amount of PBS solution was added onto the tissue surface to provide index matching. *Ex vivo* images were acquired from the luminal side at 20 frames/second with an acquisition rate of 20, 480 A-lines/second.

Figure 3.3(A) presents a cross-sectional image of colon mucosa *ex vivo*. It consists of 1024×566 pixels covering an imaging area of $872 \mu\text{m} \times 435 \mu\text{m}$. As can be seen, the detailed cellular-level microstructures, such as the crypt lumens and the individual goblet cell, were clearly resolved. For the crypt lumens, it could be observed that they have very good contrast relative to the epithelium; specifically, with an increasing imaging depth, the crypt lumen structures become less clear, which is a bit different as compared to those in the histology image. Such difference mainly comes from the tissue scattering, as well as the system aberration effects. While for the goblet cells distributed around the crypt lumens, their structures

could also be clearly resolved, and they appeared to be empty with the translucent mucin contained. Figure 3.3(B) shows a representative cross-sectional histology of the rat colon tissue. Both crypt lumen and goblet cell structures could be appreciated by μ OCT with a good correspondence to the histology, yet it shows relatively low image penetration.

FIGURE 3.3: Cross-sectional image of the normal rat colon *ex vivo*. (a) Representative cross-sectional μ OCT image ($0.872 \text{ mm} \times 0.435 \text{ mm}$). (b) Histology, hematoxylin and eosin (H & E): $100\times$. The structures of crypt lumen (red arrows), the individual goblet cell (yellow arrows), and the lamina propria (green arrows) were clearly identified.

The detailed structures of both crypt lumens and individual goblet cell could be further identified within 3-D reconstructed *en face* images. Figures 3.4(A) and (B) present *en face* images at imaging depth of $80 \mu\text{m}$ and $120 \mu\text{m}$, respectively. Both images were acquired with a pixel sampling of 1024×1024 pixels, covering an imaging area of $872 \mu\text{m} \times 872 \mu\text{m}$. As can be seen from both images, the tissue structures, e.g., crypt lumens, goblet cells as well as the lamina propria that are around the crypt lumens, could be clearly identified. When comparing Figures 3.4(A) and (B), it is also worth noting that as the imaging depth increases, the average size of the crypt lumen diameters, indicated by the black dots marked by the red arrows, becomes smaller in the images. Figure 3.4(C) shows a representative *en face* histology image of the colon tissues.

Figure 3.4(D) shows a representative 3-D view of an individual crypt lumen together with its surrounding mucin-containing goblet cells. Results show that the crypt lumen appears to be tapered, and its diameter decreases with an increasing imaging depth. Figure 3.4(E) presents a representative cross-sectional histology of a single crypt lumen together with the goblet cells and lamina propria around. Both *en face* and 3-D images correspond well to the histology images.

FIGURE 3.4: *En face* image of the normal rat colon *ex vivo*. (a) Representative *en face* μ OCT image at an imaging depth of 80 μ m. (b) Representative *en face* μ OCT image at an imaging depth of 120 μ m. Both (a) and (b) consist of 1024×1024 pixels, covering an area of $0.872 \text{ mm} \times 0.872 \text{ mm}$. The structures of crypt lumen (red arrows), the individual goblet cell (yellow arrows), and the lamina propria (green arrows) could be identified. Insets in (a) and (b) present enlarged *en face* image of a single crypt lumen at the respective imaging depth. (c) Representative *en face* histology image with hematoxylin and eosin (H & E): 100 $\times$. (d) Three-dimensional (3-D) image of the low-scattering crypt lumen (CL, red arrows) and its surrounding goblet cells (GC, yellow arrows). (e) Cross-sectional histology image of a single crypt lumen (CL, red arrows), together with its surrounding goblet cells (GC, yellow arrows) and lamina propria (green arrows).

3.3.2 *In Vivo* Imaging in Rat Colon

To test if the above-mentioned cellular-level microstructures can also be visualized *in vivo*, we conducted imaging in colon tissues of the anesthetized rats *in vivo*. The colon was opened longitudinally and flushed with PBS solution three times. Cross-sectional images were acquired for 5-10 seconds from the luminal side. The system data acquisition speed was set to be 61, 440 A-lines/second, i.e., 60 frames per second. Each cross-sectional image consists of 1024×767 pixels, covering an imaging area of $872 \mu\text{m}$ (W) $\times$ $610 \mu\text{m}$ (D).

Figure 3.5 presents a representative cross-sectional image of colon tissue *in vivo*. As can be seen, the crypt lumen delineation along the axial direction in the tissue could be clearly identified, and the goblet cells that were around the crypt lumens could also be identified as low scattering structures. The appearance of those goblet cells was because of the transparent mucins contained in the cells.

FIGURE 3.5: Cross-sectional image of the normal rat colon *in vivo*. It consists of 1024×767 pixels, covering an area of 0.872 mm (W) $\times$ 0.61 mm (D). Both the structures of crypt lumen (red arrows) and the individual goblet cell (yellow arrows) could be clearly identified.

Different from Figure 3.3, results in Figure 3.5 were obtained on living rats with blood supply, heartbeat and breath *in vivo*. The motion from heartbeat and breath brought noticeable motion artifacts, which may affect the image quality. In this

study, with the imaging speed improved to 61, 440 A-lines/second, the effect of motion artifacts was alleviated so that cellular structures such as goblets cells were visualized clearly. Although the motion artifacts were suppressed, they still had an effect on the image quality to some extent. Therefore, Figure 3.5 is not as good as that obtained from *ex vivo* imaging without motion issue, heartbeat and breath shown in Figure 3.3.

3.4 Discussion

In this study, we evaluated μ OCT for imaging of cellular-level structures in rat colon both *ex vivo* and *in vivo*. The system achieves a resolution of 2.0 μm in both transverse and axial directions, and its imaging rate reaches up to 60 frames/second for *in vivo* imaging. Both cross-sectional and 3-D images of the colon mucosa were acquired and compared to the histology. Imaging results showed that detailed tissue microstructures could be clearly identified and comparable to those in the histology. Specifically, the cross-sectional images give a clear view of the layered morphological microstructures, which consequently helps facilitate early disease diagnoses, while the high scanning speed helps capture images fast and herein reduces the influences of motion artifacts to image qualities. The main purpose of this study was to assess the image quality that could be expected from endoscopic imaging, as well as to lay a preliminary foundation for our future *in vivo* endoscopic studies.

For the OCT scanning speed, one main factor to be considered is whether the speed is fast enough to suppress the effect of motion on the image quality. During the *in vivo* imaging in this study, the imaging speed was 61, 440 A-lines/second (60 frames/second), and the rats were anesthetized and they were still alive with blood supply and breath. The imaging results in Figure 3.5 demonstrate that this imaging speed up to 61, 440 A-lines/second can help reduce the influences of motion artifacts caused by heartbeat and breath on image quality.

A major factor, however, currently limiting the application of μ OCT for *in vivo* imaging was the lack of flexible fiber-optic probe, which will be studied in the next stage. Specifically, to achieve a high transverse resolution down to 2-3 μm , the short DOF will be a key factor limiting the system performance, and makes the

tissue's areas of interest cannot be fully covered. To alleviate such an issue for *in vivo* endoscopic imaging, a candidate choice would be the tethered capsule probe design [48], as it touches the tissue surface closely when traveling through the GI tracts, which thereby helps extend the effective depth range. Furthermore, obtaining higher imaging depth while maintaining high resolutions is another important topic required to be resolved for endoscopic studies in the future. Another issue was the limited system scanning speed and software processing speed to obtain 3-D volumetric tissue images. The limited scanning speed makes *in vivo* imaging prone to motion artifacts. Future development of μ OCT for *in vivo* study will be improving the data acquisition and image processing speed.

3.5 Summary

In conclusion, we evaluated the feasibility of detecting crypts and goblet cells in rat colon using μ OCT. This is also the first demonstration of utilizing spectral domain OCT (SDOCT) as a tool for cellular-level microstructures imaging in intestinal mucosa *ex vivo* and *in vivo*. *Ex vivo* imaging results showed that μ OCT is able to clearly visualize these important anatomic structures, which are confirmed by the corresponding histology images. We also showed that such cellular-level information can be captured *in vivo* in real-time. This study proves that μ OCT system could be a powerful tool to perform “optical biopsy” in intestinal tracts, which thus justifies future development of transendoscopic μ OCT probes for cellular-resolution imaging.

Chapter 4

Endoscopic μ OCT Fiber Probe for Cellular-resolution Imaging of GI Tracts

This chapter aims to design and fabricate a flexible and miniature fiber-optic probe in an endoscopic μ OCT system and demonstrate its capability to obtain cellular-level details of GI tissues through circumferential scanning. To balance the dispersion, we employed a common-path design for the distal end optics. Unlike existing common-path designs in which an amplitude beamsplitter is used, we designed an apodizing wavefront splitter to create an annular pupil function for the sample arm and saved the center beam for the reference. To mitigate the trade-off between the high transverse resolution and relatively short DOF, the annular focusing moderately extends the DOF without the sacrifice of transverse resolution.

Section 4.1 describes an optical design for the proposed endoscopic probe. It starts with the discussion about the effect of apodization design on the DOF extension as well as the combining effect with chromatic focal shift, and then is followed by the specific probe design including the apodization and common-path design. Section 4.2 provides the experimental methods including probe fabrication, construction of endoscopic μ OCT system, system performance testing and animal imaging verification with the swine model. Section 4.3 shows the results of system performance as well as the *ex vivo* imaging in swine colon. A brief discussion is presented in Section 4.4 and a summary on this study is given in Section 4.5.

4.1 Optical Design

4.1.1 Working Principle of Annular Apodization for DOF Extension

Annular apodization is also known as center obscuration. The idea is to block the center part of the optical beam to create an annular illumination and/or detection. It is well known that an annular pupil function can create a Bessel like focus, which is characterized by extended length of the main lobe along the axial/depth direction and presence of sidelobe artifacts. To evaluate the effect of the apodization design on DOF extension, we firstly conducted a simulation employing a representative apodization design used in the previously reported desktop μ OCT system by using Zemax software (Zemax LLC, Kirkland, Washington, USA)(Figure 4.1). It is composed of a fiber collimator lens (AC050-015-B; Thorlabs Inc.), a 45 degrees rod mirror (#54-092; Edmund Optics Inc.) to redirect the central beam out and a focusing lens (AC080-016-B; Thorlabs Inc.). The focusing numeric aperture (NA) is 0.13 (1% power level).

FIGURE 4.1: Representative schematic of apodization design by focusing optics.

There are a few mechanisms that extend the DOF in the optics. In addition to the apodization, chromatic focal shift generally focuses light of different wavelength to different axial focal point, which essentially extends the DOF. In the simulation, the wavelength is in the range of 650 - 950 nm according to existing supercontinuum light source. Spherical aberrations increase as NA increases even in the aspheric doublets. The spherical aberrations of the miniaturized optics at the distal end of the probe are much larger than that of the bulky aspheric doublets. Since the axial focus degrades much faster with spherical aberrations than the transverse focus, the DOF can be effectively extended at the price of sidelobe artifacts.

By use of scalar diffraction equation of circular aperture [99], we can obtain a PSF of monochromatic light without any optical aberration (Figure 4.2(A)). The PSF demonstrates mirror symmetry and pronounced axial sidelobes due to the cropping of the Gaussian beam by the lens aperture. In Figure 4.2(B), spherical aberration of the lens and the wavelength dependent focal shift are added in the Zemax model, so that the PSF is shifted back to lens and is non-symmetric. In Figure 4.2(C), the light source is changed to broadband source, so the sidelobes are smoothed by the chromatic aberration. In Figure 4.2(D), combined with central obscuration (apodization), the DOF is extended and the central lobe transverse profile becomes narrower and the first sidelobe is pronounced.

FIGURE 4.2: Simulated point spread function (PSF) of the desktop spectral domain OCT (SDOCT) in different focusing conditions: (A) monochromatic light source ($\lambda = 810$ nm), spherical aberration free; (B) monochromatic light source ($\lambda = 810$ nm) with spherical aberration and wavelength dependent focal shift; (C) broadband source (650 nm - 950 nm) with spherical aberration and chromatic focal shift; (D) broadband source (650 nm - 950 nm) with spherical aberration, chromatic focal shift and apodization. All figures are in log scale.

Moreover, Figure 4.3 provides a further confirmation that the combination of aberration factors, including spherical aberration, chromatic focal shift and apodization, is capable to increase the DOF.

FIGURE 4.3: Axial intensity profiles under four conditions. (1) monochromatic light source ($\lambda = 810$ nm) and spherical aberration free (red); (2) monochromatic light source ($\lambda = 810$ nm) with spherical aberration and wavelength dependent focal shift (black); (3) broadband source (650 nm - 950 nm) with spherical aberration and chromatic focal shift (green); (4) broadband source (650 nm - 950 nm) with spherical aberration, chromatic focal shift and apodization (blue).

From the simulation, we can conclude that the DOF could be extended by $\sim 30\%$ with the combination of spherical aberration, chromatic focal shift and apodization.

4.1.2 Probe Design

4.1.2.1 Overview of the Probe

The endoscopic μ OCT system consists of three parts: the imaging console, the rotary joint and the fiber-optic probe (Figure 4.4). Firstly, we designed a probe with the configuration indicated in Figure 4.5, which transmits the light from the imaging console to the sample and then returns the backscattered light from the sample back to the console.

FIGURE 4.4: Schematic diagram of the endoscopic μ OCT system with common-path probe. SCL: Supercontinuum light source; FC: fiber coupler; L: achromatic lens; G: grating; CCD: charge-coupled device; PC: personal computer.

FIGURE 4.5: Schematic diagram of the common-path μ OCT probe. 1: drive-shaft; 2: single mode fiber; 3: ferrule; 4: glass spacer; 5: GRIN lens; 6: beam-splitter; 7: reference reflector; 8: outer sheath tube.

This probe is composed of a ferrule with a single mode fiber (SMF), a glass spacer, a gradient-index (GRIN) lens, a beamsplitter and a reference reflector. The ferrule is applied to protect the SMF to deliver the beam to the probe optics. The glass spacer is utilized to expand the input beam, and the GRIN lens is used to focus the beam. Through the beamsplitter, the beam is split into two paths: the central part as the reference arm and the annular part as the sample arm. In the reference arm, the beam is reflected by the end surface of the reference reflector, while in the sample arm, the beam is backscattered by the sample. The backreflected reference beam and backscattered sample beam were then recombined by the beamsplitter into the SMF along the same path. Besides, a driveshaft is connected to this probe

to transmit the motion from the rotary joint to the probe. An outer sheath is used to cover the probe to maintain the imaging lumen of the sample and protect the inner probe.

The core of this probe is the apodizing wavefront splitter similar to that reported in [25, 73], which is simple to design and ease for fabrication. Owing to a broadband light source applied for the endoscopic μ OCT, the combined effect of chromatic focal shift and apodization extends the DOF moderately.

Furthermore, the wavefront splitter also realizes a common-path interferometer. With the common-path design, the reference and sample arms almost share the same optics, and herein the material dispersion could be minimized. Besides, the common-path design also avoids the problem of polarization mismatch between reference and sample arms during circumferential scanning, consequently improving its feasibility for endoscopic imaging *in vivo*.

With regard to the outer sheath, its inner diameter (ID) and the optical probe (glass spacer and GRIN lens) forms a clearance fit so that the optical probe can rotate freely and co-axially with the sheath. The outer diameter (OD) of the sheath is designed to fully fill the luminal area so that the epithelium of the tissue is positioned within the optimal imaging range (Figure 4.5, section A-A).

4.1.2.2 Design Requirements

In this study, we chose swine tissues as the animal model to explore since the swine GI tracts resemble the human GI tracts in the structure and size. The sizes of crypt lumens and goblet cells in transverse direction are around 10 - 25 μm and 20 - 40 μm , respectively. According to the Nyquist-Shannon sampling theorem, the transverse resolution should be less than 5 μm to clearly resolve crypt lumens and goblet cells. For future *in vivo* imaging, the probe together with the outer sheath tube surrounding the probe will be placed through the colonoscopy channel of which the ID is around 2.8 - 3.2 mm. Upon this situation, one main issue is that both the sheath and the outer sheath tube should be passed through the colonoscopy channel. Therefore, the maximum OD of both the sheath and the outer sheath tube should be 2.8 mm. In this study, we chose the outer sheath with an OD of 2.8 mm. Besides, to ensure tissue's areas of interest surrounding the sheath closely to alleviate the current limited DOF, the focus area should be

slightly away from the outer sheath. As a result, the working distance, defined as the distance between the center line of the probe and the focused spot on the sample, should be slightly longer than the outer radius of outer sheath, e.g., in the range of 1.5 - 1.6 mm.

4.1.2.3 Zemax Simulation

Simulations for the sample arm were conducted by using optical software Zemax to determine specifications of the components in a try-and-error fashion. As a design input, the light source was simulated as a Gaussian beam with the NA of 0.13 and the center wavelength of 810 nm with the range from 650 nm 950 nm based on the existing light source.

To achieve the desired transverse resolution and the working distance, we adjusted the length of the spacer. In the optimized design, the refractive index is 1.517 (BK7) and the length is set to be 1.63 ± 0.07 mm, so that the beam is expanded sufficiently to acquire a high transverse resolution.

Based on the optimized spacer design mentioned above, the desired working distance can be achieved by selecting suitable GRIN lens including the length and NA. Meanwhile, the chromatic focal shift can be tailored by adjusting the length of the GRIN lens: the longer the GRIN lens, the larger the chromatic focal shift. At the same time, the NA of GRIN lens needs to meet the requirement of continuum resolution and image intensity along the chromatic focal shift. Taking all above-mentioned parameters into consideration, the NA is chosen as 0.5 and the length is 4.991 ± 0.005 mm to ensure the working distance of 1.5 - 1.6 mm and considerable chromatic focal shift. In this design, the acceptant tolerance of GRIN lens's length is much tight, because the focusing power of GRIN lens is much sensitive to its optical length.

The normalized radius of the beam at the beamsplitting interface was set to be at $1/e^2$ power level. The normalized radius of the central hole of the apodizing wavefront splitter was set to be < 0.5 to control the sidelobe artifacts and signal coupling loss.

Since there exists a small discrepancy between the simulation and final experimental results, several iterations of the probe fabrication and the relative simulation

based on the performance test results need to be conducted to optimize the optics length.

Transverse Resolution

The realistic resolution can be expected by the root-mean-square (RMS) spot radius, which gives a rough idea of the spread of all rays and takes all aberration into account. As shown in Figure 4.6, the RMS radius is $3.27 \mu\text{m}$. Hence, the transverse resolution defined as the FWHM of the focal spot is predicted as $3.27 \mu\text{m}$. This prediction is also further confirmed by the FWHM of transverse PSF at the focal plane as around $3.4 \mu\text{m}$ (Figure 4.7).

FIGURE 4.6: Zemax simulation of the spot diagram at focus.

Depth-of-focus (DOF)

DOF represents the axial distance over which the beam size at the $1/e^2$ beam is not larger than 1.414 times of that at the beam waist. As illustrated in Figure 4.8, compared to the focused spot, the spot size doubles at $\pm 110 \mu\text{m}$ away for the focal plane. Hence, the diffractive limited focal range is $\pm 77.77 \mu\text{m}$ and herein the DOF is predicted to be $155.54 \mu\text{m}$.

Strehl Ratio

FIGURE 4.7: Zemax simulation of the transverse point spread function (PSF) at focus.

FIGURE 4.8: Zemax simulation of the through focus spot diagram.

Strehl ratio, defined as a ratio of the peak diffraction intensity of an aberrated wavefront to that of the ideal wavefront, is frequently used as the maximum acceptance level of wavefront aberration for general observing. Also pointed out in Figure 4.8, the strehl ratio of the beam arriving at the sample is 0.913. Besides, the ratio of the first sidelobe to the main lobe is about 0.0707.

Chromatic Focal Shift

Illustrated in Figure 4.9, the chromatic focal shift was predicted as 97.36 μm with the wavelength range from 650 nm to 950 nm.

FIGURE 4.9: Zemax simulation of the chromatic focal shift.

4.2 Materials and Methodology

4.2.1 Probe Fabrication

In the probe presented in Figure 4.10, a Polyetheretherketone (PEEK) tube (0.50 mm ID and 1.59 mm OD; Beijing Jianxin Technology Co., Beijing, China) was utilized as a driveshaft and cemented to a glass ferrule (Accu-Glass LLC, St. Louis, Missouri, USA). The light transmits through a SMF (780HP; Thorlabs Inc.), which is protected by the ferrule with an ID of 0.126 mm and an OD of 1.8 mm, to a BK7 glass spacer (1.8 mm Dia; GrinTech GmbH, Jena, Germany) with a length of 1.625 mm for expansion. To avoid back-reflection at the fiber-spacer interface, the ferrule together with the SMF and the spacer were angle-polished at 4 degrees. After that, a GRIN lens (1.8 mm Dia; GrinTech GmbH) with a length of 4.991 mm was used to focus the beam. A beamsplitter was cemented to the free end of the GRIN lens to divide the input light into a reference beam and a sample beam.

FIGURE 4.10: Prototype of the common-path probe. (A) 3-D configuration of this fabricated probe. 1: driveshaft; 2: single mode fiber; 3: ferrule; 4: glass spacer; 5: GRIN lens; 6: beamsplitter; 7: reference reflector. (B) Photograph of the fiber-optic probe with the brown Polyether ether ketone (PEEK) tube as the driveshaft. Inset is the annular sample beam.

FIGURE 4.11: Schematic of the gold coating prism. L: Leg. H: hypotenuse. a & b: the major and minor axes of the area without coating, respectively.

The apodizing wavefront beamsplitter is composed of two right angle prisms (Changchun Boxin Photoelectric Co., Changchun, China) with both hypotenuses cemented and a reflective annular metal coating in between (Figure 4.11). The leg of prism is 1 mm and the hypotenuse is 1.414 mm. As for the uncoated area, the major axis (a) is in the range of 0.22 ± 0.02 mm and the minor axis (b) in the range of 0.16 ± 0.02 mm.

After through the beamsplitter, the center circular portion went directly through

the beamsplitter towards the reference reflector with a length of 0.80 ± 0.01 mm (1.0 mm Dia; Prime Bioscience, Singapore, Singapore), while the other annular portion was reflected by 90 degrees to the radial direction toward the sample.

We used ultraviolet cured optical adhesive (NOA 85; Edmund Optics Inc.) to cement all optical components.

4.2.2 μ OCT Imaging Console

The imaging console consists of light source, fiber coupler, spectrometer, data acquisition hardware, and computer system (Figure 4.4). A supercontinuum light source (SC; Superk Extreme OCT; NKT Photonics, Birkerød, Denmark) provides a broadband illumination over a spectral range of 650 nm - 1600 nm. The output was filtered by a short pass dichroic filter (DMSP1000; Thorlabs Inc.) to limit the source bandwidth at 650 nm - 1000 nm. We chose a 2×2 fiber coupler with a splitting ratio of 50:50 (Gould Fiber Optics, Millersville, Maryland, USA) to guide the output of the light source to the fiber-optic probe via a rotary junction. The illumination power delivered to the sample from the probe was measured to be 15.6 mW.

The interference signals returning from the probe were collected by a spectrometer: the returning signals were collimated by a collimation lens L (AC127-030-B-ML; Thorlabs Inc.), dispersed by a diffraction grating G (960 lines/mm at 840 nm; Wasatch Photonics Inc.), and then focused by a camera lens (Nikon AF Nikkor 85 mm f/1.8D) onto a line-scan CCD camera (E2V, AViiVA EM4-EV71YEM4CL2014-BA9). The detected spectrograms were finally transferred from the camera to a computer through camera link cables and an image acquisition card (KBN-PCECL4-F; Bitflow Inc.) at 12-bit resolution. During the experiments, the camera was synchronized by a triggering signal of 20k Hz generated by the computer, thereby, the system line-scanning rate was 20k lines/second.

4.2.3 Outer Sheath

Since the diameter of both glass spacer and GRIN lens is 1.8 mm, a transparent glass tube with 1.818 mm ID (2.8 mm OD; Thorlabs Inc.) was used in place of the

commonly used polymer sheath to maintain a circular lumen and center the distal end optics with regard to the tissue lumen.

The glass tube was immobilized by a thin heat shrink tube which was connected to the metal housing of the probe. During the circumferential scanning, the outer sheath was immobilized to translation stages (HFF001, APY001/M and MBT616D; Thorlabs Inc.) while the inner probe was rotated.

4.2.4 Rotary Joint

Illustrated in Figure 4.12, the rotary joint comprises of a motorized rotation stage (URB100CC; Newport, Irvine, California, USA), a fiber rotary joint (Princetel Inc., Hamilton Township, New Jersey, USA), and a customized timing belt pulley to transmit the rotation motion from the motorized stage to the probe.

FIGURE 4.12: Photograph of the fiber-optic probe connected to a rotary joint. FRJ: fiber rotary joint; MRS: motorized rotation stage; TBP: timing belt pulley. TS: translation stages.

For *in vivo* application, only the distal end of the fiber probe will be inserted into the internal organs. The rotary stage is used to rotate the probe at the proximal end of the fiber probe so it will be outside the body. The translation stages displayed in Figure 4.12 are applied to support the tissues during current *ex vivo* experiment, and they will be not utilized during the *in vivo* experiment since there is no need to use additional setup to support the imaging luminal tissues at that time. Thus, the effect of translation stages can be omitted in future *in vivo* application.

4.2.5 System Characterization

The axial resolution of the system was measured by detecting the FWHM of the axial PSF with a glass reflector as the sample. We used a tilted glass reflector (with an attenuation of 33.7 dB) in the focal plane of the sample arm to characterize the axial resolution and sensitivity.

To testify the transverse resolution, we used a laser beam profiler (LBP2-HR-VIS2; Newport) together with an objective (50 $\times$ DRY Plan Fluorite Objective; Nikon) to capture the in-focus sample beam. With respect of the DOF, we also utilized this laser beam profiler to measure. To further confirm the transverse resolution and DOF measured by above-mentioned method, we conducted imaging experiments to measure the FWHM of the transverse PSF with a home-made microparticle phantom as the sample consisting of the micro-beads (the standard size of 2 μm ; Polyscience Inc., Warrington, Pennsylvania, USA) mixed with the agarose solution (No. PC0701-100g; Vivantis Inc., Oceanside, California, USA).

4.2.6 Imaging Protocol and Swine Tissue Imaging *Ex Vivo*

To validate the capability of endoscopic imaging, *ex vivo* imaging experiments with this fiber-optic endoscopic μOCT system were conducted on freshly explanted swine colon tissues. We collected the swine colon tissues for endoscopic μOCT imaging from a slaughterhouse in Singapore. We opened up the tissue lumen and dissected the tissues into rectangular shape of 7.0 mm by 10.0 mm before attaching each tissue to the outer surface of the sheath. During circumferential scanning, the imaging rate was 1 revolution/second which was limited by the speed of the motorized rotation stage. When finishing the experiments, we marked the regions of interest using tissue marking dye and then fixed the tissues using 4% neutral buffered formaldehyde for histological analysis in comparison with μOCT images.

During imaging processing, we performed 2-frame averaging (time-lapse averaging) and software-based speckle reduction [136, 137] to reduce the speckle of the images and enhance the cellular-level structural contrast.

4.3 Results

4.3.1 Performance Test

4.3.1.1 Axial Resolution and Sensitivity

The result in Figure 4.13(A) indicates that the axial resolution was $2.48 \mu\text{m}$ in air, and the corresponding resolution in tissue was $1.81 \mu\text{m}$ (assuming the refractive index $n=1.37$). The measured SNR of the -33.7 dB reflector was 65.1 dB (Figure 4.13(B)), so that the sensitivity of this system was tested to be 98.8 dB .

FIGURE 4.13: Characterization of axial resolution and sensitivity of the endoscopic μ OCT system. (A) Measured axial profile of the constructed endoscopic μ OCT system. (B) Measured axial profile acquired with a glass reflector (red curve) vs. measured noise floor with reference light only (blue curve). The noise floor was averaged over 256 A-lines. The signal power was plotted in a log scale.

4.3.1.2 Transverse Resolution and DOF

Illustrated in the Figure 4.14(A), the spot size of the beam at the focal plane (Depth = $215 \mu\text{m}$) was measured to be $4.8 \mu\text{m}$ by the laser beam profiler, which is also confirmed by the transverse profile of a microparticle (Figure 4.14(B)). The irregular intensity distribution of the measured PSFs in Figures 4.14(A) was caused by fabrication defects in the annular metal coating of the beamsplitter. Similarly, a small discrepancy of the transverse resolution between the predicted and measured values might be also resulted from the above-mentioned fabrication defects. Besides, the DOF of the fabricated probe was measured to be $\sim 150 \mu\text{m}$, which is almost consistent with the simulation results.

FIGURE 4.14: Characterization of transverse resolution and depth-of-focus (DOF) of the endoscopic μOCT system. (A) 2-D images of the beam profile at three different axial depths. Transverse point spread function (PSF) and lateral PSF were displayed in bottom and left sides, respectively. Intensity is scaled in color bar. (B) μOCT image of the microparticle phantom (upper left) and measured transverse PSF at three different axial depths obtained from the μOCT image.

4.3.1.3 Focal Distance

The path length difference between the reference and the sample focus was maintained to be 215.3 μm . The working distance, which is between the sample focus

and the center of the probe, was about 1.516 mm so that the focus was 116 μ m away from the outer surface of the sheath with a radius of 1.4 mm.

4.3.1.4 Power Efficiency of the Sample Light

Power efficiency of the sample light is calculated as the ratio of the optical power between the probe sample beam and the probe input beam. During the experiments, I fabricated 5 probes in total. For each probe, I measured the optical power in the positions illustrated in Figure 4.15. The testing results and calculated power efficiency have been summarized in Table 4.1.

FIGURE 4.15: Illustration of positions of the three points for power measurement during the probe fabrication.

TABLE 4.1: Measurement of μ OCT probe power.

Probe input power: 35.3 mW	Probe 1	Probe 2	Probe 3	Probe 4	Probe 5
Beam before splitting (mW)	24.5	23.8	24.7	25.2	25.6
Sample beam after splitting (mW)	15.6	15.2	16.4	15.5	16.7
Power efficiency of sample light	44.2%	43.1%	46.2%	43.9%	47.3%

We can conclude that the average of more than 44.9% optical power was delivered to the sample. About 36.9% power was directed to the reference beam and the reminding 18.2% power was lost among the coupling between the fiber and glass spacer and absorbing by the gold coating between the prisms.

4.3.2 *Ex Vivo* Imaging in Swine Colon

In representative cross-sectional images of swine colon mucosa (Figure 4.16 and 4.17), in addition to the layered structures of the mucosa, the crypt profiles including crypt lumens (white arrows, Figure 4.16(B-D) and 4.17(B-D)) can also

be clearly visualized. Owing to the improved spatial resolution, we are able to pick up cellular-level microstructures, such as the mucus-laden goblet cells (yellow arrow heads, Figure 4.16(B-D) and 4.17(B-D)), which are consistent with the previously published results using a desktop μ OCT system [138]. More importantly, when comparing the endoscopic μ OCT images with corresponding histology image (Figure 4.16(E)), a good correlation between μ OCT images and the histology can be clearly observed, convincingly confirming the above-mentioned findings.

In Figures 4.16(A) and 4.17(A), the central white part refers to the space occupied by the probe (so that there was no image), while the annular images show the cross-sectional views of swine colon wall. During the imaging, the data set was saved as the two-dimensional array in the Cartesian coordinates with the depth direction corresponded to the column direction and the tangent direction corresponded to the row direction. During the post-processing, I converted the data from Cartesian coordinates to the polar ones as shown in Figures 4.16(A) and 4.17(A).

4.4 Discussion

The improved spatial resolution achieved by the proposed endoscope enables visualization of cellular-level structures in GI tracts, which has never been reported to the best of our knowledge. The results underscore the critical role of high transverse resolution in visualizing cellular-level details in comparison with those obtained with lower transverse resolution systems [59]. According to the previous results obtained using a desktop μ OCT system [138], the ideal transverse resolution is $\sim 2 \mu\text{m}$ or below. The current transverse resolution of $4.8 \mu\text{m}$ is still not enough for resolving more cellular-level details, such as columnar epithelial cells.

The common-path probe with apodization design adopted can extend the DOF moderately while maintaining the high transverse resolution [25, 73]. Further improvement in transverse resolution requires DOF extension techniques such as phase modulation for producing quasi-Bessel beams through a cylindrical waveguide or a phase mask [75, 139]. Our further study will focus on endoscopic μ OCT probe with higher extended DOF without the compromise of high resolution for clinical application.

FIGURE 4.16: Cross-sectional images of a normal swine colon *ex vivo* by the endoscopic μ OCT system. (A) Representative cross-sectional μ OCT image during circumferential scanning: it consists of 900 pixels (axial, 893.7 μm) $\times$ 6200 pixels (circumferential, minimum circle: 8375.5 μm). Red arrow indicates the conjugated profile of the inner surface of the transparent sheath covering the probe. The cellular structures such as crypt lumens and goblet cells can be obviously captured in the indicated areas by red boxes. (B-D) Respective zoomed-in views of three red boxes in (A) showing crypt lumens (white arrows) and goblet cells (yellow arrow heads). Image Size: 475.5 μm (circumferential, minimum circle) $\times$ 484.6 μm (axial). (E) Representative histology, hematoxylin and eosin (H & E) staining. Black arrows indicate crypt lumens, and the yellow arrow heads indicate goblet cells. Scale bar: 100 μm .

FIGURE 4.17: Cross-sectional images of a normal swine colon *ex vivo* by the endoscopic μ OCT system. (A) Representative cross-sectional OCT image during circumferential scanning: it consists of 900 pixels (axial, 893.7 μm) $\times$ 6200 pixels (circumferential, minimum circle: 8375.5 μm). Red arrow indicates the conjugated profile of the inner surface of the transparent sheath covering the probe. The cellular structures such as crypt lumens and goblet cells can be obviously captured in the indicated areas by red boxes. (B-D) Respective zoomed-in views of three red boxes in (A) showing crypt lumens (white arrows) and goblet cells (yellow arrow heads). Image Size: 475.5 μm (circumferential, minimum circle) $\times$ 484.6 μm (axial). Scale bar: 100 μm .

We employed a rigid glass tube as the outer sheath to mitigate the issue of limited DOF so that crypts structures were properly maintained around the relatively small focal region. However, this glass tube may not be a viable option for clinical applications. Moving forward, this glass tube can be replaced by a polymer capsule, similar to the previously reported tethered capsule design [48]. The OD of the capsule can be tailored according to the GI tract to be imaged. For smaller lumens such as pancreatic and bile ducts, a diameter of 1.5 - 2 mm might be suitable.

During the experiments, the inner surface of the transparent outer sheath would present strong back-reflection when the sample light goes through the air-glass

interface. Therefore, to alleviate its influences, we applied water to fill the space (air) between the beamsplitter and the inner surface of the outer sheath. Even though the back-reflection of the inner surface of the outer sheath was mitigated by utilizing the water to filling the air gap between the beamsplitter and the inner surface, it is still highly stronger than the reflection by the scattered tissues (swine colon in this study). Hence, the conjugated profile of the inner surface of the transparent tube is still clearly displaced in Figure 4.16(A) and 4.17(A). Our next-step work is to remove or reduce it to a sustainable level to minimize influences on the imaging results, and a possible solution could be to slightly adjust the angle between the sample beam and the inner surface of the tube.

We also found that the contrast of cellular-level details is very sensitive to speckle noise as shown in Figure 4.18. Of course, inter-frame averaging will be very difficult to realize simply due to the motion artifacts caused by the sample and probe motion. To solve this issue, multifiber angular compounding OCT [140] may be a candidate choice in the future, since it is able to achieve inter-A-line averaging, which is significantly less sensitive to motion artifacts. Besides, the current rotational speed may not be fast to suppress the motion effect in *in vivo* imaging, so we plan to replace the current motorized rotation stage by a new one with a faster speed (more than 60 circles/second) to alleviate the motion effect.

FIGURE 4.18: Comparison of μ OCT images with and without frame averaging and speckle reduction algorithm. (A-C) μ OCT images with frame averaging and speckle reduction algorithm. (A'-C') Original μ OCT images without frame average and speckle reduction algorithm corresponding to (A-C). Scale bar: 100 μm .

The proposed high resolution of endoscopic μ OCT can capture more subtle morphological features and would be beneficial diagnosing cancers at an early stage, and thus could pave the way for the surveillance, detection and treatment of GI tracts diseases.

4.5 Summary

In summary, we designed and fabricated a flexible endoscopic μ OCT probe with cellular resolution for visualization swine colon *ex vivo*. The cellular-level microstructures such as the intestinal crypts and goblet cells can be clearly resolved, demonstrating that this probe-based μ OCT system is capable of imaging the cellular-level morphology and thus provides the probability of evaluating the pathological lesions of GI cancers at an early stage. Furthermore, the flexibility of this probe renders it traverse through the inner lumen of the tissues for circumferential visualizing. Additionally, the usage of the outer sheath can mitigate the issue of limited DOF so that crypts structures were properly maintained around the relative small focal region. And in the future, a polymer capsule would be employed to replace the outer sheath for clinical applications.

Chapter 5

Imaging Cellular Structures of Coronary Arteries by a Dual-functional Probe-based μ OCT

In this chapter, we explored a dual-functional μ OCT technique by combining the probe-based μ OCT system and spectroscopic OCT for intravascular use. With the high resolution, the probe-based μ OCT can delineate not only the layered structures of arterial wall but also the cellular-level anatomical structures of atherosclerotic plaques including foam cells and smooth muscle cells. Combined with spectroscopic OCT, the collagen fibers could be clearly detected by the improved contrast.

In Section 5.1, we first give a background to visualize the cellular compositions in coronary arteries. Section 5.2 introduces the study methods in details. After that, the *ex vivo* imaging results in normal swine coronary arteries and human atherosclerotic coronary arteries are shown in Section 5.3. Section 5.4 discusses the experimental results and Section 5.5 finally summarizes this study.

5.1 Background

Development and progression of atherosclerotic lesions is mediated by a number of cellular components [6, 9, 10], which are not readily visualized using the current clinical investigation tools. Besides, collagen fibers and elastic fibers play a major role in maintaining the cap stability and diminished collagen fibers may produce a tendency to plaque rupture [12, 13]. Therefore, visualizing these cellular and extracellular components *in situ* and *in vivo* may allow us to redefine and expand the definition of vulnerable plaque, with implications for CAD therapy and for the prevention of AMI.

Spectroscopic OCT, an extension of the traditional OCT, can provide additional true color contrast [141–143] using the same data acquired from conventional OCT without additional hardware cost. Specifically, we found that collagen fibers selectively scatter more light of longer wavelength than that of shorter, which is very different from the spectroscopic behaviors of other structures and thus enables collagen fibers to be directly quantified in color by spectroscopic OCT. Consequently, this geometric specificity opens a new avenue to evaluate collagen fibers with contrast enhancement but without altering hardware of the system.

In this chapter, we employed a dual-functional probe-based μ OCT to acquire the cellular and extracellular compositions by conducting *ex vivo* imaging experiments, and testified its feasibility of intravascular μ OCT imaging. Specifically, color-encoded μ OCT images by spectroscopic OCT enhance the imaging contrast of collagen. It is the first time, to the best of our knowledge, to verify the capability of probe-based μ OCT for visualizing microstructures of coronary arteries at the cellular level and imaging the collagen in structural color.

5.2 Methodology

5.2.1 Probe-based μ OCT

The construction of the probe-based μ OCT system used in this study is shown in Figure 5.1(A), including an imaging console and a common-path fiber-optic probe for beam delivery toward the sample, and the details have been previously reported

in Chapter 4. Briefly, the system applied a broadband light source centered at ~ 810 nm with ~ 250 nm spectral range FWHM by a supercontinuum light source (SC; Superk Extreme OCT; NKT Photonics) together with a following short pass dichroic filter (DMSP1000; Thorlabs Inc.). A 2×2 fiber coupler (Gould Fiber Optics) with a splitting ratio of 50:50 was utilized to direct the light source toward the fiber-optic probe via a rotary joint. The rotary joint consists of a rotation stage (URB100CC; Newport) with a speed of $360^\circ/\text{second}$ and a fiber rotary joint (Princetel Inc.) for the rotation transmission from the motorized stage to the probe.

Depicted as Figure 5.1(B), in the illumination path, after transmitted by a SMF (780HP; Thorlabs Inc.), the beam was then expanded by a BK7 glass spacer with the diameter of 1.8 mm and the length of 1.63 ± 0.07 mm (GrinTech GmbH) and then focused by a GRIN lens (GT-LFRL-180-50; GrinTech GmbH) with the length of 4.991 mm and the NA of 0.5. After that, a beamsplitter, comprised of two right angle prisms (Changchun Boxin Photoelectric Co.) with an apodizing coating in between, was used to divide the beam into a center circular beam and an annular beam. The center circular beam as the reference beam was guided to a glass rod (Prime Bioscience) with a length of 0.80 ± 0.02 mm and a gold coating on its end surface to reflect the beam, while the annular beam as the sample beam was redirected by 90° outside to the imaging sample and then backscattered, similar to the previous studies in [25, 73]. The incident power for the sample was measured as 12.8 mW. In the detection path, the reflected beam and backscattered beam interfered and returned along the same path into the SMF.

In addition, during the imaging, a transparent outer sheath tube (51-2800-1800; Thorlabs Inc.) was served as a barrier to protect imaging tissues from rotating probe. The OD of the sheath of 2.8 mm fully fills the lumen of coronary arteries to ensure areas of interest properly maintained around the relative small focal region.

The interferometric signals returning from the probe were guided into a spectrometer through the fiber coupler. The spectrometer consists of an achromatic lens (AC127-030-B-ML; Thorlabs Inc.) to collimate the beam, a diffractive grating with 960 lines/mm at 840 nm (Wasatch Photonics Inc.) to disperse the beam, a camera lens (Nikon AF Nikkor 85 mm f/1.8D) to focus the beam, and a line-scan CCD camera (E2V, AViiVA EM4-EV71YEM4CL2014-BA9) to detect the signal. The detected spectrum was then digitalized at a 12-bit resolution and

FIGURE 5.1: Construction of the probe-based μ OCT. (A) Schematic of the probe-based μ OCT system. SCL: Supercontinuum light source; DF: dichroic filter; L1-L3: achromatic lens; FC: fiber coupler; G: grating; L4: camera lens; CCD: charge-coupled device; IMAQ: imaging acquisition. (B) 3-D configuration of the probe. 1: driveshaft; 2: single mode fiber; 3: ferrule; 4: glass spacer; 5: GRIN lens; 6: beamsplitter; 7: reference reflector. (C) Photograph of the fabricated probe compared with a one-cent coin.

transferred to a computer through an imaging acquisition card (KBN-PCECL4-F; Bitflow Inc) and camera link cables. At the meanwhile, the computer generates a

triggering signal of 20k Hz to synchronize the camera via a data acquisition board (PCI-6221, National Instruments, Austin, TX, USA), and consequently, the system scanning rate was 20k lines/second. During image post-processing, a fast Fourier transform was performed to reconstruct the depth-resolved images of the sample. Furthermore, 2-frame averaging (time-lapse averaging) and software based speckle reduction [136, 137] were conducted to reduce the image speckle and improve the cellular-level imaging contrast.

5.2.2 System Performance

To testify the axial resolution and sensitivity of the system, we placed a glass surface at the focus and tilted the surface to prevent saturation; therefore, the total attenuation of this tilted surface was 33.7 dB. The measured axial PSF in Figure 5.2 demonstrated the axial resolution of 2.1 μm in air, and correspondingly of 1.53 μm in tissue assuming the refractive index of 1.37. Besides, the SNR was tested to be approximately 64.3 dB, and herein, the sensitivity was estimated as 98.0 dB.

FIGURE 5.2: Measured axial point spread function (PSF) of the system.

To characterize the transverse resolution and DOF, a laser beam profiler (LBP2-HR-VIS2; Newport) and an objective (50 $\times$ DRY Plan Fluorite Objective; Nikon) were applied to capture the sample beam. The transverse resolutions were tested to be 4.8 μm . By adjusting the position of probe along the axial direction, we could measure the beam with different depth position and thereby test the DOF. The diffractive limited DOF was demonstrated to be ~ 150 μm . And these measurements were further confirmed by additional imaging experiment upon a home-made

microparticle phantom including the micro-beads with the standard size of 2 μm (Polyscience Inc.).

5.2.3 Imaging Procedure

We collected freshly explanted swine coronary artery tissues from a slaughterhouse in Singapore. Fresh human atherosclerotic coronary artery tissues were extracted from a cadaver heart. We inserted the probe together with outer sheath into the coronary artery lumen so that the imaging tissues surround the outer surface of sheath tightly. In addition, when finishing image acquisition, the human coronary artery tissues were fixed using 4% neutral buffered formaldehyde and then stained respectively using Masson's trichrome and hematoxylin and eosin (H & E) for histological analysis in comparison with μOCT images.

The study using human arterial tissues was approved by the Institutional Review Board at Nanyang Technological University (IRB-2014-12-004). The study using animal was approved by the Institutional Animal Care and Use Committee (IACUC) of Nanyang Technological University, Singapore (ARF-SBS/NIE-A0312).

To estimate the high resolution of this probe-based μOCT , we compared the obtained μOCT images with the corresponding images processed by a $\sim 10 \mu\text{m}$ axial resolution in air and a $\sim 20 \mu\text{m}$ transverse resolution which is the highest resolution of current OCT for intravascular imaging to date [17, 18, 27]. This procession was achieved by two steps: the first step is to apply two Gaussian-shape coherence functions with an axial FWHM of $\sim 10 \mu\text{m}$ in air and a transverse FWHM of $\sim 20 \mu\text{m}$ to convolute with our detected imaging data, respectively; then the second step is to combine the above convoluted results.

5.2.4 Spectroscopic OCT

During the post-processing, we applied a spectroscopic imaging technology, termed spectroscopic OCT, into analysis to detect the natural "color" of collagen fibers. Spectroscopic OCT is a stain-free imaging tool to evaluate the spectroscopic contrast of aligned fibrous structures in arteries.

We discovered that aligned cylindrical scatterers generate characteristic spectral centroid shifts towards the longer wavelengths in the spectral window of 760-920 nm, while microstructures consist of nano-spheres exhibit shifts towards the shorter end. Based on this, we put forward a novel model to predict the spectral shifting trends of cylinders in the near infrared region, and predicted a characteristic spectral centroid shift in the backscattered near infrared (NIR) fields of aligned cylinders, with respect to those of other geometric arrangements.

The basic idea of spectroscopic OCT is to use the time-frequency analysis method to obtain the spectral centroid shift in the backscattered light field at each sample point. The most widely used time-frequency analysis method is the short time Fourier transform (STFT), which can be considered as the result of passing a signal through a bank of band-pass filters with constant bandwidth.

5.3 Results

5.3.1 Morphological Characteristics of Normal Swine Coronary Arteries

In a representative cross-sectional μ OCT image (Figure 5.3(A)), layers of media and adventitia can be clearly distinguished. Additionally, shown in Figures 5.3(C, D), within the media, both the circumferentially oriented elastic laminae with high reflectivity and alternative low-scattered smooth muscle can be frequently and evidently observed in regular arrangement. Specifically, the most media layer is featured in cyan shown in Figure 5.3(B), consistent with the existence of a large amount of elastic fibers as well as surrounding collagen fibers.

The inner elastic lamina (IEL), a layer of elastic tissue as the outermost part of the thin intima, is a flexible barrier between the arterial intimal and medial layers, and it may have an effect on atherosclerosis via its modulation of diffusion across the artery wall [144–147]. By imaging the normal swine artery, regular IEL is acquired as a bright and very thin structure (Figure 5.3(D)).

FIGURE 5.3: Cross-sectional images of a normal swine coronary artery *ex vivo*. (A) A representative cross-sectional μ OCT image during circumferential scanning showing anatomical information of media (M), adventitia (Ad) and inner elastic lamina (IEL). It consists of 1024 pixels (axial, 1016.8 μm) $\times$ 5188 pixels (circumferential, minimum circle: 8375.5 μm). (B) Corresponding color-coded geometry specific μ OCT image showing elastic fibers (cyan) featuring the most areas of media. (C) Magnified view indicating the multiple signal-rich elastic laminae and alternative signal-poor smooth muscle within the media. Image Size: 1037.0 μm (circumferential, minimum circle) $\times$ 637.5 μm (axial). (D) Magnified view depicting the signal-rich IEL as a thin hyper-reflective band just beneath the inner luminal surface of tissues (red arrow head), and the underlying multiple elastic laminae and smooth muscle within the media. Image Size: 1585.7 μm (circumferential, minimum circle) $\times$ 637.5 μm (axial). (C'-D') Respectively corresponding images of (C) and (D) by OCT with an axial resolution of ~ 10 μm in air and a transverse resolution of ~ 20 μm . Red arrows in (A) indicate the conjugated profile of the inner surface of the outer sheath. Scale bars: 1 mm in (A, B); 200 μm in (C, D) and (C', D').

5.3.2 Morphological Characteristics of Human Atherosclerotic Plaques

Previous histopathological studies have revealed that atherosclerotic plaque progression is commonly featured by grossly thickened intima and irregular layered structures [144–147]. The μ OCT image of human atherosclerotic plaques (Figure

5.4) illustrates the cross-section of arterial wall with intimal thickening, in accordance with the histopathological characteristics.

Foam cells, usually appeared as fat-laden engorged macrophages, serve as a hallmark of plaque build-up and subsequent atherosclerosis formation [148–152]. In a μ OCT image (Figures 5.4(E)), the foam cells derived from macrophages can be evidently distinguished and the accumulation of these foam cells is manifested as clusters of punctate highly-scattering spots with backward shadow. The corresponding histological findings (Figure 5.4(E), bottom inset) present a consensus on the μ OCT images. Besides, we also visualized another foam cells with similar scattering intensity of aforementioned foam cells but with spindle shape, which may be derived from smooth muscle cells (Figures 5.4(D)).

Within the progression of atherosclerotic plaque, smooth muscle cells migrate and proliferate from the media into the intima [6, 10, 147]. In μ OCT images, smooth muscle cells can be visualized as spindle-shaped cells, which have the signal-rich interior and signal-poor surrounding (Figures 5.4(D, F)). These findings are supported by the corresponding histological images (Figure 5.4(F), bottom inset). Accompanying with the existence of smooth muscle cells, collagen fibers produced by smooth muscle cells also can be detected within the intima (Figure 5.4(B)) and the corresponding histological image in Figure 5.4(C) supports this finding.

5.4 Discussion

With the improved axial and transverse resolutions, the probe-based μ OCT provides a possibility to capture cellular-level microstructures. Compared Figures 5.3(C, D) with Figures 5.3(C', D'), the microstructures of elastic laminae and smooth muscle can be clearly detected thanks to the enhancement of spatial resolution. Similarly, the cellular components such as foam cells and smooth muscle cells can be clearly resolved and distinguished, demonstrated by the comparisons between Figures 5.4(D-F) to Figures 5.4(D'-F').

The current study has a few limitations with respect to a clinically viable technology. First of all, the proposed fiber-optic probe does not have enough axial imaging depth for intravascular imaging *in vivo*. This issue may be resolved by use of DOF extension techniques [111, 112, 153, 154]. Secondly, we did not include

FIGURE 5.4: Coronary atherosclerotic images acquired by the probe-based μ OCT depicting thickened intima. Human cadaver heart tissue. (A) A representative cross-sectional μ OCT imaging showing lipid pools as areas with shallow light penetration and graduate decay in the reflectance signal along the radial direction, spindle-shaped foam cells derived from smooth muscle cells (D, white arrows), “cotton ball” like foam cells derived from macrophages (E, black arrows), and smooth muscle cells (D and F, blue arrow heads). It consists of 1024 pixels (axial, 1016.8 μm) $\times$ 5184 pixels (circumferential, minimum circle: 8375.5 μm). (B) Corresponding geometry specific image showing collagen fibers (densely packed aligned cylinders coded in cyan) in contrast to the pink-coded cell-dense tissue (spherical structures). (C) Trichrome stained histology confirming the findings in (B). (D) Magnified view depicting the spindle-shaped foam cells (white arrows) and the smooth muscle cells (blue arrow heads). Image Size: 2742.0 μm (circumferential, minimum circle) $\times$ 794.4 μm (axial). (E) Magnified view depicting the “cotton ball” like foam cells (black arrows), and representative hematoxylin and eosin (H & E) staining of “cotton ball” like foam cells (inset). Image Size: 1252.2 μm (circumferential, minimum circle) $\times$ 794.4 μm (axial). (F) Magnified view depicting the smooth muscle cells (blue arrow heads), and representative (H & E) staining (inset). Image Size: 783.4 μm (circumferential, minimum circle) $\times$ 794.4 μm (axial). (D'-F') Respectively corresponding images of (D), (E) and (F) by OCT with an axial resolution of $\sim 10 \mu\text{m}$ in air and a transverse resolution of $\sim 20 \mu\text{m}$. LP: lipid pool. Red arrows in (A) indicate the conjugated profile of the inner surface of the outer sheath. Arrowheads in (B) and (C): collagen fibers; Asterisks in (B) and (C): cell-dense tissue. Scale bars: 1 mm in (A-C); 200 μm in (D-F) and (D'-F').

a polymer sheath in our study so that the influence of the polymer sheath on the spatial resolution and sensitivity need to be tested in future. Thirdly, we used a relative low imaging speed for image acquisition which is not enough to suppress motion artifacts *in vivo* . The simple solution is to improve the image acquisition and the current spectrometer can support a high speed A-line rate up to 250k Hz.

5.5 Summary

In conclusion, we investigated the feasibility of intravascular imaging by use of a circumferentially scanning μ OCT fiber-optic probe. The results from human atherosclerotic coronary arteries demonstrate the capability of the μ OCT fiber-optic probe to identify key cellular structures within the plaques. Further development of a μ OCT intravascular catheter will provide more accurate assessment of plaque vulnerability and hold promises for the early diagnosis of atherosclerotic lesions, thus paving the way toward clinical implementation.

Chapter 6

Design and Fabrication of a DOF-extended Probe with Calcite-based Polarization Design

In this chapter, I proposed and fabricated a novel fiber-optic probe, with a calcite-based polarization design and multiple aperture synthesis (MAS) function, aimed to achieve a high transverse resolution along an extended DOF. Section 6.1 gives a brief background on the study motivation. Section 6.2 describes the details about current study methods on the imaging system including the probe design and fabrication as well as the MAS technique. Finally, a summary on current study is given in Section 6.3.

6.1 Background

High transverse resolution of OCT generally results in relatively short DOF and thereby limits its clinical application for volumetric imaging. As presented in the previous chapters, I have fabricated a fiber-optic probe to moderately extend the DOF by use of the apodizing wavefront splitter and validated its ability to visualize cellular structures of colon and coronary arteries, whereas the extended DOF is still insufficient.

The MAS technique we proposed in [112] has been demonstrated to enable DOF extension along high resolution in desktop μ OCT system. It contains two steps in this design. The first step is the beam separation during the imaging: the Gaussian output beams with different spatial or angular frequency components are guided to the sample, and the respectively returning Gaussian beams are detected separately. The second step is to correct the phase offset or shift during the post-processing: by independently correcting the phase offset or shift of distinct apertures and then coherently summing detected signals with different spatial frequencies, a digitally synthesized image can be acquired with an obvious DOF extension and high resolution but without the signal loss. Owing to the merits of considerable DOF extension and simple manipulation on the probe, it is a powerful candidate for probe-based μ OCT to extend the DOF.

In this study, I developed a novel fiber-optic probe by utilizing the calcite-based polarization design, which is suitable for probe-based μ OCT to implement the MAS technique to extend the DOF while maintaining the same transverse resolution. At the first stage of this study, I focused on the design and fabrication of the optical imaging system with a fiber-optic probe to realize the first step of the MAS technique: the beam separation. At the next stage, imaging experiments on the microparticle phantom as well as the animal model will be conducted in the future.

6.2 Imaging System Design and Fabrication

6.2.1 Probe Design

6.2.1.1 Overview of the Probe

Illustrated in Figure 6.1, this probe is composed a ferrule with a SMF, a glass spacer, a GRIN lens (GRIN lens 1) followed by a birefringent calcite and a polarization analyzer, another GRIN lens (GRIN lens 2), and a prism.

The ferrule is used to protect the SMF transmitting the light into the probe optics. The spacer and GRIN lens 1 are employed to collimate the beam. When the beam normally incidents to the calcite, it is divided into two rays: one is the ordinary ray (o-ray, blue ray in Figure 6.1), and other is the extraordinary ray (e-ray, red

ray in Figure 6.1). Followed by the calcite, the polarization analyzer is introduced to rotate the polarization direction of the ordinary and extraordinary rays. In the pupil plane of GRIN lens 2, the apertures occupied by the o-ray and e-ray are different, forming two distinct apertures. By a coating prism after the GRIN lens 2, these two beams are redirected to the sample for imaging.

FIGURE 6.1: Schematic diagram of the μ OCT probe with the calcite-based polarization design. Dotted line accounts for the optical axis of calcite: α is the angle between optical axis and interface of calcite. Blue and red rays indicate the optical paths of o-ray and e-ray, respectively. SMF: single mode fiber.

The key design for the probe is to divide the wavefront within the probe which is the basis for applying the MAS algorithm during the post-processing. The core element to achieve wavefront division is the calcite according to its birefringent property, since the refractive index of calcite is 1.46 and 1.65 for o-ray and e-ray at 810 nm, respectively.

Shown in Figure 6.2(A), when passing through the calcite, o-ray still follows the Snell's law and propagates along the central axis of calcite perpendicular to the interface plane, and it has a polarization direction perpendicular to the paper plane. At the same time, since the optical axis of calcite lies at an angle α compared to the o-ray propagation direction, e-ray propagates with a walk off angle different from the o-ray propagation, and it has a polarization direction in the paper plane but perpendicular to its propagation direction.

After passing through the calcite, o-ray and e-ray go through the polarization analyzer with a specific polarization direction pointed out in Figure 6.2(B). The analyzer rotates the polarization direction of both o-ray and e-ray in the illumination path, so that after passing through the polarization analyzer, they both have the same polarization directions as the optical axis of analyzer.

FIGURE 6.2: Principle of multiple aperture division and synthesis based on polarization design. (A) Side view of the probe in the illumination path. α is the angle between optical axis of calcite and interface of calcite. (B) Cross-sectional view of analyzer showing the polarization direction of the analyzer (black arrow) and that of o-ray (blue arrow). (C) Schematic of four round-trip light paths based on the illumination and detection paths. Blue and red rays indicate the optical paths of o-ray and e-ray, respectively.

In the illumination path, the light can be divided into two paths and then backscattered by the sample. Analogously, the returning light could also be divided into two paths in the detection path. Consequently, as far as both illumination and detection paths are concerned, the light paths can be divided into four parts depicted in Figure 6.2(C):

- (1) The light comes from the illumination path of o-ray and returns (is scattered) along the same illumination path of o-ray but the opposite direction is called as OO light, and the round trip light path of OO light is called as OO path;
- (2) The light comes from the illumination path of o-ray and returns (is scattered) along the illumination path of e-ray but the opposite direction is called as OE light, and the round trip light path of OE light is called as OE path;
- (3) The light comes from the illumination path of e-ray and returns (is scattered) along the illumination path of o-ray but the opposite direction is called as EO light, and the round trip light path of EO light is called as EO path;

- (4) The light comes from the illumination path of e-ray and returns (is scattered) along the same illumination path of e-ray but the opposite direction is called as EE light, and the round trip light path of EE light is called as EE path.

Light signals from all four light paths are coupled back into the SMF for detection. Due to the refractive index difference of calcite between o-ray and e-ray, the optical path length among these light paths is different. According to the optical path length through the calcite, it can be divided into three different apertures:

- (1) The round-trip path length of OO light;
- (2) The round-trip path length of OE or EO light, of which the round-trip path is approximately $\Delta L * (n(o) - n(e))$ longer than that of OO light;
- (3) The round-trip path length of EE light, of which the round-trip path is approximately $2 * \Delta L * (n(o) - n(e))$ longer than that of OO light;

where ΔL is the physical length of calcite, and $n(o)$ and $n(e)$ are the refractive index of calcite for o-ray and e-ray, respectively.

The path length differences enable it possible to encode the light signals from different paths and realize path length division as well as the following detection. This ability to detect the light signals separately in path length domain facilitates the implementation of MAS technique for DOF extension.

6.2.1.2 Optical Design by Use of Zemax

I designed the optical specifications and evaluated the performances of this probe using software Zemax in a try-and-error fashion. As a design input, the light source is simulated as a Gaussian beam with the center wavelength of 810 nm and the range from 650 nm to 950 nm. The NA of Gaussian beam is 0.13 (1% power level). The optimized optical design is depicted in Figure 6.3.

In the glass spacer (BK7) with the refractive index of 1.517 and the length of 6.95 ± 0.05 mm, the incident beam is expanded at the end interface to meet the requirement of the effective objective lens NA. In combination of GRIN lens 1 with the NA of 0.5 and the length of 1.23 ± 0.005 mm, the beam is focused as

FIGURE 6.3: Layout of the μ OCT probe by Zemax simulation. Blue and red rays indicate the optical paths of o-ray and e-ray, respectively. Magenta rays indicate the common area of o-ray and e-ray. α is the angle between optical axis of calcite and interface of calcite.

collimating beam before spread into the calcite. With respect to the calcite, the length is set to be 7.4 ± 0.01 mm under the assumption of $\alpha = 15^\circ$, to separate two distinct apertures as large as possible. Meanwhile, all aperture need to be trapped within all the optics of probe. Followed by the calcite, an analyzer with the length of 1.7 ± 0.05 mm is utilized to rotate the polarization direction of rays. After that, GRIN lens 2, which share the same property as GRIN lens 1 except the length of 2.6 ± 0.005 mm, is used as the objective lens to focus the rays to achieve a working distance of 1.52 ± 0.02 mm. To realize side-view imaging, a right angle prism is applied to redirect the beams by 90° for the imaging sample.

Transverse Resolution

In case of o-ray illumination, the RMS radius is $3.03 \mu\text{m}$ (Figure 6.4(A)), while it is $4.11 \mu\text{m}$ in case of e-ray illumination (Figure 6.4(B)).

Depth-of-focus (DOF)

In case of o-ray illumination only (Figure 6.5(A)) or e-ray illumination only (Figure 6.5(B)), the spot size doubles at $\pm 60 \mu\text{m}$ away for the focal plane. Thus, the diffractive limited focal range is $\pm 42.42 \mu\text{m}$.

Chromatic Focal Shift

When taking only o-ray into consideration (Figure 6.6(A)), the chromatic focal shift was predicted as $83.73 \mu\text{m}$ with the wavelength range from 650 nm to 950 nm. And in case of e-ray illumination (Figure 6.6(B)), the chromatic focal shift is approximately $54.20 \mu\text{m}$.

FIGURE 6.4: Spot diagram at the focus of sample arm simulated by Zemax. (A) Situation with only o-ray. (B) Situation with only e-ray.

6.2.2 Probe Fabrication

Shown in Figure 6.7, a SMF (780HP; Thorlabs Inc.) protected by a glass ferrule (Accu-Glass LLC) transmits the light to a BK7 glass spacer (2.0 mm Dia; Grin-Tech GmbH, Jena, Germany) for expansion. The ferrule and the glass spacer were cemented with the interface at an angle-polished of 4 degrees in case of fiber end reflection. Both GRIN lens 1 and GRIN lens 2 are in a diameter of 2.0 mm (GrinTech GmbH). With respect to the calcite (Shanghai Daheng Optics and Fine Mechanics Co., Ltd., Shanghai, China) and the polarization analyzer (Wuhan Union Optic Inc, Wuhan, China), both the cross-section are squares with 2 mm long. The Leg of the right angle prism is 1.3 mm (Changchun Boxin Photoelectric Co.). During the fabrication, we cement all optical components by using ultraviolet cured optical adhesive (NOA 71; Norland Optical Inc., Cranbury, New Jersey, USA).

FIGURE 6.5: Zemax simulation of the through focus spot diagram. (A) Situation with only o-ray. (B) Situation with only e-ray.

6.2.3 μ OCT Imaging Console

The probe is connected to an imaging console to form a probe-based system for imaging (Figure 6.8). The light source is a supercontinuum light source (SC; Yangtze Soton Laser Co., Ltd) with a broadband spectrum covering a wavelength range of 480 nm – 2200 nm. A short pass dichroic filter (DMSP1000; Thorlabs Inc.) was used to set the imaging bandwidth at 650 nm – 1000 nm. Then a 2×2 fiber coupler with a splitting ratio of 50:50 (Gould Fiber Optics) was applied to split the light into two paths: one is used as reference beam, while the other is guided to the probe as the sample beam.

The light backscattered from the sample interfered with the light backreflected from the reference arm. Then the interferometric signals were collected by a spectrometer consisting of a collimation lens L5 (AC127-030-B-ML; Thorlabs Inc.), a diffraction grating G (1200 lines/mm at 840 nm; Wasatch Photonics Inc.), a camera lens L4 (Nikon AF Nikkor 85 mm f/1.8D) and a line-scan CCD camera (E2V, AViiVA EM4-EV71YEM4CL2014-BA9). The detected spectrum was digitalized

FIGURE 6.6: Zemax simulation of the chromatic focal shift. (A) Situation with only o-ray. (B) Situation with only e-ray.

FIGURE 6.7: Prototype of the μOCT probe with the calcite-based polarization design. (A) 3-D configuration of this fabricated probe. 1: SMF: single mode fiber; 2: ferrule; 3: glass spacer; 4: GRIN lens 1; 5: calcite; 6: polarization analyzer; 7: GRIN lens 2; 8: prism. (B) Photograph of the fabricated probe.

at 12-bit resolution and transmitted to a computer for processing via camera link cables and an image acquisition card (KBN-PCECL4-F; Bitflow Inc.).

FIGURE 6.8: Schematic diagram of the probe-based μ OCT system with the corresponding probe. SCL: Supercontinuum light source; DF: dichroic filter; L1-L5: achromatic lens; FC: fiber coupler; PC: polarization controller; W: window; M: mirror; G: grating; L6: camera lens; CCD: charge-coupled device; IMAQ: imaging acquisition.

During the imaging, the probe was connected to two linear stages (M-VP-25XA; Newport) to complete linear scanning. A triggering signal of 20k Hz was applied to synchronize the camera, thus, imaging was conducted on a rate of 20k lines/second.

In order to alleviate the influence of dispersion between the sample and reference arms, I utilized ZF12 flint glass (Yixing Purshee Optical Elements Co., Ltd, Yixing, China) with specific length as the window in the reference arm to carefully match the materials in the sample arm. Besides, another dispersion compensation method proposed in [86] was also adopted to obtain optimal axial resolution.

6.2.4 MAS Algorithm

As derived in [112], the primary goal of MAS algorithm used in the post-processing is to correct the phase offset of each distinct aperture, and then to coherently summate the multiple images from distinct apertures to reconstruct a new refocused image.

The core step is to correct the phase offset, which is generally originated from the optical path difference through the calcite and defocus-induced wavefront curvature. In frequency domain OCT (FDOCT), the detected spectral interferogram contains three components: direct-current (DC) term, cross-correlation (CC) term

and auto-correlation (AC) term, and can be expressed as a function of spatial frequency k :

$$I(k) = S(k) \left[\text{DC} + 2 \int_{-\infty}^{+\infty} \sqrt{I_r(k)I_s(k)} \cos(2kz) dz + \text{AC} \right]. \quad (6.1)$$

Here, $S(k)$ is the power spectral density of light source and decided by the source. $I_r(k)$ and $I_s(k)$ are the electric field reflected from the reference and sample arms, respectively. z represents the path length difference between the reference and sample arms: $z = z_r - z_s$. DC term is considered as DC component which is mainly dependent upon the power spectral density and the reflectivity of reference. AC term is generated by the interference between the backscattered light from different sample reflectors. For a given OCT system, $S(k)$ and DC term are seen as constant, and AC term is far smaller than CC term, therefore, they can be omitted in the following analyzation for simplicity. In this calcite-based polarization design, it totally contains three apertures in the detection path, and thereby, the CC term can be described as:

$$I(k) = \sqrt{I_r(k)I_s(k)} \left\{ \sum_{m=1}^3 \exp[\exp(i2kz) + ik(m-1)\Delta z] + C.C. \right\}, \quad (6.2)$$

where $C.C.$ accounts for the complex conjugate and also can be omitted. Δz is the optical path difference between two adjacent apertures, and it contains two terms: $\Delta z = \Delta z_{cal} + \Delta z_{curve}$. Here, Δz_{cal} is the optical path difference through the calcite and the details is introduced in Section 6.2.1.1; while Δz_{curve} indicates the small extra optical path difference deriving from the defocus-induced wavefront curvature.

Defining the frequency k value as $k = k_0 + \Delta k$, with k_0 of the spatial frequency corresponding with central wavelength λ_0 ($k_0 = 2\pi/\lambda_0$) and Δk of the spatial frequency bandwidth of the light source, the Eq. (6.2) can be rewritten as:

$$I(k) = \sqrt{I_r(k)I_s(k)} \left\{ \sum_{m=1}^3 \exp[\exp(i2kz) + i\alpha_m + i\beta_m] \right\}, \quad (6.3)$$

where $\alpha_m = k_0(m-1)\Delta z$ is an constant phase independent on k -sapce, and $\beta_m = \Delta k(m-1)\Delta z$ is an additional oscillation phase depending on k -sapce.

As pointed out in Eq. (6.3), there are two phase-shifts (α_m and β_m) and it needs two steps to manipulate the phase correction in sequence. The first is to correct the depth-shift α_m by multiplying a constant phase coefficient $\exp(-i\alpha_m)$ based on the Fourier shift theorem. The optimal value of α_m can be achieved when $\sum_{m=1}^3 I(k) \cdot \exp(-i\alpha_m)$ reaches its maximum value. Analogously, the second is to correct β_m by multiplying an oscillation phase $\exp(-i\beta_m)$. The optimal value of β_m can be obtained when $\sum_{m=1}^3 I(k) \cdot \exp(-i\alpha_m) \cdot \exp(-i\beta_m)$ achieves its maximum value.

By coherently summing the distinct sub-images, the digitally synthesized image can be obtained as the following equation:

$$I_{MAS}(k) = \sum_{m=1}^3 I(k) \cdot \exp(-i\alpha_m) \cdot \exp(-i\beta_m), \quad (6.4)$$

6.2.5 Probe Performance Justification by Numerical Analysis

As credited to [112], numerical simulation was conducted to characterize the performance of MAS technique compared to other selective methods of a full aperture and an apodizing aperture, and therefore, before fabricating the probe, I conducted the relative numerical analysis to justify the performance of MAS technique on this calcite-based polarization design probe (Figure 6.9).

As for the MAS method with the calcite-based polarization design, there are two different apertures in the illumination path: the o-ray and the e-ray. Thus, the number of apertures used in the MAS is set to 2 in the simulation (Figure 6.9(C)).

FIGURE 6.9: Numerical simulation results. (A-C) Pupil functions (PFs) of a full aperture, an apodizing aperture, and a multiple aperture synthesis (MAS) method with the calcite-based polarization design, respectively. (D-F) 2-D Point spread functions (PSFs) of the full aperture, the apodizing aperture, and the MAS as a function over the transverse (x) and axial (z) directions. (G) Transverse PSFs at the focus ($z = 0$). (H) Transverse PSFs at an out-of-focus plane of $z = b$, $1.3b$, and $2b$, respectively. b is the confocal parameter. Red, green and blue rays indicate the cases of the full aperture, the apodizing aperture and the MAS, respectively.

As for the 2-D PSFs (Figure 6.9(D-F)), the MAS produces larger DOF than that by a full aperture, and less sidelobes than that by an apodizing aperture.

DOF represents the axial distance over which the beam size at the $1/e^2$ beam is not larger than 1.414 times of that at the beam waist. Figure 6.9(G) shows the

transverse PSFs of a full aperture, an apodized aperture, and a MAS at the focus where the axial distance z is defined as 0.

In Figure 6.9(H), for the full aperture depicted as the red line (at the axial depth of $z = b$), the FWHM of the transverse PSF becomes 1.414 times larger than that at the focus ($z = 0$), where b refers to the confocal parameter. Consequently, the DOF of full aperture is $2b$ due to the symmetry of the beam. For the apodized aperture, when the axial depth becomes $1.3b$ ($z = 1.3b$), the FWHM of the transverse PSF becomes 1.103 times (less than 1.414 times) larger than that at the focus, which indicates that the DOF of apodized aperture should be larger than $2.6b$. For the MAS method, at the axial depth of $z = 2b$, the FWHM of the transverse PSF becomes 1.175 times larger than compared to that at the focus, indicating that the DOF of MAS method should be larger than $4b$. The DOF of these three approaches has been summarized in Table 6.1, indicating that the DOF can be extended by apodized aperture and MAS approaches.

TABLE 6.1: Depth-of-focus (DOF) of approaches with a full aperture, an apodized aperture and a multiple aperture synthesis (MAS).

Approach	A full aperture	An apodized aperture	A MAS
DOF	$2b$	$> 2.6b$	$> 4b$

6.3 Summary

In the existing stage of this study, I designed and fabricated a fiber-optic probe with the calcite-based polarization design, which is designed to combine the existing MAS algorithm to alleviate the limitation of short DOF. The simulation results demonstrate the effect of this probe design on DOF extension. In the next stage, I will further justify its performance on the tissues.

Chapter 7

Modeling of Mechanical Stress Exerted by Cholesterol Crystallization on Atherosclerotic Plaques

Using the μ OCT fiber-optic probe fabricated in the above-mentioned works (Chapters 4 and 5), we planned to acquire images of atherosclerotic plaques in 10 intact human cadaver hearts and analyze images with cholesterol crystal accumulation, in an effort to evaluate the mechanical effect of cholesterol crystal on the vulnerability of atherosclerotic plaques. The images from the first human cadaver hearts were presented in Chapter 5, which did not contain cholesterol crystal. We were instructed by the funding agency (NRF) to rescope our project work before we continue with the second heart. Specifically, all the human studies were removed by NRF which stopped image acquisition. In order to complete the work, we used μ OCT images acquired by using the desktop imaging system before the project rescope.

Based on the geometric information of cholesterol crystals measured by μ OCT, this chapter conducts a finite element (FE) analysis to evaluate the effect of cholesterol crystallization on mechanical stability or the vulnerability of atherosclerotic plaques. Section 7.1 gives a background on the study motivation originated from the improved resolution of μ OCT. Section 7.2 describes study methods including

the FE modeling and cholesterol crystal measurement. In section 7.3, the measuring and analyzing results are presented in detail. After that, Section 7.4 presents a discussion upon the results and Section 7.5 finally summarizes this chapter.

7.1 Background

Plaque rupture is the critical cause of cardiovascular thrombosis, whereas the detailed mechanisms are not fully understood. Recent studies by Abela et al. have indicated that cholesterol crystals may play a significant role in plaque rupture [155]. The crystallization of cholesterol in an atheromatous plaque leads to the rapid accumulation of these crystals in a limited space, and consequently, the crystals extrude through or protrude into membranes, damaging the fibrous cap and increasing the potential of plaque rupture [155–157]. This proposal is supported by histological observations of abundant cholesterol crystals around plaque rupture sites in human pathologic specimens and *in vitro* experimental demonstrations of up to a 45% volume increase of cholesterol crystals during crystallization [155–157]. This hypothesis is also supported by a histological study by Frink, which showed the correlation between parallel cholesterol crystals and the sites of plaque rupture [158].

The physical stress on the cap can be conveniently predicted by finite element (FE) modeling [159–164]. However, cholesterol crystals, potential physical risk factors of plaque rupture, have not been included in previous mechanical models; therefore, how cholesterol crystals affect atherosclerotic plaques remains elusive. Cholesterol crystals are dissolved in tissue sections embedded in paraffin during standard histological processing and leave “clefts” in the histology sections, which may not faithfully reflect the geometry of the cholesterol crystals *in vivo*. IVOCT imaging can detect cholesterol crystals *in vivo* [17, 18, 165]. However, the typical spatial resolution of IVOCT is too coarse to accurately measure cholesterol crystals. μ OCT can clearly characterize cholesterol crystals in intact arterial tissues *ex vivo* with 1–3 μ m spatial resolution [25, 26, 166], and thereby opens the possibility for modeling cholesterol crystallization in necrotic cores and exploring the relationship between cholesterol crystals and plaque rupture.

This study aims to investigate the stress exerted by cholesterol crystal growth through the finite element method (FEM). We modified the existing 2-D plaque models by adding a cholesterol crystal model based on the μ OCT measurement. We chose the peak circumferential stress (PCS) as the primary risk factor in our mechanical model because a large proportion (58%) of plaques ruptures at points of PCS [160, 163, 167, 168]. Our study discovered a link between PCS and the magnitude of crystal expansion and a correlation between PCS and the spatial distribution of cholesterol crystals. The new mechanical plaque model proposed in this study improves our understanding of the physical role of cholesterol crystals in plaque rupture and creates the possibility to preventively evaluate the mechanical risk factor based on high-resolution μ OCT images.

7.2 Methodology

7.2.1 Geometry of the Plaque Model

We propose an idealized, 2-D FEM of the cross-section of an atherosclerotic coronary artery based on the morphology and geometry established in previous studies [159, 162]. As illustrated in Figure 7.1, the lumen was modeled as a circular hole bearing the blood pressure. Cholesterol crystals are frequently observed in atheromatous plaques and are usually present in the necrotic core. The fibrous cap, the thin part between the lumen and necrotic core, is vulnerable to damage, which easily induces plaque rupture. During crystallization, cholesterol crystals expand in the confined space (necrotic core) and then protrude through or penetrate into the thin fibrous cap. To reproduce cholesterol crystals and their dynamics in our model, we measured the geometry of cholesterol crystals using μ OCT imaging, followed by increasing the size of the cholesterol crystals in the depth direction.

The morphologic parameters of the artery were adopted from a previous study [6]. The mean thickness of the fibrous cap is 91 μm , with a range from 25 μm to 370 μm . The mean lumen area is 5.77 mm^2 . The arc angle and mean area of the necrotic core are 84.65 degrees and 2.22 mm^2 , respectively, with a relative mean core area of 19.17% and a relative core thickness of 49.41%. These geometrical dimensions are in close agreement with other investigations [9, 169]. In this condition, the stenosis severity is approximately 70.53%.

FIGURE 7.1: Diagram of an idealized cross-section of an atherosclerotic coronary artery. (A) Structure of the overall atherosclerotic coronary artery. (B) Magnified view of the thin fibrous cap with the cholesterol crystals illustrated in the red box in (A).

7.2.2 Measurement of Cholesterol Crystal Geometry Using μ OCT

The detailed construction of the μ OCT imaging device used in this study was reported in a previous study [84]. In brief, the spatial resolution of μ OCT was 1.3 μ m (axial) in air by 2.5 μ m (transverse). The size of the B-scan (cross-sectional) images was 0.872 mm (width) by 1.4 mm (height). The image speed was 20 frames per second with 512 A-lines per frame. We examined formalin-fixed and non-identified aorta specimens from patients with abdominal aortic aneurysm treated by vascular surgery. The specimens were stored in 4% neutral buffered formaldehyde. We identified the regions of interest by gross visual inspection and acquired μ OCT images from the luminal surface of the aortas.

In the cross-sectional μ OCT images, the length of cholesterol crystals was measured as the horizontal distance between the left and right boundaries of the cholesterol crystals, and the thickness was measured as the vertical distance between the top and bottom surfaces under the assumption that the refractive index was 1.37. The Institutional Review Board at Nanyang Technological University (IRB-2014-12-004) approved the studies using human arterial tissues.

7.2.3 Material Properties

In the idealized model, the arterial wall and the fibrous plaque were made of transversely isotropic materials with linear elastic properties, sharing similar mechanical properties in the circumferential (Θ) and axial (z) directions for each tissue. The material properties of the arterial and fibrous cap were adopted from previous studies [159, 160, 167] and are shown in Table 7.1: Young's moduli E_r , E_Θ and E_z (in the radial, circumferential and axial directions, respectively), Poisson ratios $\nu_{r\Theta}$ and $\nu_{\Theta z}$ (in the r - Θ and Θ - z planes, respectively), and shear modulus $G_{r\Theta}$ (in the r - Θ plane). The fibrous cap and fibrous plaque were assumed to be continuous and shared the same material properties. The necrotic core was estimated to be a very soft (Young's modulus $E = 1$ kPa) and incompressible (Poisson ratio $\nu=0.49$) isotropic material [159–162, 170]. Cholesterol crystals are incompressible but rigid solids; thereby, the Young's modulus and Poisson ratio of cholesterol crystals were assumed as 100 kPa and 0.49, respectively.

TABLE 7.1: Material properties of the plaque and artery used in the structural analyses.

	E_r (kPa)	$E_\Theta = E_z$ (kPa)	$\nu_{r\Theta}$	$\nu_{\Theta z}$	$G_{r\Theta}$ (kPa)
Plaque	50	1000	0.01	0.27	500
Artery	10	100	0.01	0.27	50

7.2.4 Structural Analysis

The FE analysis was conducted to calculate the continual stress and strain of the fibrous cap and to investigate PCS as a result of expanded cholesterol crystals using Ansys 15.0 (Ansys, Inc., Canonsburg, Pennsylvania, USA). Due to the symmetry of this idealized model, we simulated half of the cross-section for analysis, with the nodes along the center line restricted to moving in the radial (r) direction. The various regions of the artery, plaque and lipid core were meshed with 1224 eight-node quadrangular elements. The models were solved under the assumption of plane strains.

Additionally, a static pressure of 110 mmHg (14.6 kPa) acted along the lumen wall, representing the mean intracoronary blood pressure adopted from previous studies [159, 160, 170]. For simplicity, we placed only one cholesterol crystal in each

location, and the effects of multiple cholesterol crystals were simulated by increasing the magnitude of expansion. This simplified model enables us to investigate the effects of the magnitude and the spatial distributions of cholesterol crystal growth on PCS.

7.2.5 Locations and Loading of Cholesterol Crystals

We chose three representative locations at the cap: the cap shoulder, the cap center, and halfway between the shoulder and the cap center (Figure 7.1). Cholesterol crystal growth was implemented by expanding the cholesterol crystal in the depth (thickness) direction.

7.3 Results

To quantitatively characterize the effects of cholesterol crystal growth on PCS, we investigated the PCS changes caused by their expansion and spatial distributions. We used the idealized model without cholesterol crystals as the reference. In this case, PCS was located at the shoulder of the cap and the entire cap was expanded toward the abluminal side by the luminal blood pressure, which agrees well with previous studies (Figure 7.2) [159–161]. A PCS of 275.6 kPa was the pre-loaded peak offset pressure, and deviations from this offset caused by crystal loading would indicate either stabilizing or destabilizing effects of the cholesterol crystals. We found that the loading of concentrated crystals at the cap shoulder imposed the highest risk of plaque rupture by proportionally increasing PCS, whereas the evenly distributed crystal loading along the cap mitigated this risk by exerting less PCS than concentrated crystals.

7.3.1 Identification and Measurement of Individual Cholesterol Crystals Using μ OCT

In a representative μ OCT image of a human aortic atherosclerotic plaque (Figure 7.3), almost every cholesterol crystal in the necrotic core near the cap could be identified by its reflections from the top and bottom surface. We measured the

FIGURE 7.2: Stress and strain of the coronary artery without cholesterol crystals. (A) Stress distribution of the overall coronary artery. (B) Contour plot of the stress on the fibrous cap (yellow dashed box in A). (C) Contour plot of the strain on the fibrous cap. The dashed line is the original contour before loading, and the meshed section is the deformed cap after loading. PCS: peak circumferential stress.

length and thickness of 20 different cholesterol crystals from the image presented in Figure 7.3, and the average length and thickness are $269.1 \mu\text{m}$ and $3.0 \mu\text{m}$, with standard deviations of $80.16 \mu\text{m}$ and $0.33 \mu\text{m}$, respectively.

7.3.2 Peak Circumference Stress Is Proportionally Dependent on the Cholesterol Crystal Growth

To quantitatively characterize the relationship between cholesterol crystal growth and PCS, we examined PCS during a crystal expansion of $400 \mu\text{m}$ in thickness at the cap shoulder. As expected, PCS increased as the displacement of the cholesterol crystals increased (Figure 7.4). PCS was almost linearly dependent on the cholesterol crystal growth within the expansion range of $0\text{-}400 \mu\text{m}$ with a slope of $0.442 \text{ kPa}/\mu\text{m}$.

FIGURE 7.3: A μ OCT image of a human aortic atherosclerotic plaque revealing the geometries of cholesterol crystals. ChCs: cholesterol crystals. NC: necrotic core. Scale bar, 50 μ m.

FIGURE 7.4: Peak circumferential stress (PCS) with varying expansion of one cholesterol crystal at the cap shoulder.

7.3.3 Cholesterol Crystals at the Cap Shoulder Impose the Highest Peak Circumference Stress

We also investigated PCS changes caused by cholesterol crystal growth at three different locations: the shoulder (Figure 7.5), halfway between the shoulder and the cap center (Figure 7.6), and the cap center (Figure 7.7). At each location, cholesterol crystal growth of 2 μ m caused a significant increase in PCS: 78.85% at the shoulder (Figure 7.5), 69.85% halfway between the shoulder and the cap

center (Figure 7.6), and 44.30% at the cap center (Figure 7.7). The locations of the PCS coincided with the positions of the cholesterol crystal growth, indicating that the crystal growth was the primary risk factor in this idealized model. The crystal growth also caused significant cap deformation toward the lumen center at the shoulder, halfway between the shoulder and the cap center, and the cap center.

FIGURE 7.5: Stress and strain of the artery with one cholesterol crystal at the cap shoulder. (A) Stress distribution of the overall coronary artery. (B) Contour plot of the stress on the fibrous cap (yellow dashed box in A). (C) Contour plot of the strain on the fibrous cap. The dashed line is the original contour before loading, and the meshed section is the deformed cap after loading. PCS: peak circumferential stress. ChC: cholesterol crystal.

7.3.4 Effect of the Spatial Distribution of Cholesterol Crystals on the Peak Circumference Stress

There are typically multiple expanding cholesterol crystals distributed along the cap. To understand the effect of the spatial distribution of cholesterol crystals on PCS, we conducted simulations to investigate the simplest case: two cholesterol crystals located in any two of the three above-mentioned cap positions. The results summarized in Figure 7.8 show that the distributed cholesterol crystal loading may

FIGURE 7.6: Stress and strain of the artery with one cholesterol crystal halfway between the shoulder and the cap center. (A) Stress distribution of the overall coronary artery. (B) Contour plot of the stress on the fibrous cap (yellow dashed box in A). (C) Contour plot of the strain on the fibrous cap. The dashed line is the original contour before loading, and the meshed section is the deformed cap after loading. PCS: peak circumferential stress. ChC: cholesterol crystal.

increase PCS (384.6 kPa in Figure 7.8(A) and 349.2 kPa in Figure 7.8(B)) but to a lesser extent than in the cases of concentrated loading (Figures 7.5-7.7) or may even maintain the PCS (273.8 kPa; Figure 7.8(C)) compared with the control case (275.6 kPa; Figure 7.2). The corresponding maximum cap deformation (Figures 7.8(A'), 7.8(B') and 7.8(C')) was also significantly smaller than those of the concentrated loading cases.

The results of three cholesterol crystals distributed at three locations confirmed the findings in the case of two cholesterol crystals (Figure 7.9(A)), but the PCS was even smaller (164.7 kPa) and was collocated with the cholesterol crystal near the shoulder of the cap. Because the three cholesterol crystals were distributed more uniformly than the case of two cholesterol crystals, the strain of the cap (Figure 7.9(B)) was smaller and was almost the same as in the control case. These results indicate that the spatial distribution of the cholesterol crystal loading may alter the magnitude and location of PCS: evenly distributed cholesterol crystal loading

FIGURE 7.7: Stress and strain of the coronary artery with one cholesterol crystal at the cap center. (A) Stress distribution of the overall coronary artery. (B) Contour plot of the stress on the fibrous cap (yellow dashed box in A). (C) Contour plot of the strain on the fibrous cap. The dashed line is the original contour before loading, and the meshed section is the deformed cap after loading.

PCS: peak circumferential stress. ChC: cholesterol crystal.

may balance the stress caused by blood pressure and reduce the PCS magnitude.

7.3.5 Sensitivity Analysis

The sensitivity analysis of PCS on the cap was performed for all models by changing the Young's modulus of cholesterol crystals from -50% to 1000% . Under consideration of such variations, the value of the PCS on the cap altered within 2.16% , demonstrating reasonable errors in the Young's modulus of cholesterol crystals with minimal influence on the conclusions of this study.

FIGURE 7.8: Stress and strain of the cap with two cholesterol crystals at any two random positions. (A) Contour plot of the stress on the fibrous cap when cholesterol crystals are located at the shoulder and halfway between the shoulder and the cap center. (A') Corresponding strain contour of the cap of A. (B) Contour plot of the stress on the fibrous cap when cholesterol crystals are located halfway between the shoulder and the cap center and at the cap center. (B') Corresponding strain contour of the fibrous cap of B. (C) Contour plot of the stress on the fibrous cap when cholesterol crystals are located at the shoulder and at the cap center. (C') Corresponding strain contour of the fibrous cap of C. The dashed line is the original contour before loading, and the meshed section is the deformed cap after loading. PCS: peak circumferential stress. ChC: cholesterol crystal.

FIGURE 7.9: Stress and strain of the cap with three cholesterol crystals at three positions. (A) Contour plot of the stress on the fibrous cap. (B) Corresponding strain contour of the fibrous cap in A. The dashed line is the original contour before loading (control case), and the meshed section is the deformed cap after loading. PCS: peak circumferential stress. ChC: cholesterol crystal.

7.4 Discussion

Cholesterol crystals have long been identified in histology images as clefts in the necrotic core around the cap [171]. Abela et al. proposed that cholesterol crystals physically penetrate into the fibrous cap, increasing the likelihood of plaque rupture [155–157]. However, due to the technical challenge of detecting individual crystals in the native tissue state, this potential risk factor was not investigated in previous coronary atherosclerotic models [159–164].

In this study, we used μ OCT imaging to obtain high-resolution measurements of individual cholesterol crystals in intact human arterial tissues. With the geometric information obtained by μ OCT, the physical risk factor of cholesterol crystals was investigated, for the first time, in an idealized plaque model. The results of this work provide new insight into the role of cholesterol crystals in the physical mechanism of plaque rupture.

We found that PCS collocated with the cholesterol crystals when they were concentrated in one location of the cap in the idealized plaque model (Figures 7.5–7.7), and PCS was proportionally dependent on the cholesterol crystal expansion, placing the vessel wall at a higher risk of plaque rupture. Moreover, cholesterol crystal growth compromises cap stability by causing cap deformation (Figures 7.5–7.7). Previous studies predicted that PCS often occurs on the shoulder of the cap, whereas the histological study by Maehara et al. found that 63% of plaque ruptures among 254 patients were on the shoulder, and the other 37% were on the center of the cap [172]. Our results point out that PCS occurs at positions other than the shoulder with the presence of cholesterol crystals, which agrees with the histological study by Maehara et al.

This research revealed that the magnitude of PCS changes significantly depends on the distribution of the cholesterol crystals. When all cholesterol crystals were concentrated at one position (Figures 7.5–7.7), PCS was significantly higher than in the cholesterol crystal-free model (Figure 7.2) because the region of the cap in contact with the concentrated cholesterol crystals bears the combined stress of luminal blood and cholesterol crystal expansion, whereas the rest of the cap is only under luminal blood pressure.

The situation was different when the cholesterol crystals were distributed at multiple locations rather than concentrated at one location on the abluminal side of the cap. Evenly distributed cholesterol crystals with an expansion of 2 μm acted against the stress loaded by the blood pressure and consequently reduced PCS with regards to the concentrated cholesterol crystals or to an even lower PCS than that in the cholesterol crystal-free control case. This finding indicates, as far as mechanical risk factors are concerned, that the plaque was less vulnerable to rupture because as the cholesterol crystals continued to expand, the stress exerted by the cholesterol crystals eventually counteracted the blood pressure to the extent that PCS was lower than the cholesterol crystal-free control.

7.4.1 Effect of Nonlinearity of Material Properties

To test whether the linear solution assumed by our current study may be a good approximation of the biomechanical system under investigation, we conducted another analysis by applying the non-linear solutions into the all idealized models of artery without and with cholesterol crystals, in which the artery, plaque and necrotic core were assumed as Mooney-Rivlin materials with non-linear properties [173]. We found that the results (Figure 7.10) are agreed with those obtained using the linear solution well, except that PCS values resulted from the non-linear properties were reduced compared to those obtained using the linear properties. Therefore, assumption of non-linear material properties (artery, plaque and necrotic core) does not change the conclusion that cholesterol crystals at the cap shoulder impose the highest PCS. This result suggests that, as far as the current study is concerned, the linear solution may be enough to characterize the effect of cholesterol crystallization on PCS and the mechanical risk factor of cholesterol crystals.

7.4.2 Response of Peak Circumferential Stress with Respect to Intracoronary Blood Pressure

To investigate whether intracoronary pressure plays a role in the effect of cholesterol crystal expansion on PCS, we varied the lumen pressure P from 70 mmHg (9.3 kPa) to 200 mmHg (26.6 kPa). We found that the PCS location corresponded to the

FIGURE 7.10: Summary of the cap stress in eight idealized models with non-linear solutions. (1) Cap stress without cholesterol crystals. (2) Cap stress with one cholesterol crystal at the cap shoulder. (3) Cap stress with one cholesterol crystal halfway between the shoulder and cap center. (4) Cap stress with one cholesterol crystal at the cap center. (5) Cap stress with cholesterol crystals located at the shoulder and halfway between the shoulder and cap center. (6) Cap stress with cholesterol crystals located halfway between the shoulder and cap center and at the cap center. (7) Cap stress with two cholesterol crystals at the cap shoulder and at the cap center. (8) Cap stress with three cholesterol crystals located at three positions. PCS: peak circumferential stress.

location of the concentrated cholesterol crystal regardless of luminal blood pressure change. The results listed in Figure 7.11 also show that, although intracoronary blood pressure increase elevates the baseline PCS (when crystal expansion = 0), it did not have any effect on the linear relationship between crystal expansion and PCS (Figure 7.11).

FIGURE 7.11: Effect of crystal expansion at the cap shoulder on peak circumferential stress with varying blood pressure. P: intracoronary blood pressure.

7.4.3 Sensitivity Limitation

The current study was conducted using a previously reported idealized cross-section of the coronary artery [159]. The mechanical and/or structural differences from the actual situation may result in prediction errors. In addition to tangentially arranged cholesterol crystals, randomly oriented cholesterol crystals were observed. In the current study, we developed a simple model to investigate the effect of tangentially arranged cholesterol crystal growth on PCS without covering the effect of crystal orientation.

7.5 Summary

This study investigated the effects of the magnitude and locations of cholesterol crystal growth on PCS in an idealized plaque model, which is related to plaque rupture of the coronary artery. Cholesterol crystal growth concentrated at one location of the fibrous cap increases the risk of plaque rupture; we demonstrated that the magnitude of PCS was collocated with and proportional to the expansion of cholesterol crystals. In particular, the shoulder region of the fibrous cap is more susceptible to erosion/rupture induced by cholesterol crystal growth than other locations; the closer the cholesterol crystals are from the shoulder, the higher the PCS. Additionally, the spatial distribution of the stress plays a significant role in the overall effect of the cholesterol crystal expansion on PCS: the evenly distributed cholesterol crystals exert less PCS on the cap than the concentrated crystals.

Chapter 8

Conclusion and Future Work

8.1 Conclusion of Work

In this thesis, we have considered the high-resolution visualization related to GI tracts and coronary arteries by the probe-based μ OCT. It consists of five major contributions.

The first contribution is to develop a desktop high-resolution μ OCT system to capture cellular-level morphological microstructures of the large intestine *ex vivo* and *in vivo*. Due to the high resolution, both goblet cells and crypt lumens can be clearly distinguished, of which the appearance could be a characteristic of intestinal metaplasia as an indicator of colorectal cancer. Therefore, visualization of intestinal microstructure changes in cross-sectional view in such high resolution would assist early disease diagnosis and its treatment. Such capability of μ OCT for cellular-level scanning renders it become potential to be an alternative *in vivo* imaging tool for the early detection of GI cancers.

The second contribution is the development of an endoscopic μ OCT system especially the fiber-optic probe, which is critical to translate the μ OCT techniques from the lab research to clinical application. The specific technical advances are: 1) with the annular apodization design, this endoscopic μ OCT can extend the DOF by $\sim 30\%$ without compromising high resolution; 2) an outer sheath is applied to allow more areas of interest within the focal ranges so that the issue of limited DOF

could be mitigated; 3) the common-path design of the probe can minimize the dispersion and polarization mismatch to enhance imaging contrast to some extent. Upon the successful fabrication of this probe-based μ OCT system, we exploited it to visualize intestinal microstructures by circumferential scanning, and it is the first work to conduct cellular-level GI tracts imaging towards clinical application to the best of our knowledge.

The third contribution is to investigate the capability of a dual-functional probe-based μ OCT on the visualization of cellular and extracellular compositions of coronary atherosclerosis at high resolution and color contrast. The imaging results in this study demonstrated the feasibility of cellular-level imaging in atherosclerotic coronary arteries using μ OCT.

The fourth contribution is the design and fabrication of the second μ OCT fiber probe with a calcite-based polarization design and MAS technique. I demonstrated the advantages of this second probe with regard to the first probe theoretically: 1) the second probe may generate 2 times larger DOF; 2) the coupling efficiency is optimal which is superior over that of the first probe.

The fifth contribution is the development of a mechanical model and analysis method to evaluate the effect of cholesterol crystallization in the atherosclerotic plaques on mechanical stability or plaque vulnerability. I established a mechanical model of atherosclerotic plaques containing expanding cholesterol crystals. It is the first time, to the best of our knowledge, to take cholesterol crystallization into account thanks to the required native geometrical information of cholesterol crystals by using μ OCT in human. This simulation model allows us to preventively evaluate the plaque vulnerability induced by cholesterol crystal growth and deepen our understanding for mechanisms of plaque rupture, which would assist in the prevention of atherosclerosis.

8.2 Outlook for Future Studies

8.2.1 Imaging by Probe-based μ OCT

In previous studies, we have developed the probe-based μ OCT system and testified its ability for imaging GI tracts and coronary arteries. However, there are still

several limitations and we intend to develop the following steps to improve the performance.

8.2.1.1 Imaging Verification by Calcite-based Polarization Design Probe

To further verify the DOF extension performance of the second probe, namely the calcite-based polarization design probe, I will conduct the imaging experiments on the microparticle phantom and the animal model by using the corresponding probe-based μ OCT system.

8.2.1.2 Helical Scanning

In probe-based OCT system for GI tracts and arteries imaging, the helical scanning of the probe is designed to acquire multiple A-scan signals to rebuild the final 3-D images of biological samples, which can be realized through the combination of rotation and pullback of probe. Currently, we have rotated the probe for 360° scanning by connecting the probe with a rotary joint. Next, the pulling back of the whole probe is designed to combine with rotation to realize helical scanning.

8.2.1.3 Animal Imaging Experiment Verification

After the helical scanning mechanics are developed, we will test this fabricated probe-based μ OCT system by carrying out animal imaging experiment including *ex vivo* and *in vivo* to justify its effectiveness of real-time imaging. During *in vivo* experiments, motion artifacts are a major issue predominantly resulting from heartbeat and breath. The usage of outer sheath discussed in Section 4.2.3 can fix the probe in a relative position and help to relieve this issue as a result. Besides, high-speed imaging is also able of alleviating the motion artifacts, and consequently, we will increase the imaging speed in the next stage.

8.2.2 Angular Frequency Array Probe for Speckle Reduction

Speckle noise is another problem for cellular-level μ OCT imaging that may dramatically degrade the image contrast. Angular compounding is the most effective method for speckle reduction in which by averaging the amplitude of backscattered signals from the samples in different angles [174]. To solve this problem for endoscopic μ OCT system, we will develop a multi-channel probe with new design named angular frequency array to achieve speckle reduction by using angular compounding in which the incident light is divided into three parts at different incident angles.

FIGURE 8.1: Schematic of the probe-based μ OCT system with angular frequency array probe. (A) Diagram of an angular frequency array probe with three-channel fiber. (B) Diagram of probe-based μ OCT system. AFA1-2: angular fiber array 1-2. BS: beamsplitter. M: mirror. SMF: single mode fiber. FC1-5: fiber coupler 1-5. FA: fiber array. L: lens. G: grating. PC: personal computer.

Shown in Figure 8.1(A), the core element of this proposed probe is a customized angle fiber array to implement beam separation by physically dividing the illumination beams at three different propagating angles. And in the detection path, the dividing beams can be detected individually. By incoherently averaging the detected signals from different angles, the random speckle noise is suppressed while

the signal intensity remains unchanged, thus resulting in an improvement of SNR as well as the image quality. After probe fabrication, the probe is connected with a μ OCT system console for imaging indicated as Figure [8.1\(B\)](#).

Author's Publications

Journal Articles

- **Yuemei Luo**, En Bo, Haitao Liang, Xianghong Wang, Xiaojun Yu, Xin Ge, Xinyu Liu, Jianhua Mo, and Linbo Liu, “Imaging Cellular Structures of Atherosclerotic Coronary Arteries Using Circumferentially Scanning Micro-Optical Coherence Tomography Fiber Probe,” Submitted to *IEEE Access* (Under review).
- **Yuemei Luo**, Dongyao Cui, Xiaojun Yu, En Bo, Xianghong Wang, Nanshuo Wang, Cilwyn Shalitha Braganza, Shufen Chen, Xinyu Liu, Qiaozhou Xiong, Si Chen, Shi Chen, and Linbo Liu, “Endomicroscopic Optical Coherence Tomography for Cellular Resolution Imaging of Gastrointestinal Tracts,” *Journal of Biophotonics* 11(4), e201700141 (2018).
- **Yuemei Luo**[†], Xiaojun Yu[†], Xinyu Liu, Si Chen, Xianghong Wang, Shi Chen, and Linbo Liu, “Towards High Speed Imaging of Cellular Structures in Rat Colon Using Micro-Optical Coherence Tomography,” *IEEE Photonics Journal* 8 (4), 3900308 (2016).
(Xiaojun Yu and Yuemei Luo contributed equally to this work.)
- **Yuemei Luo**, Dongyao Cui, Xiaojun Yu, Si Chen, Xinyu Liu, Hongying Tang, Xianghong Wang, and Linbo Liu, “Modeling of Mechanical Stress Exerted by Cholesterol Crystallization on Atherosclerotic Plaques,” *PloS one* 11 (5), e0155117 (2016).
- En Bo, **Yuemei Luo**, Si Chen, Xinyu Liu, Nanshuo Wang, Xin Ge, Xianghong Wang, Shufen Chen, Shi Chen, Jinhan Li, and Linbo Liu, “Depth of Focus Extension in Optical Coherence Tomography via Multiple Aperture Synthesis,” *Optica* 4 (7), 701-706 (2017).

- Xiaojun Yu, Qiaozhou Xiong, **Yuemei Luo**, Nanshuo Wang, Lulu Wang, Hong Liang Tey, and Linbo Liu, “Contrast Enhanced Subsurface Fingerprint Detection Using High-Speed Optical Coherence Tomography,” *IEEE Photonics Technology Letters* 29, 70-73 (2017).

Conference Proceedings

- **Yuemei Luo**, Nanshuo Wang, Dongyao Cui, Xiaojun Yu, En Bo, Xianghong Wang, Xinyu Liu, Jinhan Li, Shufen Chen, Si Chen, Shi Chen, and Linbo Liu, “Micro-Optical Coherence Tomography Endoscopic Imaging of Rat Colon Ex Vivo,” In *CLEO-PR, OECC and PGC 2017*, Aug. 1st-3rd, 2017, Singapore.
- **Yuemei Luo**, Nanshuo Wang, Dongyao Cui, Xianghong Wang, Qiaozhou Xiong, Xiaojun Yu, En Bo, Jinhan Li, Xinyu Liu, Si Chen, Shi Chen, and Linbo Liu, “Micro-OCT Endoscopic Probe for Gastrointestinal Tracts Imaging,” In *SPIE BiOS 2017*, 28 Jan- 2 Feb, 2017, San Francisco, CA, USA.
- **Yuemei Luo**, Xinyu Liu, Si Chen, Dongyao Cui, Xianghong Wang, Linbo Liu, “Mechanical Modeling of Cholesterol Crystallization in Atherosclerotic Plaques base on Micro-OCT Images,” In *SPIE BiOS*, pp. 96892P-96892P. International Society for Optics and Photonics, 2016.
- **Yuemei Luo**, and Linbo Liu, “Modeling of Cholesterol Crystals in Necrotic Core based on Micro-OCT Images,” In *Photonics Global Conference*, Singapore, July 1, 2015.

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