Development of cellular resolution optical coherence tomography

Bo, En

2018

Bo, E. (2018). Development of cellular resolution optical coherence tomography. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/81264>

<https://doi.org/10.32657/10220/47468>

Development of Cellular Resolution Optical Coherence Tomography

En BO

School of Electrical & Electronic Engineering

A thesis submitted to the Nanyang Technological University
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.

10/Jan/2019

Date

----- -----

En BO

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

10/Jan/2019

Date

Linbo LIU

Authorship Attribution Statement

This thesis contains material from 6 papers published in the following peer-reviewed journals where I was the first author.

Chapter 3 is published as

1. En Bo, Yuemei Luo, Si Chen, Xinyu Liu, Nanshuo Wang, Xin Ge, Xianghong Wang, Shufen Chen, Shi Chen, Jinhan Li, Linbo Liu, Depth of focus extension in optical coherence tomography via multiple aperture synthesis, *Optica* 4(7), 701-706 (2017). DOI: 10.1364/OPTICA.4.000701.
2. En Bo, Xin Ge, Lulu Wang, Xuan Wu, Yuemei Luo, Shufen Chen, Si Chen, Haitao Liang, Guangming Ni, Xiaojun Yu, Linbo Liu, Multiple aperture synthetic optical coherence tomography for biological tissue imaging, *Optics express* 26(2), 772-780 (2018). DOI: 10.1364/OE.26.000772.

The contributions of the co-authors are as follows: Linbo Liu supervised the overall project and participated in writing the manuscript. Si Chen and Shufen Chen prepared the experimental tissues. All other co-authors contributed to the imaging studies. All authors read and edited the manuscript.

Chapter 4 is published as

1. En Bo, Xin Ge, Xiaojun Yu, Jianhua Mo, Linbo Liu, Extending axial focus of optical coherence tomography using parallel multiple aperture synthesis, *Applied optics* 57(13), 3556-3560 (2018). DOI: 10.1364/AO.57.003556.

The contributions of the co-authors are as follows: En Bo and Xin Ge developed the imaging system, conducted the imaging studies and participated in writing the manuscript. Linbo Liu supervised the overall project and participated in writing the manuscript. All other co-authors contributed to the imaging studies. All authors read and edited the manuscript.

Chapter 5 is unpublished:

1. En Bo, Xin Ge, Yuemei Luo, Xuan Wu, Si Chen, Haitao Liang, Shufen Chen, Xiaojun Yu, Ping Shum, Jianhua Mo, Nanguang Chen, and Linbo Liu, Cellular resolution tomography in turbid tissue in vivo through digital aberration compensation, 2018.

The contributions of the co-authors are as follows: En Bo, Xin Ge, Nanguang Chen and Linbo Liu conceived the study. En Bo and Xin Ge developed ADAS-OCT method, conducted the imaging studies and participated in writing the manuscript. Linbo Liu supervised the overall project and participated in writing the manuscript. All other co-authors contributed to the imaging studies. All authors read and edited the manuscript.

Chapter 6 is published as

1. En Bo, Xinyu Liu, Si Chen, Xiaojun Yu, Xianghong Wang, and Linbo Liu, Spectral domain optical coherence tomography with dual-balanced detection for auto-correlation artifacts reduction, Optics Express 23(21), 28050-28058 (2015). DOI: 10.1364/OE.23.028050.

The contributions of the co-authors are as follows: Linbo Liu supervised the overall project and participated in writing the manuscript. All other co-authors contributed to the imaging studies. All authors read and edited the manuscript.

Chapter 7 is published as

1. En Bo, Si Chen, Dongyao Cui, Shi Chen, Xiaojun Yu, Yuemei Luo, Linbo Liu, Single-camera full-range high-resolution spectral domain optical coherence tomography, Applied optics 56(3), 470-475 (2017). DOI: 10.1364/AO.56.000470.

The contributions of the co-authors are as follows: Linbo Liu supervised the overall project and participated in writing the manuscript. All other co-authors contributed to the imaging studies. All authors read and edited the manuscript.

10/Jan/2019

Date

En BO

Acknowledgements

- I would like to express my sincere gratitude to my supervisors, Prof. Liu Linbo, for all the knowledgeable instructions and valuable discussions throughout my PhD study. I am sincerely grateful for the opportunity and the independence he provided. His keen insights to find problems and novel ideas to solve problems always impress me a lot.
- I am particularly grateful for the kind support provided by my Thesis Advisory Committee members, Prof. Chen Nanguang, and Prof. Wei Lei during the candidature.
- I also take the opportunity to thank: Dr. Ge Xin for many fruitful discussions, valuable suggestions, technical assistances and experimental cooperations; Ms. Chen Si, Ms. Chen shufen and Mr. Liang Haitao for their help in biological tissue preparation; Dr. Liu xinyu for helpful discussions about dispersion compensation; Finally, I would like to thank the rest of my colleagues in Liu Linbo Group at Fiber Technology Lab, Nanyang Technological University for good interactions.
- I am incredibly lucky to have the support of my considerate parents: Bo Linhe and Wang Yongpu. Most importantly, I want to thank my wife, Nie Pin for being with me through all of this and for being my trusted friend. Pin has helped me at every step of my PhD career, and listens to all my frustrations and pushes me to go forward when experiments don't go well. She is an amazing person who is both a persevering theoretical researcher and gentle caring person. I am lucky to meet her.

“The unexamined life is not worth living.”

—Socrates

To my families, mentors and friends

Summary

Diagnosis of most mucosal diseases including epithelial cancers relies on white-light endoscopy/visual inspection followed by random biopsy and histologic examination. These current standard-of-care tools are associated with many issues such as sampling errors, tissue destruction, and processing artifacts. In addition, it is too traumatic to take a biopsy from tissues like eyes and coronary arteries. Being high resolution, high speed, and non-invasive, optical coherence tomography (OCT) has emerged as an endoscopic and intravascular imaging tool that provides valuable anatomic information complementary to the standard-of-care techniques. However, it has a typical resolution of $\sim 10\ \mu\text{m}$, which is too coarse to visualize most cellular structures. Micro-optical coherence tomography (μOCT) provides spatial resolutions of $1\sim 2\ \mu\text{m}$ to visualize microstructures at the cellular and sub-cellular level. One of the major inherent issues with the current μOCT imaging systems is the limited depth of focus (DOF). The DOF, defined as the confocal parameter in the classical theory of Fourier domain OCT (FD-OCT), is proportional to the square of the transverse resolution. Consequently, a trade-off occurs between the transverse resolution and the DOF in which a lower transverse resolution results in a larger DOF and vice versa. Another two of the major inherent issues with current μOCT imaging systems are the auto-correlation artifacts (ACA) and complex conjugate artifacts (CCA). ACA originates from the interference between sample reflectors or scatters of different depths; CCA arises from the fact that the current μOCT system can only detect the real part of the complex interferometric signal.

The main objective of this research is to develop novel optomechanical techniques and apparatus to overcome the above-mentioned issues with OCT to facilitate its clinical applications.

The main contribution of this thesis is the development and validation of multiple aperture synthesis (MAS) techniques and imaging systems to overcome the aberrations of the imaging system such as defocus and those from the turbid tissue. MAS is a kind of digital refocusing technique, which is analogous to synthetic aperture radar (SAR). The key technology of MAS is the implementation of aperture division and

the multiplexing apparatus. In the first attempt, a micro cylindrical lens driven by a piezoelectric transducer (PZT) is employed to demonstrate the feasibility of DOF extension without signal loss and sidelobe artifacts, but at the cost of imaging speed. In the second attempt, a mirror with two surfaces coated is employed to achieve the same DOF extension purpose without sacrificing the imaging time. In the third attempt, I design and develop a calcite beam displacer based MAS apparatus for overcoming defocus and aberrations induced by turbid tissue, which has the potential to scale down and be miniaturized in an endoscopic fiber probe. The proposed MAS techniques may ultimately overcome the inherent trade-off between DOF extension, signal loss/sidelobe artifacts/imaging speed in high-resolution OCT.

Additionally, I develop a novel homemade dual-channel spectrometer by employing two lines of a three-line camera to suppress ACA and CCA. For ACA suppression, two interferometric spectra with a phase difference of π are detected by the dual-channel spectrometer simultaneously. ACA is suppressed by counterbalance signals obtained from dual channels. For CCA suppression, two interferometric spectra with a phase difference of $2\pi/3$ are detected by the dual-channel spectrometer simultaneously. The complex interferometric signal is reconstructed by trigonometric manipulation of two real interferometric spectra, and then ACA is suppressed by use of inverse Fourier transform.

The problems of limited DOF, turbid tissue induced aberration, and fundamental ACA and CCA are addressed in this thesis, which enables us to visualize imperceptible changes in cellular and sub-cellular resolution, and evaluates pathological lesion in an early period by characterizing the cellular and sub-cellular morphology of human tissues. All the novelties enable this project to play a potential role in the clinical practice of disease diagnosis and treatment, especially for gastrointestinal cancer, epithelial cancer, and atherosclerosis.

Contents

Acknowledgements	vii
Summary	xi
Table of Contents	xvi
List of Figures	xvii
List of Tables	xix
List of Abbreviations	xxi
1 General Introduction	1
1.1 Background of the study	2
1.2 Motivation and objective	5
1.3 Significance and novelty	6
1.4 Thesis organization	8
2 OCT Theory	11
2.1 Theory of SD-OCT	12
2.2 Main system parameters	16
3 Depth of Focus Extension in OCT via Serial Multiple Aperture Synthesis	17
3.1 Introduction	18

3.2	Multiple aperture synthesis	21
3.3	Numerical analysis	25
3.4	Experimental methods	29
3.4.1	Optical coherence tomography	29
3.4.2	Micro-cylindrical lens fabrication	31
3.4.3	Phantom preparation	32
3.5	Experimental results	33
3.5.1	DOF extension verification in phantom	33
3.5.2	DOF extension in biological tissue imaging	36
3.6	Discussions and conclusions	39
4	Extending Axial Focus of OCT using Parallel Multiple Aperture Synthesis	43
4.1	Introduction	44
4.2	Materials and methods	46
4.2.1	Multiple aperture synthesized OCT system	46
4.2.2	Multiple aperture synthesis	48
4.3	Experimental results	51
4.3.1	DOF extension in microparticle phantom imaging	51
4.3.2	DOF extension in biological tissue imaging	53
4.4	Discussions and conclusions	55
5	Cellular Resolution <i>in vivo</i> Tomography in Turbid Tissue via Digital Aberration correction	57
5.1	Introduction	58

5.2	Working principle of ADAS-OCT	62
5.2.1	Concept of ADAS-OCT	62
5.2.2	Theory of ADAS-OCT	64
5.2.3	Experimental setup	68
5.3	Experimental results	71
5.3.1	DOF extension verification using microparticle phantom	71
5.3.2	Correction of sample-induced aberrations <i>ex vivo</i>	73
5.3.3	Swine esophagus imaging <i>ex vivo</i>	74
5.3.4	<i>In vivo</i> red blood cells imaging	76
5.3.5	Scalable imaging performance	77
5.4	Discussions and conclusions	79
6	Spectral Domain OCT with Dual-balanced Detection for Auto-correlation Artifacts Reduction	83
6.1	Introduction	85
6.2	Theory	86
6.3	Experimental methods	87
6.3.1	SD-OCT system configuration	87
6.3.2	Sensitivity analysis	90
6.4	Experimental results	91
6.4.1	Optimal spectral matching	92
6.4.2	Auto-correlation artifacts reduction verification	94
6.4.3	Auto-correlation artifacts reduction in stent and cornea imaging	95
6.5	Discussions and conclusions	98

7	Single-camera Full-range High-resolution Spectral Domain OCT	101
7.1	Introduction	103
7.2	Theory	104
7.3	Experimental methods	107
7.4	Experimental results	109
7.4.1	Wavelength dependent coupler splitting ratio	109
7.4.2	CCA suppression verification	111
7.4.3	Sensitivity analysis	113
7.4.4	CCA suppression performance in imaging	115
7.5	Discussions and conclusions	116
8	Conclusions and future developments	119
8.1	Contributions to the field of OCT	119
8.2	Future development of MAS OCT	121
8.2.1	Ranging depth of MAS OCT	121
8.2.2	MAS OCT probe	122
8.2.3	Dual-channel spectrometer design	125
	Author's Publications	127
	Bibliography	129
	Appendix	143

List of Figures

1.1	μ OCT of superficial arterial morphology.	5
2.1	Principle of OCT	13
2.2	Schematic of a Michelson interferometer used in OCT	14
2.3	Schematic of a Michelson interferometer used in OCT	16
3.1	Working principles of multiple aperture synthesis	21
3.2	Numerical simulation results of multiple aperture synthesis	27
3.3	Schematic of the SD-OCT system with multiple aperture synthesis . . .	29
3.4	PZT assembly and adjustment	32
3.5	Stepwise image sequence of the phantom in linear scale	34
3.6	Images of fresh grape samples to verify DOF extension performance . .	36
3.7	Images of rat adipocyte samples to verify DOF extension performance .	38
4.1	Parallel multiple aperture synthesized OCT	47
4.2	DOF extension in microparticles phantom imaging	52
4.3	Cross-sectional images of extracted lemon slices	54
5.1	Concept of aberration correction by ADAS in turbid tissue	63
5.2	Schematic of cellular resolution interferometric tomography with ADAS	65

5.3	Schematic of the ADAS-OCT	69
5.4	Measured axial PSFs with different depth delays	71
5.5	DOF extension verification using microparticle phantom	72
5.6	Correction of sample-induced aberrations	74
5.7	Aberration correction in swine esophagus imaging <i>ex vivo</i>	75
5.8	Aberration correction in red blood cells imaging <i>in vivo</i>	76
5.9	Scalable imaging performance	78
6.1	Optical configuration of SD-OCT system with dual-balanced detection	88
6.2	Optimal spectral matching	93
6.3	Auto-correlation artifacts reduction of a glass plate	94
6.4	Auto-correlation artifacts reduction of a drug-eluting stent	96
6.5	Cross-sectional images of the central area of a swine cornea	97
7.1	Schematic of the instantaneous complex conjugate resolved SD-OCT	107
7.2	Wavelength dependent coupler splitting ratio	110
7.3	Complex conjugate artifacts suppression performance	112
7.4	CCA-suppressed A-lines with different depth delays	113
7.5	Full-range imaging of the anterior segment of a rat eye	115
8.1	Schematic of the polarization encoded probe	122
8.2	Principle of polarization-based multiple aperture division and synthesis	123

List of Tables

1.1	Comparison between OCT, μ OCT and histology	4
4.1	Comparison of FWHMs and transverse resolution captured by standard and parallel multiple aperture synthesized OCT	53

List of Abbreviations

2D	two dimensional	MAS	multiple aperture synthesis
3D	three dimensional	MCL	micro cylindrical lens
2D	two dimensional	MFD	mode field diameter
3D	three dimensional	MRI	magnetic resonance imaging
AC	auto correlation	NA	numerical aperture
ACA	auto correlation artifact	NIR	near-infrared
AO	adaptive optics	OCM	optical coherence microscopy
CC	cross correlation	OCT	optical coherence tomography
CCA	complex conjugate artifact	OPD	optical path difference
CSM	confocal scanning microscopy	PL	path length
CT	computed tomography	PRA	pixel reassignment
CTF	coherence transfer function	PSF	point spread function
DBD	dual-balanced detection	PZT	piezoelectric transducer
DC	direct current	SAR	synthetic aperture radar
DFT	discrete Fourier transform	SD	single detection
DOF	depth of focus	SD-OCT	spectral domain OCT
FD-OCT	Fourier domain OCT	SIM	structured illumination microscopy
FT	Fourier transform	SLD	super luminescent diode
FWC	full well capacity	SMF	single mode fiber
FWHM	full width at half maximum	SNR	signal to noise ratio
IVUS	intravascular ultrasound		

Chapter 1

General Introduction

1.1 Background of the study

Gastrointestinal cancers and standard of care

Colorectal, stomach, and esophageal cancers claims nearly 1.8 million lives and more than 2.5 million new cases per year worldwide, and these cancers are also the most diagnosed cancers in Singapore [1–3]. Since early cancer may be curable and screening of gastrointestinal (GI) tracts is an effective method to detect cancer at an early stage, it is vital for cancer prognosis as early as possible. Neoplasia of the GI tract which easily develops into cancer usually derives from the epithelium of the mucosa. Precise assessment of mucosal features like dysplasia, intestinal metaplasia, mucosal and inter-mucosal carcinoma are therefore of great importance to detect lesions of the GI tract before they progress to an advanced stage [4, 5].

For screening and surveillance of intraepithelial tumor in GI tracts, white light endoscopy (WLE) and random biopsy are two standard-of-care techniques. However, detection and diagnosis of early neoplastic changes in GI tracts is extremely difficult with standard WLE and random biopsy, as the early stage lesions are often confined within the lower half of the epithelium and may not be visible under WLE [7, 8]. This situation is even worse in Barrett’s esophagus and ulcerative colitis where the lesions have a patchy distribution throughout the entire organ [9]. WLE and random biopsy is also limited in guiding endoscopic interventions, especially for colorectal cancer. Colorectal cancer is generally accepted as a progression from hyperplasia (benign adenoma), and thus removing adenomatous polyps can likely reduce the risk of developing malignant tumors [4, 5]. Colonoscopic polypectomy is routinely utilized as colorectal polyps 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 (95% confidence interval) [10], 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 nonneoplastic polyps to avoid unnecessary polypectomies with subsequent colonoscopy and attendant costs [7, 8].

In addition, as histopathologic analysis of biopsy tissues 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 [11, 12].

State-of-the-art endoscopic techniques

Many approaches are being investigated to address these issues, including chromoscopy, narrow-band imaging (NBI), Fluorescence endoscopy, Confocal laser endomicroscopy (CLE), and spectral domain optical coherence tomography (SD-OCT). Magnifying chromoendoscopy, NBI and Fluorescence endoscopy have been shown to enable detailed observation of pit patterns and vascular structures, which is very useful for the qualitative diagnosis of colorectal lesions [6–8]. SD-OCT is a reflectance imaging tool that provide cross-sectional images of human tissue with 10 μm spatial resolution and ~ 2 mm penetration depth. Three-dimensional SD-OCT imaging of the entire distal esophagus has been demonstrated by use of high-speed helical scanning of balloon-centering catheters [13–15]. Although chromoscopy, NBI, and SD-OCT can provide additional information of the imaged areas, cellular features are not well appreciated because of the resolution limit. CLE is one technology that is capable of providing images that contain cellular and subcellular features of the esophageal [9], gastric [16], and colorectal epithelium [17]. Although CLE shows promise, its field size is usually limited to less than 500 μm [17, 18]. Thus, confocal endoscopic imaging may be

subject to sampling errors similar to those of endoscopic biopsy [19]. More importantly, fluorescein-based CLE does not display nuclei, because of the pharmacokinetic properties of fluorescein. Lastly, comparison with standard histopathologic analysis is limited because the fibered confocal microscope produces images of horizontal planes, parallel to the surface of the GI tract, while vertical sections are displayed in routine histopathologic analysis.

Micro-optical coherence tomography

Micro-optical coherence tomography [20, 21], termed μ OCT, demonstrates capability of $1\sim 2\ \mu\text{m}$ resolution imaging, which is more than one order of magnitude higher than the standard OCT technology, which provides an axial (depth) resolution of approximately $10\ \mu\text{m}$ and a transverse resolution of $30\sim 40\ \mu\text{m}$ [23–32] (Table 1.1). This

Table 1.1: Comparison between OCT, μ OCT and histology

Modality	Spatial resolution (x,y,z)	DOF	Histological information
OCT	$30\mu\text{m}\times 30\mu\text{m}\times 10\mu\text{m}$	2-3 mm	Tissue level microscopic <i>in vivo</i>
μ OCT	$2\mu\text{m}\times 2\mu\text{m}\times 1\mu\text{m}$	200 μm	Cellular level microscopic <i>ex vivo</i>
Histology	$500\text{nm}\times 4\mu\text{m}\times 500\text{nm}$	$\sim 5\ \text{mm}$	Subcellular level microscopic <i>ex vivo</i>

breakthrough of μ OCT in spatial resolution brings about a quantum leap in acquired information including cellular and subcellular details (Fig. 1.1) [20–22]. The fact that μ OCT brings about a quantum leap in acquiring information encourages us to continue to push it towards histopathology *in vivo*. However, μ OCT is still an *ex vivo* technology and cannot be used for clinical diagnosis as of now, because μ OCT is still not capable of providing a sufficient depth of focus (DOF) to stably acquire cellular and subcellular information of biological tissues *in vivo*. The limited DOF is mainly induced by aberrations of imaging systems and turbid tissues. Besides, there are two inherent artifacts: auto-correlation artifacts (ACA) and complex conjugate artifacts (CCA) need to be suppressed before we capture good quality images.

Figure 1.1: μ OCT of superficial arterial morphology. (a) Three-dimensional rendering of the swine coronary artery *ex vivo*, showing a pattern of raised cells that are consistent with endothelial “pavementing”. (b) A cell with scant cytoplasm, consistent with a lymphocyte (yellow arrow). (c) A cell with an indented, bean-shaped nucleus (green arrow) characteristic of a monocyte. (d) A cell, possibly a neutrophil (blue arrow), with a multilobed nucleus, is shown attached to the endothelial surface. This figure refers to [20].

1.2 Motivation and objective

The main objective of this research is to develop novel optomechanical techniques and apparatus to overcome the DOF limitation and inherent ACA and CCA issues to facilitate the clinical application of this new generation of OCT technology. The specific aims of the thesis include:

- 1) Design, develop, and validate novel multiple aperture synthesis (MAS) techniques and apparatus for overcoming the aberrations of imaging systems and turbid tissues;
- 2) Design, develop, and validate a low-cost and compact dual-channel spectrometer by employing two lines of a three-line camera to suppress ACA and CCA.

1.3 Significance and novelty

The main significance of this thesis is to enable the cellular resolution OCT (both transverse and axial resolution are $\sim 2 \mu\text{m}$) as a practical imaging tool for clinical applications. The specific significances of the thesis include:

1) MAS technique achieves ~ 10 times DOF extension in cellular OCT, and compensates the aberration induced by the imaging system and turbid tissue. This breakthrough in DOF extension and aberration compensation overcomes the two major technical obstacles toward cellular resolution image *in vivo*: limited DOF and resolution/signal degradation due to aberrations. The technical advances made in this these work may have the following important applications in future:

a. Enable cellular resolution imaging throughout the full thickness of epithelium ($100 \mu\text{m}$) for detection of intraepithelial neoplasms at early stages. The typical epithelial thickness is in the range of hundreds of micrometers, which cannot be covered by a Gaussian DOF of cellular transverse resolution. In the out of focus regions, the resolution and signal intensity is degraded so that missed diagnosis is inevitable. The extended DOF made possible by MAS is the key to achieve full thickness detection for precancerous changes at cellular level.

b. Enable noninvasive high throughput blood cell count. Accurate white blood cell count by high resolution OCT requires screening through a large volume of blood, which is not possible with the limited DOF of the Gaussian beam. Extending the DOF by 10 times enables in-focus imaging of 10 times larger blood volume. In addition, for those blood cells in relatively deep tissue, correction of the aberrations caused by the turbidity of overlying mucosa is required to clearly identify individual cells.

2) MAS implemented by calcite beam displacers may enable, for the first time, cellular resolution endoscopic OCT *in vivo*. The calcite beam displacer based MAS apparatus has the potential to be scaled down to an endoscopic fiber probe. In endoscopic and

intracoronary imaging, in order to maintain the tissue of interest in focus, a large DOF (>2 mm) is required due to the motion of the fiber probe and the tissue caused by the respiration and heartbeat. The extended DOF will make it practical to perform cellular resolution imaging *in vivo* in gastrointestinal tracks and coronary arteries.

3) ACA and CCA are inherent artifacts in OCT system. The dual-channel spectrometer is compact and of low cost, and can also facilitate the clinical application of this new generation of OCT technology.

This thesis has two main innovations: 1) MAS to address the fundamental problem of limited DOF and aberrations induced by turbid tissue in high resolution OCT. 2) A compact dual-channel spectrometer to suppress the fundamental artifacts, including ACA and CCA.

The main significant innovation of this thesis is to achieve a practicable MAS technique for DOF extension and compensation of turbid tissue induced aberrations *in vivo*. Unlike all previous DOF-extended techniques, which used non-Gaussian beams, this technique physically separates the illumination and detection aperture apart, which is analogous to SAR. Then by correcting the optical path difference and defocus-induced wavefront curvature, images with distinctive aperture are coherently summed together. This system digitally refocuses the sample and obtains a brand-new image with nearly the same transverse resolution over an extended DOF, which is 10 times more than the confocal parameter. Most importantly, the MAS technique can compensate the turbid tissue induced aberrations when performing *in vivo* imaging. The capability of DOF extension and aberration compensation will greatly facilitate the clinical application of this new generation of OCT technology.

Another innovation is to develop a novel home-made dual-channel spectrometer by employing two lines of a three-line camera to suppress ACA and CCA. For ACA suppression, two interferometric spectra with a phase difference of π are detected by the

dual-channel spectrometer simultaneously. By cancelling the identical components between dual-balanced spectrometer channels, this OCT system achieves a signal-to-noise ratio (SNR) increase of ~ 2.9 dB, ACA reduction up to ~ 9 dB and direct current term suppression up to ~ 30 dB. For CCA suppression, two interferometric spectra with a phase difference of $2\pi/3$ are detected by the dual-channel spectrometer simultaneously. The complex interferometric signal is reconstructed by trigonometric manipulation of two real interferometric spectra, and then ACA suppression is up to ~ 25 dB by use of inverse Fourier transform. These advantages including ACA and CCA suppression significantly improve the OCT images with regard to the single-channel images. The novel MAS together with dual-channel spectrometer in this thesis, enable us to visualize imperceptible changes in cellular and sub-cellular resolution, and evaluate pathological lesion in an early period by characterizing cell type, cellularity, cell polarity, cell viability, nucleus morphology. All the novelties enable this thesis to play a potential role in the clinical practice of disease diagnosis and treatment, especially for gastrointestinal cancer, epithelial cancer, atherosclerosis and coronary artery disease.

1.4 Thesis organization

This thesis consists of 7 chapters.

Chapter 1 introduces the background of this study firstly, including clinical needs and general diagnostic techniques. Then the motivation and objective of this thesis is introduced, including what will be done and can be achieved at the end of this thesis. The significance and novelty of proposed techniques is elaborated at Section 1.3 in details.

Chapter 2 introduces the basic theory of spectral domain OCT (OCT) and main performance parameters, including axial resolution, transverse resolution and DOF.

Chapter 3 reports a novel technique—MAS for overcoming the DOF limitation in high resolution OCT. The aperture division and multiplexing are implemented by employ-

ing a piezoelectric (PZT) driven cylindrical lens. The DOF extension is experimentally validated to be 10 times of the Confocal parameter. Experimental results demonstrate the feasibility and efficacy of high-resolution biological tissue imaging with a dramatic DOF extension.

Chapter 4 proposes a novel parallel MAS OCT by employing a mirror with two surfaces coated without sacrificing imaging speed. The proof-of-concept experiments demonstrate the feasibility address the fundamental problem of limited DOF in high resolution OCT.

Chapter 5 illustrates a novel MAS OCT by employing a calcite beam displacer. This novel design overcomes defocus and aberrations induced by turbid tissue, which will greatly facilitate the clinical application of this new generation of OCT technology. In my opinion, this novel design has the potential to scale down and is the most practical technique potential to be miniaturized for endoscopic applications.

Chapter 6 reports a SD-OCT to reduce ACA using dual-balanced detection. This new design is of lower cost and enables better ACA suppression, compared with the dual spectrometer design reported previously.

Chapter 7 demonstrates a SD-OCT using a dual-channel spectrometer for CCA suppression. The quality of CCA-suppressed images is significantly improved over prior arts.

Chapter 8 summarizes the work presented in this thesis and discusses the future work.

Chapter 2

OCT Theory

Being high resolution, high speed, and non-invasive, OCT has been broadly applied in ophthalmology and endoscopy imaging of transparent and turbid tissue [23]. Since Spectral domain OCT (SD-OCT) was introduced [29, 101–103], researchers have made significant progress in vivo retinal imaging [104, 105], 3D volumetric imaging [106], ultra-high resolution imaging [20, 55, 107], Doppler blood flow determination in the human retina [108, 109], and so on.

2.1 Theory of SD-OCT

The basic schematic of a general SD-OCT imaging system is depicted Fig. 2.1(a). Light from a broad-band source is directed into a 2×2 fiber optic coupler, and a simple Michelson interferometer is built. The coupler is assumed to split the incident optical power into sample and reference arms by ratio of 50:50. Light exiting the reference fiber is incident upon a reference delay and redirected back into the same fiber. Light exiting the sample fiber is incident upon a scanning mechanism, which is designed to scan the biomedical sample in two transverse directions. The light backscattered or reflected from the sample is interfered with the reference arm light in the fiber coupler, and projected on a photo detector. The detected interference signals are processed by computer to generate an A-scan, which represents the profile along the axial direction corresponding to some fixed point of the biomedical tissue.

SD-OCT performs cross-sectional imaging by measuring the magnitude and echo time delay of backscattered light. Cross-sectional images are captured by performing multiple measurement of echo time delay along the axial direction, and scanning the biomedical tissue transversely [33]. Multiple A-scans are arrayed to generate a cross-sectional image. In the same way, multiple cross-sectional images are arrayed to generate three-dimensional volume data sets, as shown in Fig. 2.1(b).

Consider the Michelson interferometer shown in Fig. 2.2, The interferometer is illu-

Figure 2.1: Principle of OCT. (a) Schematic of a general SD-OCT system, (b) OCT generates A-scan, and cross-sectional or three-dimensional images[33].

minated by a plane wave whose electric field expressed in complex form as E_i . Here, $s(k, \omega)$ is the electric field amplitude as a function of the wavenumber $k = 2\pi/\lambda$, and angular frequency $\omega = 2\pi\nu$, which are respectively the spatial and temporal frequencies of each spectral component of the field having wavelength λ . The wavelength λ and frequency ν are coupled by the index of refraction $n(\lambda)$. The beam splitter is assumed to have an achromatic (wavelength-independent) power splitting ratio of 0.5. The reference reflector is assumed to have electric field reflectivity r_R and power reflectivity $R_R = |r_R|^2$. The sample is assumed to have electric field reflectivity r_S and power reflectivity $R_S = |r_S|^2$. The distance from the beam splitter to the reference reflector and sample is respectively z_R and z_{Sn} . Arbitrarily setting $z = 0$ at the surface of the beam splitter, the detector current can be donated as

Figure 2.2: Schematic of a Michelson interferometer used in OCT[33].

$$I_D(k, w) = \frac{\rho}{2} \langle \left| \frac{s(k, \omega)}{\sqrt{2}} r_R e^{i(2kz_R - \omega t)} + \frac{s(k, \omega)}{\sqrt{2}} \sum_{n=1}^N r_{Sn} e^{i(2kz_{Sn} - \omega t)} \right|^2 \rangle \quad (2.1)$$

where ρ is defined as the responsivity of the detector, the factor 2 represents round-trip of each field through the beams-plitter, and the angular brackets denote integration over the response time of the detector[33]. $S(k)$ is substituted using $|s(k, \omega)|^2$. Expanding the magnitude squared functions in Eq. (2.1) eliminates the terms dependent upon the temporal angular frequency $\omega = 2\pi\nu$. Using Eulers rule to simplify Eq. (2.1) generates a real result for the detector current as a function of wave-number, commonly known as the spectral interferograms

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

As shown in Eq. (2.2), there are three components: direct current (*DC*) terms, auto-correlation *AC* terms and cross-correlation (*CC*) terms. *DC* term is a pathlength-independent bias, and its amplitude is proportional to the sum of the power from reference and sample arms. *AC* term is mainly composed of the interference signals

between different sample tissue depths, and served as artifacts in OCT images. AC term cannot be neglected especially for tissues with layered structures. CC term that encodes depth-resolved information is necessary component for OCT imaging. Generally, the detected interference signal in k -space can be expressed in simplified formulation.

$$I_D(k) = S(k)[DC + 2 \int_{-\infty}^{+\infty} a(z) \cos(2knz + \phi) dz + AC] \quad (2.3)$$

where $S(k)$ is the spectral intensity distribution of the light source, $a(z)$ is the amplitude of the elementary waves backscattered from the sample tissue and reference mirror at depth of z . n is the refractive index of the sample tissue. ϕ is the phase shift of the interferometric signal.

The sample reflectivity profile $r_S(z_S)$ is estimated from the inverse Fourier transform (FT) of $I_D(k)$. Define the inverse FT of $S(k)$ as $\gamma(z)$. The inverse FT of Eq. (2.2) may be calculated as

$$\begin{aligned} I_D(k) = & \frac{\rho}{8} \{ \gamma(z) [R_R + R_{S1} + R_{S2} + \dots + R_{Sn}] \} & \text{“DC”} \\ & + \frac{\rho}{4} \{ \gamma(z) \bigotimes \sum_{m \neq n \neq 1}^N \sqrt{R_R R_{Sn}} \delta[z \pm (z_{Sn} - z_{Sm})] \} & \text{“AC”} \\ & + \frac{\rho}{8} \{ \gamma(z) \bigotimes \sum_{n=1}^N \sqrt{R_R R_{Sn}} \delta[z \pm (z_R - z_{Sn})] \} & \text{“CC”} \end{aligned} \quad (2.4)$$

Because only the CC encodes depth-resolved information for OCT imaging, DC and AC terms are not took into consideration. For a certain depth z_0 , the inverse FT of Eq. (2.3) may be calculated and expressed in simplified formulation, then the depth-resolved profile can be obtained.

$$i_D(z) = \gamma(z) \bigotimes \sum_{n=1}^N a(z) [\delta(z + z_0) + \delta(z - z_0)] \quad (2.5)$$

2.2 Main system parameters

The axial resolution (Δz) is determined by the bandwidth of the light source and is equal to the coherence length of the light source.

$$\Delta z = \frac{2 \ln 2}{\pi} \frac{\lambda_0^2}{\Delta \lambda} \quad (2.6)$$

where λ_0 and $\Delta \lambda$ are the center wavelength and the wavelength bandwidth of the laser source, respectively.

The transverse resolution (Δx) and depth of focus (DOF) are both determined by the the numerical aperture ($NA = d/2f$) of the objective lens as shown in Fig. 2.3. d is the beam size of the laser source and f is the focal length of objective lens. λ_0 is the central wavelength of the light source.

$$\begin{cases} \Delta x = \frac{2\lambda_0}{\pi} \frac{1}{NA} \\ DOF = \frac{4\lambda_0}{\pi} \frac{1}{NA^2} \end{cases} \quad (2.7)$$

Figure 2.3: Schematic of OCT focusing beam in sample arm. The low and high NA optics geometries show a trade-off between transverse resolution and depth of focus for an OCT system [33].

Chapter 3

Depth of focus extension in optical
coherence tomography via serial
multiple aperture synthesis

Abstract

In this work, we report a novel technique for overcoming the depth of focus (DOF) limitation in optical coherence tomography (OCT). Using confocal optics on a sample arm, we scanned the illumination beam across the under-filled objective lens pupil plane by steering the beam at the pinhole using a micro-cylindrical lens. The detected interferometric signals from multiple distinctive apertures were digitally refocused, which is analogous to synthetic aperture radar (SAR). Using numerical simulations and phantom imaging experiments, we verified that this technique can maintain a diffraction-limited transverse resolution along a DOF that is ~ 10 times larger than the confocal parameter. In this study, we applied this technique for the first time to image biological tissue. The ability to extend the DOF without signal loss and sidelobe artifacts may ultimately overcome the DOF limitation in high-resolution OCT.

3.1 Introduction

OCT is a cross-sectional and three-dimensional (3D) imaging technique that provides non-invasive, high-speed and high-resolution images of scattering media, such as inhomogeneous biological tissues [23]. Up until now, this technique has been widely applied in ophthalmology [34] and cardiology [35]. In OCT, the transverse resolution Δx is determined

by the diffraction-limited spot size of the sample focused beam and defined as the beam waist diameter under the assumption of a Gaussian input beam. This parameter can be expressed as $\Delta x = 2\lambda/\pi NA$, where $NA = d/2f$ is the numerical aperture (NA) of the objective lens. The corresponding depth of focus (DOF) can be defined as the confocal parameter and is expressed as $DOF = 4\lambda/\pi NA^2$, which represents the axial distance between the values of z (axial depth) where the size at the $1/e^2$ beam is two times larger than that at the beam waist. According to the definitions stated above, the transverse resolution is inversely proportional to the NA of the objective lens, and the DOF is proportional to the square of the transverse resolution. Consequently, a trade-off occurs between the transverse resolution and the DOF in which a lower transverse resolution results in a larger DOF and vice versa.

To achieve a high transverse resolution over an extended DOF, various approaches have been proposed. Axicon lenses and phase and amplitude pupil filters can generate a focus with Bessel beam characteristics, including a more uniform main lobe diameter in the transverse direction over an extended DOF compared with the confocal parameter [20, 36]. However, in the spatial frequency domain, Bessel beams suffer a major loss of spatial frequency components in the coherence transfer function (CTF) compared with that of Gaussian beams, which results in a significant decline of sensitivity and sidelobe artifacts in the OCT images [37]. Although these problems can be mitigated by dark-field

Gaussian detection, the fundamental problems of a suboptimal CTF must still be resolved [37–39]. Digital refocusing has drawn considerable research interest because an optimal CTF can be maintained, which in principle prevents reductions in sensitivity and sidelobe issues [40–43]. A previously reported digital refocusing approach, interferometric synthetic microscopy (ISAM), computationally reconstructs diffraction-limited transverse resolutions by solving the inverse scattering problem. However, the ISAM approach requires absolute phase stability during 3D image acquisition, which is difficult to realize *in vivo*, especially in endoscopic applications [40]. Recently, a novel approach named depth-encoded synthetic aperture has been reported, and it extends the DOF by synthesizing different apertures and correcting the wavefront curvature caused by the defocus effect [41–43]. This approach does not require a phase stabilizing setup, and it has low computational costs and good compatibility with conventional Fourier domain OCT (FD-OCT) hardware. However, an inherent issue of signal coupling loss caused by the mismatch between the NA of the fiber pinhole and apertures generated by the wavefront divider/splitter is observed, and an overall 50% signal loss can be attributed to the implementation of aperture division and the multiplexing apparatus [42, 43]. Here, we demonstrate a multiple aperture synthesis (MAS) technique for achieving a digital refocusing function without signal loss or sidelobe artifacts.

3.2 Multiple aperture synthesis

Figure 3.1: Working principles of multiple aperture synthesis. (a) Schematic with a micro-cylindrical lens centered at the fiber pinhole. (b) Schematic with a micro-cylindrical lens transversely shifted by a step size of ΔL . (a-b) Magnified views of the optical beam indicated by the dashed boxes in (a-b). (c) Five apertures generated by the transversely shifted micro-cylindrical lens and the aperture of the objective lens. (d) Focusing beams of the five apertures in (c). (e) Optical axes of the focusing beams of the five apertures in (c). SMF, single mode fiber; MCL, micro-cylindrical lens; PZT, piezoelectric transducer; L1, collimating lens; L2, focusing lens; LF, low spatial frequency; and HF, high spatial frequency. Δz is the total optical path difference between A-lines acquired in (a) and (b), and composed of two components: Δz_s induced by the transverse shift of the MCL and the defocusing term Δz_c . ΔL is the transverse shifting step size of the micro-cylindrical lens.

The MAS technique extends the DOF in a spectral domain OCT (SD-OCT), which is analogous to synthetic aperture radar (SAR). In Fig. 3.1(a) and 3.1(a), a micro-cylindrical lens (MCL) is positioned $\sim 10 \mu\text{m}$ away from the tip of the sample fiber with an axisymmetric configuration along the optical axis. All light paths of the spatial (angular)

frequencies originating from the fiber pinhole are refracted sequentially by MCL, L1 and L2 and intersect at the focal point of L2 with a uniform phase or optical path length. Under this ideal condition, a maximal constructive interference occurs at this focal point, which results in a diffraction-limited transverse point spread function (PSF). When the MCL is linearly shifted along a transverse direction perpendicular to the cylindrical axis by a piezoelectric transducer (PZT) (Fig. 3.1(b) and 3.1(b)), the spatial or angular frequency of the sample beam with respect to the full aperture of the focusing lens L2 is swept as the incidence angle at the curved surface of the MCL changes. The aberration caused by the cylindrical lens is not noticeable and the resolution in the x and y transverse dimensions is the same.

Fig. 3.1(c) depicts the effective optical apertures in the objective lens (L2) pupil plane. Apertures 1-5 denote five apertures generated by transversely shifting the MCL. Fig. 3.1(d) illustrates the spatial or angular frequency of focused beams 1-5, which correspond to apertures 1-5 in Fig. 3.1(c) in the focal plane of the objective lens L2. During image acquisition, we chose to acquire an axial line (A-line) at each of the five equally spaced spatial frequencies and synthesize five apertures together as shown in Fig. 3.1(e). Compared with the standard SD-OCT, more high spatial frequency light signals of the sample reflectivity can be coherently synthesized, which will theoretically and experimentally generate a larger aperture and an extended DOF.

The total optical path difference (OPD) between A-lines acquired in Fig. 3.1(a) and (b) is defined as $\Delta z = \Delta z_s + \Delta z_c$ and composed of two components: Δz_s , which is induced by the transverse shift of the MCL; and Δz_c , which is the defocusing term with $\Delta z_c = 0$ at the focus of L2. For scatter located away from the focal point, when these OPDs are corrected to 0, the A-lines with distinctive apertures can be coherently summed together to form a digitally synthesized aperture.

In classical theory of Fourier domain OCT, the detected interference signal $I(k)$ in k -space, including DC, AC and CC terms, was presented in Eq. (2.3). Because only the CC encodes depth-resolved information for OCT imaging, $S(k)$, DC and AC can be omitted in the following derivation. The original CC term for the n -th cross-sectional image is as follows:

$$I_n(k) = \sqrt{I_r(k)I_s(k)}[\exp(i2kz_n) + C.C.] \quad (3.1)$$

where $C.C.$ is the abbreviation for the complex conjugate, which will also be omitted in the following derivation. The subscript n represents the sequence number of distinctive apertures. To correct the OPDs and the defocus term, we define $k = k_0 + \Delta k$ and $z = z_n + \Delta z_n$, where z is the corrected optical path length for rendering a perfect focus. Then, Eq. (3.1) is rewritten as follows:

$$I_n(k) = \exp(i\alpha_n)\sqrt{I_r(k)I_s(k)}[\exp(i2kz_n)\exp(i\beta_n)] \quad (3.2)$$

where $\alpha_n = -k_0 \cdot \Delta z_n$ and $\beta_n = -\Delta k \cdot \Delta z_n$. Eq. (3.2) includes a constant phase α_n and an oscillation phase β_n , which are induced by multiple transverse shifts of the MCL and the defocusing term. The MAS process consists of two main steps to correct α_n and β_n in sequence.

First, according to the Fourier shift theorem, we correct the axial-delayed OPDs and axially shift them to the aligned depth by multiplying the cross-sectional image by $\exp(-i\alpha_n)$. This step is called the axial shift. The n -th axial-shifted cross-sectional image is calculated as follows:

$$I_n^{ax}(k) = I_n(k) \cdot \exp(-i\alpha_n) \quad (3.3)$$

where the superscript *ax* represents the axial-shift operation. When the criterion $|\sum_{n=1}^m I_n(k) \cdot \exp(-i\alpha_n)|$ reaches its maximum value, α_n achieves its optimal value α_n^{op} , which is not depth-dependent and is constant for each cross-sectional images; here, the superscript *op* represents the optimal value and m represents the total number of apertures.

Second, the constant phase β_n can be eliminated by multiplying the Fourier spectrum of Eq. (3.3) by a constant phase coefficient $\exp(-i\beta_n)$. This step is called the defocusing correction. The n -th defocusing-corrected cross-sectional image is calculated as follows:

$$I_n^{de}(k) = I_n^{ax}(k) \cdot \exp(-i\beta_n) \quad (3.4)$$

where the superscript *de* represents the defocusing-correction operation.

To precisely correct the defocusing term, we must obtain the optimal β_n at depth z for each cross-sectional image. The optimal $\beta_n(z)$ is strictly contained in the phase set $\{\pi/2, \pi, 3\pi/2, 2\pi\}$. When the criterion $\max\{|\sum_{n=1}^m I_n^{ax}(k) \cdot \exp[-i\beta_n(z)]|\}$ reaches its maximum value, $\beta_n(z)$ achieves its optimal value $\beta_n^{op}(z)$. Combining Eqs. (3.3) and (3.4), the digitally refocused cross-sectional image is calculated as follows:

$$I_{MAS} = \sum_{n=1}^m I_n(k) \cdot \exp(-i\alpha_n^{op}) \cdot \exp[-i\beta_n^{op}(z)] \quad (3.5)$$

This MAS achieves optimal constructive interference at the center of the scatter and destructive interference away from the center, which not only increases the total backscattered intensity but also preserves the diffraction-limited transverse resolution over a DOF extended by multiple times.

3.3 Numerical analysis

The image formation in an OCT system can be described by a CTF, which is the Fourier transform of the effective PSF [44–47]. Here, we define $p(m, n)$ as the pupil function (PF) of the objective lens. m and n are the corresponding radial spatial frequencies in two transverse directions. Because the light passes the objective lens twice, the two-dimensional (2D) CTF can be obtained by convolving PFs of the illumination and

detection aperture [47–50].

$$C(m, n) = p(f\lambda m, f\lambda n) \otimes \otimes p(f\lambda m, f\lambda n) \quad (3.6)$$

where $\otimes \otimes$ represents the 2D convolution operation. f and λ represent the focal length of the objective lens and the wavelength of the illumination light. The PSFs are the Fourier transform of the CTFs in two transverse directions and are calculated as follows:

$$F(x, y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} C(m, n) \exp[-2\pi i(mx + ny)] dm dn \quad (3.7)$$

where x and y denote the variables on the plane perpendicular to the optical axis and z is the axial variable.

We performed numerical simulations using MATLAB software (MathWorks, Natick, MA, USA) to characterize PFs, PSFs and CTFs of the MAS technique with respect to available methods. Here, we consider three aperture types in the illumination and detection paths: full aperture, annular apodized aperture and MAS. We define the radius of the full aperture as 1 (Fig. 3.2(a)) and the radii of the annularly apodized aperture as 0.7 (Fig. 3.2(b)). In the case of MAS, five apertures were equally spaced with a transverse spacing of 0.25 as indicated by the bold numbers 1-5 in Fig. 3.2(c). Under the condition of Gaussian illumination, CTFs (Fig. 3.2(d-f)) corresponding to PFs (shown in Fig. 3.2(a-c)) were obtained by convolving PFs of the illumination and de-

Figure 3.2: Numerical simulation results. (a-c) PFs of a full aperture, an annular apodized aperture and an MAS. (d-f) Two-dimensional coherent transfer functions (CTFs) of a full aperture, an annular apodized aperture and an MAS in the focal plane as a function over the transverse (m and n) spatial frequencies. (g-i) Two-dimensional CTFs of a full aperture, an annular apodized aperture and an MAS as a function over the transverse spatial frequency (m) and axial (z) dimensions. (j-l) Two-dimensional PSFs of a full aperture, an apodization and an MAS as a function over the transverse (x) and axial (z) dimensions. (m-n) Transverse CTFs in the focal plane and at an out-of-focus plane of $z = b$, respectively. b is the confocal parameter. (o-p) Transverse PSFs in the focal plane at an out-of-focus plane of $z = b$ and $10b$. Intensities in all figures are normalized by their maximum values.

tection aperture. The 2D CTFs (Fig. 3.2(d-f)) show that although the annular apodized aperture produces the largest frequency range, the cut-off spatial frequency of the MAS and full aperture is much greater than that of the apodization. The MAS technique has a better transverse CTF than the full aperture in the focal plane (Fig. 3.2(m)), even has a similar transverse CTF as the full aperture at an out-of-focus plane of $z = b$ (Fig. 3.2(n)), where b is the confocal parameter. 2D PSFs (Fig. 3.2(j-l)) can be obtained via the Fourier transform of CTFs in two transverse directions. The apodized aperture produces the largest DOF but the worst sidelobes (-17.5 dB, Fig. 3.2(k)). Both transverse PSFs of the full aperture (Fig. 3.2(j)) and MAS (Fig. 3.2(l)) have insignificant sidelobes (< -61.9 dB and -46.4 dB, respectively), compared with the sidelobe of -20 dB in Ref. [39]. However, the DOF of the MAS (Fig. 3.2(l)) is much greater than that of the full aperture (Fig. 3.2(j)). The full-width-at-half-maximum (FWHM) transverse spot size of the full aperture doubles at depth b , whereas that of the apodized aperture and MAS are expanded by 13.9% and 73.0% (Fig. 3.2(p)) at depth $10b$, respectively. These results indicate that the MAS technique can extend the DOF by a value that is more than 10 times that of the full aperture.

3.4 Experimental methods

3.4.1 Optical coherence tomography

The schematic of a MAS SD-OCT system is illustrated in Fig. 3.3. A super luminescent diode (SLD) array (T850-HP, Superlum, County Cork, Ireland) provides a broadband spectrum with an FWHM bandwidth of 165 nm centered at 850 nm. The light source, whose total output power is 18.0 mW, is split into a sample light and reference light by a 2×2 wideband fiber-optic coupler (TW850R2A2, Thorlabs, Newton, NJ, USA) with a splitting ratio of 90:10. The light beam in the reference arm is collimated by lens L1 (AC050-010-B-ML, Thorlabs, Newton, NJ,

Figure 3.3: Schematic of the MAS SD-OCT system. SLD, super luminescent diode; OC1-2, 90:10 fiber-optic coupler; L1, L3, L5 and L6, collimating lens; L2 and L4, focusing lens; L7, camera lens; NDF, neutral density filter; RM, reference mirror; MCL, micro-cylindrical lens; PZT, piezoelectric transducer; BS, beam splitter; GS, galvo scanner; G, transmission diffraction grating; IMAQ, image acquisition; C, computer; and PC, polarization controller.

USA), transmitted through a neutral density filter (NDL-10C-4, Thorlabs, Newton, NJ, USA) and focused by lens L2 (M Plan Apo NIR 20 $\times$, Mitutoyo, Takatsu-ku, Kawasaki, JP) onto the reference mirror RM (PF10-03-P01, Thorlabs, Newton, NJ, USA). L2 and RM move together to match the path lengths in the sample and reference arms. The light beam in the sample arm is collimated by lens L3 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA), projected onto the galvo scanner (GVS002, Thorlabs, Newton, NJ, USA), and then focused by the objective lens L4 (M Plan Apo NIR 20 $\times$, Mitutoyo, Takatsu-ku, Kawasaki, JP). The mode field diameter (MFD) of the fiber (780 HP, Thorlabs, Newton, NJ, USA) is $5.0 \pm 0.5 \mu\text{m}$ (defined by the $1/e$ field) at 850 nm. The MCL is driven by a closed-loop PZT (AE0505D16F, Thorlabs, Newton, NJ, USA). A beam splitter (BS; BS017, Thorlabs, Newton, NJ, USA) is inserted in the sample arm to build up a dark field apparatus. The backscattered light from the sample is focused by lens L5 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA) and guided to the other 2×2 wideband fiber-optic coupler (TW850R2A2, Thorlabs, Newton, NJ, USA) with a splitting ratio of 90:10. The light beams from two arms are combined in the coupler and directed into the spectrometer after being collimated by the achromatic lens L6 (AC254-040-B-ML, Thorlabs, Newton, NJ, USA). The spectrometer consists of a 1765 line/mm diffraction grating (PING-Sample-020, Ibsen Photonics, Farum, Denmark), a camera lens (85 mm, f/1.4, Zeiss, Oberkochen, Germany) and a 4096-pixel camera (AViiVA EM4, e2V, Chelmsford, UK). The detected spec-

trum is digitized at a 12-bit resolution and transferred to a computer via an image acquisition board (KBN-PCE-CL4-F, Bitflow, Woburn, MA, USA). Two-directional transverse scanning is implemented by two galvanometer-mounted mirrors driven with saw-tooth pulses, which are generated by a 16-bit analog output of a data acquisition (DAQ) board (PCI-6221, National Instruments, Austin, TX, USA). Image acquisition and galvo mirror scanning are synchronized by the external trigger from a DAQ digital output. A discrete Fourier transform is performed on each frame of the 1024 A-lines obtained by the camera to resolve the axial depth profile of the sample.

3.4.2 Micro-cylindrical lens fabrication

We selected a closed-loop PZT with four embedded strain gauges and a travel range of 15.7 μm . The output diverging beam of the sample fiber has a full angular range (defined by the $1/e^2$ power) of 7.45 ($= 4\lambda/\pi\Delta x$) degrees, which is determined by the spot diameter Δx of the focused beam. The desired radius of the MCL is 60~70 μm based on the travel range of the PZT and fiber NA. The MCL is precisely grinded from the coreless silica termination fiber (FG125LA, Thorlabs, Newton, NJ, USA). Firstly, the coreless fiber with a diameter of 125 μm is cured in a V-groove mould, with two halves of the coreless fiber in and out of the V-groove. Secondly, the V-groove with coreless fiber cured on is grinded until the diameter of the coreless fiber needs to be measured to

be $110\ \mu\text{m}$. As shown in Fig. 3.4(a), the PZT is mounted on a 2-axis goniometer (GNL20, Thorlabs, Newton, NJ, USA) and a 3-axis compact stage (MBT616D, Thorlabs, Newton, NJ, USA). The inset provides a magnified view, which shows that two MCLs are cured on the silica. The cross-sectional width of the MCL is measured at $110\ \mu\text{m}$ as shown in Fig. 3.4(b). Fig. 3.4(c) is a side view showing the position of the MCL relative to the tip of the sample arm fiber.

Figure 3.4: PZT assembly and adjustment. (a) PZT is mounted on a 2-axis goniometer stacked on a 3-axis compact stage. Inset: the magnified view shows that two MCLs are cured on the silica. (b) Cross-sectional width of the micro-cylindrical lens. (c) Alignment of the MCL and the tip of the sample fiber.

3.4.3 Phantom preparation

A phantom of polystyrene calibration microparticles was constructed by mixing agarose solution (No. PC0701-100g, Vivantis, Oceanside CA, USA) with polystyrene microparticles (No. 64090-15, nominal size 6

μm , Sigma-Aldrich, St. Louis, Missouri, USA). This mixture was stored in a vial and placed in an ultrasonic bath for ten minutes to remove residual clusters. The sample (10 g) was poured into a cell culture dish, cured for 30 minutes at 100 °C and then cured for 24 hours at room temperature.

3.5 Experimental results

3.5.1 DOF extension verification in phantom

To demonstrate the performance of the DOF extension using the MAS technique, we conducted imaging experiments using the microparticle polystyrene calibration sample. Five cross-sectional images ($m = 5$) were acquired using the MAS SD-OCT system, and they corresponded to five transverse shifts of the MCL with five distinctive apertures. One of cross-sectional images was dispersion compensated using the algorithm in Ref. [51] and is shown in Fig. 3.5(a), which indicates that the cross-sectional images suffered from defocusing beyond the focus. According to Eqs. (3.3) and (3.4), the MAS process consisted of two steps: an axial shift and a defocusing correction. The results of the axial shift and defocusing correction are shown in Fig. 3.5(b) and (c), respectively. In Fig. 3.5(a-c), magnified views of two calibration microparticles are indicated by the dashed boxes. We measured the transverse FWHMs of 50 microparticles at different depths in Fig. 3.5(a) and (c) and performed

Figure 3.5: Stepwise image sequence of the phantom in linear scale: (a) Dispersion-compensated cross-sectional image from one of five apertures; (b) Resulting cross-sectional image of the axial-shift operation, which is the first step of the MAS; (c) Resulting cross-sectional image of the defocusing correction operation, which is the second step of the MAS. (a-c) Magnified view of two calibration beads indicated by the dashed black boxes in (a-c). (d) Transverse FWHMs of 50 calibration beads at variable depths.

the 10-order polynomial-fitting as shown in Fig. 3.5(d). Because the MFD (defined by the $1/e$ field) was $5.0 \pm 0.5 \mu\text{m}$, the theoretical transverse FWHM of the PSF at focus was estimated at $2.92 \mu\text{m}$ and the corresponding DOF was $16.01 \mu\text{m}$ at the center wavelength of 850 nm . The finest transverse FWHM of the refocused microparticle was $9.06 \mu\text{m}$; thus, the finest nominal transverse FWHM of the PSF was $3.06 \mu\text{m}$, which corresponded to a DOF of $17.30 \mu\text{m}$. The experimental results are consistent with the theoretical results. The DOF was estimated within a depth range from $910 \sim 927 \mu\text{m}$, where two fitted lines crossed in Fig. 3.5(d). Over the full axial depth range of $1030 \mu\text{m}$ ($n = 1.33$), the maximum, averaged, and minimum transverse FWHMs of the microparticles in Fig. 3.5(a) were $39.34 \mu\text{m}$, $20.16 \mu\text{m}$ and $9.26 \mu\text{m}$, respectively; therefore, the maximum, averaged, and minimum transverse FWHMs of the PSFs were $33.34 \mu\text{m}$, $14.16 \mu\text{m}$ and $3.26 \mu\text{m}$, respectively. In contrast, the maximum, averaged, and minimum transverse FWHMs of the microparticles in Fig. 3.5(c) were $13.64 \mu\text{m}$, $11.21 \mu\text{m}$ and $9.06 \mu\text{m}$, respectively; therefore, the maximum, averaged, and minimum transverse PSFs were $7.64 \mu\text{m}$, $5.21 \mu\text{m}$ and $3.06 \mu\text{m}$, respectively. Within the depth range of $760 \sim 940 \text{ m}$, the measured transverse PSF is smaller than 2-times of the diffraction-limited transverse PSF. Thus, the MAS SD-OCT system appears to preserve the diffraction-limited transverse resolution over the full axial depth, and it obtained a DOF extension of ~ 10 -fold, which is consistent with the results of the numerical simulation.

3.5.2 DOF extension in biological tissue imaging

Figure 3.6: Images of fresh grape samples to verify DOF extension performance. (a) Dispersion-compensated cross-sectional image (intensity in logarithmic grayscale), captured from one of five apertures; (b) Digitally refocused cross-sectional image via MAS; (c-d) Two boxed areas away from the focus by $329\ \mu\text{m}$ and $760\ \mu\text{m}$ are indicated in orange and green colors and magnified by two times. The transverse profiles indicated by the dashed lines show that the defocused cell wall was well digitally refocused.

To demonstrate the DOF extension performance in biological tissues, we conducted two imaging experiments: the fresh grape and the rat adipocyte *ex vivo*. Figure 3.6 shows the resultant images of the fresh grape sample, including the dispersion-compensated cross-sectional image (Fig. 3.6(a)) and digitally refocused cross-sectional image (Fig.

3.6(b)) with a size of $1747\ \mu\text{m}$ (transverse) $\times$ $1069\ \mu\text{m}$ (axial). Two local areas at depths of $329\ \mu\text{m}$ and $760\ \mu\text{m}$ away from the focus were selected and magnified by two times (Figs. 3.6(c) and 3.6(d)). After applying MAS, the blurred cell walls were digitally refocused and became sharper and brighter. The transverse profiles indicated in orange and green dashed line showed that the cell wall widths were sharpened from $27.7\ \mu\text{m}$ and $23.3\ \mu\text{m}$ to $5.7\ \mu\text{m}$ and $7.4\ \mu\text{m}$, respectively. Anisotropy was an effective indicator of image sharpness, and increased as the blur diminished [52–54]. To quantitatively determine the image quality and assess its improvement, the anisotropy ratio of the digitally refocused cross-sectional image to the dispersion-compensated cross-sectional image was calculated over the whole depth range (Fig. 3.6(e)). Over the depth range of $130 \sim 920\ \mu\text{m}$, the anisotropy ratio was above 1, and fluctuated around 1.2, which meant the transverse resolution was dramatically improved. Over the depth range of $1 \sim 130\ \mu\text{m}$, the anisotropy ratio was below 1 for the sake of strong reflection on the tissue surface. Two valleys of the anisotropy ratio at the depth of $930\ \mu\text{m}$ and $980\ \mu\text{m}$ was generated for the sake of visible line artifacts in the transverse direction and limited image intensity. Over the whole depth range of $30 \sim 1069\ \mu\text{m}$, MAS had a dramatic refocusing performance.

To further demonstrate this DOF advantage, the other experiment was conducted on the rat adipocyte sample *ex vivo*. The resultant cross-sectional image of application of MAS to the rat adipocyte tissue with a

Figure 3.7: Cross-sectional images of rat adipocyte samples to verify DOF extension performance. (a) Dispersion-compensated cross-sectional image (intensity in logarithmic grayscale), captured from one of five apertures; (b) Digitally refocused cross-sectional image via MAS; (c-d) Two boxed areas away from the focus by $435\ \mu\text{m}$ and $516\ \mu\text{m}$ are indicated in green and orange colors and magnified by four times. The transverse profiles indicated by the dashed lines show that the defocused cell boundary was well digitally refocused.

size of $1747\ \mu\text{m}$ (transverse) $\times$ $684\ \mu\text{m}$ (axial) (Fig. 3.7(b)). Over most of the whole depth range, the transverse resolution was improved as cell boundaries became sharper and brighter, and detailed features were clearly reconstructed in comparison to the original dispersion-compensated cross-sectional image (Fig. 3.7(a)). Intact features of adipocyte cell structures in the bottom of cross-sectional images were hard to be recovered because of limited laser power. Two local areas at depths of 516

μm and $453 \mu\text{m}$ above the focus were selected and magnified by four times (Figs. 3.7(c) and 3.7(d)). The transverse profiles indicated in the zoom-in local area using dashed lines showed that the cell boundaries widths were sharpened from $15.3 \mu\text{m}$ and $27.7 \mu\text{m}$ to $8.5 \mu\text{m}$ and $9.2 \mu\text{m}$, respectively. The anisotropy ratio of the digitally refocused cross-sectional image to the dispersion-compensated cross-sectional image was also calculated over the whole depth range (Fig. 3.7(e)). Over the whole depth range, the anisotropy ratio was almost all above 1, and fluctuated around 1.05, indicating a transverse resolution improvement. Several valleys of the anisotropy ratio generated for the sake of artifacts induced by strong reflection at cell junctions. This experiment demonstrated that MAS achieved a good DOF extension performance when imaging the rat adipocyte sample.

3.6 Discussions and conclusions

We developed the MAS technique to address the fundamental problem of limited DOF in high transverse resolution FD-OCT. MAS is free from signal loss and sidelobe artifacts, which are caused by a suboptimal CTF function and inherent in the currently available methods. These merits of the MAS method have been demonstrated theoretically and experimentally in this work. Therefore, the MAS technique has the potential to provide a sufficient DOF to stably acquire sub-cellular-resolution images *in vivo*. Moreover, the ability to manipulate the complex PF makes

MAS a powerful tool that can eliminate various types of optical aberrations beyond the defocus induced by the sample or focusing optics, such as aberrations induced by human eye optics. However, the current study has three limitations. First, we used transverse priority scanning, which is susceptible to motion artifacts. This limitation can be easily resolved by changing to angular frequency priority scanning. Second, the current MAS form is implemented at the cost of scanning speed. In this work, we coherently sum five A-lines with five distinctive apertures together to obtain one DOF extended A-line, which decreases the imaging speed by five times. This limitation may be mitigated by the use of faster spectrometers or an ultra-high-speed swept source. Third, the spectrum passing the diffraction grating is partially confined by the camera lens (85 mm, f/1.2, Canon Inc., Tokyo, JP) resulting in a deteriorated axial resolution of $3.0\ \mu\text{m}$. A camera lens with a shorter focal length may not confine the diffracted spectrum but would result in a limited ranging depth. This limitation can ultimately be overcome by using two spectrometers to acquire two halves of the light spectrum and then recombining them [55]. Moreover, a phase ramp could be digitally created along the fast scanning direction in the PF to achieve digital transverse scanning. Finally, we acknowledged that there would be 3 dB detection sensitivity penalty induced by the additional beam splitter inserted in the sample arm. The proposed optical apparatus is applicable to desktop OCT imaging systems for DOF extension but a significant research effort is required to develop a miniaturized optical design for

endoscopic and intravascular applications. In conclusion, the proposed MAS technique overcomes the inherent trade-off between DOF extension and signal loss/sidelobe artifacts and achieves dramatic DOF extension performance in complex tissues whose refractive index varies with depth (e.g., rat adipocyte tissue) and has strong potential for providing a sufficient DOF for cellular-resolution imaging *in vivo*.

Funding: The National Research Foundation Singapore (NRF-CRP13-2014-05), the Ministry of Education Singapore (MOE2013-T2-2-107), the National Natural Science Foundation of China (Grant No. 61705184), the National Medical Research Council Singapore (NMRC/CBRG/0036/2013) and the NTU-AIT-MUV program in advanced biomedical imaging (NAM/15005).

Chapter 4

Extending axial focus of optical coherence tomography using parallel multiple aperture synthesis

Abstract

Compromising the inherent trade-off between transverse resolution and depth of focus (DOF) remains a long-standing issue in optical coherence tomography (OCT). In this paper, we report a novel technique — parallel multiple aperture synthesis (pMAS) to simultaneously generate multiple optical apertures in an OCT sample arm by employing a two-surface coated mirror. In the proposed pMAS, the DOF is extended by a factor of 16.49 without sacrificing the transverse resolution for proof-of-concept experiments when multiple distinctive apertures are digitally synthesized. The microparticles and tissue experiments demonstrate the feasibility of pMAS to address the fundamental problem of limited DOF in high resolution OCT.

4.1 Introduction

The inverse relationship between transverse resolution and DOF in OCT [23, 56–59] hinders direct observations of cellular structures in thick specimens. Thus, imaging with extended DOF is of great interest in the fields of biology and medicine. Various techniques based on either tailored illumination [20, 38, 39], multiple aperture synthesis (MAS) [41–43, 60, 61], multi-focusing [62], scanning focusing [63], complex wavefront shaping [64] and computational methods, including algorithm processing (deconvolution) [65] and theoretical solutions (interferometric syn-

thetic aperture microscopy) [40, 54] in OCT imaging are currently being developed to provide an extended DOF. These new approaches have created unprecedented new possibilities to investigate thicker biological tissues at the expense of optical aberration, loss of speed, phase instability or tedious post-processing. Therefore, a simple, reliable, and transplantable method is strongly demanded for the DOF extension in most OCT modalities. The performance of MAS in OCT demonstrates that it is the most promising method for overcoming the DOF limit by first generating multiple apertures and then properly combining them. This process is first achieved by introducing a pizeo-electrical transducer (PZT)-driven cylindrical lens, which separates illuminated light into different apertures thereby recovering transverse resolution at the defocus plane. However, system stability is highly required because the circular motion of the cylindrical lens can induce an unexpected aberration induced by minor vibrations in the optical path, and a simple and robust MAS design would be welcomed.

In this work, we propose a novel approach—pMAS, which is analogous to synthetic aperture radar—to preserve the diffraction-limited transverse resolution over a multiple-fold-extended DOF. This approach creates multiple reflections on a two-surface coated mirror and then generates multiple distinctive optical apertures that are encoded by a constant optical path difference along the z-axis (depth dimension). Multiple sub-images are separated by equal distances along the z-axis in

one cross-sectional image. Multiple sub-images are thereafter corrected and synthesized to generate a brand-new image with a multiple-times-extended DOF. In the post-processing approach, we applied the previous dispersion compensation algorithm and the digital refocusing algorithm [60, 61], thereby enabling an extended depth of field. Most importantly, a fiber-based system makes pMAS more flexible and portable without compromising features.

4.2 Materials and methods

4.2.1 Multiple aperture synthesized OCT system

Fig. 4.1(a) illustrates the constructed fiber-based SD-OCT system for DOF extension via pMAS. The power source is a supercontinuum source (SuperK Extreme OCT, NKT Photonics A/C, Birkerød, Denmark), filtered by a dichroic mirror (DMSP950, Thorlabs, Newton, NJ, USA), resulting in a center wavelength of 890 nm and a 3-dB bandwidth of 180 nm. The light from the light source is split into the sample arm (50%) and the reference arm (50%) by a 2×2 coupler (TW850R5A2, Thorlabs, Newton, NJ, USA). In the reference arm, the light is collimated by a lens L1 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA) and focused by a lens L2 (M Plan Apo NIR 20 $\times$, Mitutoyo, Takatsu-ku, Kawasaki, JP) onto the reference mirror M1 (PF10-03-P01, Thorlabs, Newton, NJ, USA). The light beam in the sample arm is collimated by a lens L3

Figure 4.1: Parallel multiple aperture synthesized OCT system. (a) Schematic of the DOF-extended SD-OCT system. (b) Magnified view of the optical beams generated by the MASE. (c) Illumination apertures and detection apertures of three beams indicated in (b), and the aperture of the objective lens. SLD, super luminescent diode; OC, fiber optic coupler; PC, polarization controller; L1, L3, L5, collimating lens; L2, L4, focusing lens; L6, camera lens; NDF, neutral density filter; M1-2, mirror; GS, galvo scanner; MASE: multiple aperture synthesis element; G, transmission diffraction grating; IMAQ, image acquisition; C, computer; B1-2, two beams generated by multiple reflection on the front and back surfaces of MASE; A1-2, two optical apertures generated by the MASE and the optical aperture of the objective lens. Δd is the transverse distance between two adjacent illumination beams.

(AC050-010-B-ML, Thorlabs, Newton, NJ, USA) and focused by an objective lens L4 (M Plan Apo NIR 20 $\times$, Mitutoyo, Takatsu-ku, Kawasaki, JP). The backscattered light from the sample is recombined with the light from the reference arm, and finally directed into the spectrometer. The spectrometer consists of a lens collimation lens L5 (AC050-010-B-

ML, Thorlabs, Newton, NJ, USA), 1500 lines/mm diffraction grating (PING-1500/915-980-030, Ibsen Photonics, Farum, Denmark), a camera lens (85 mm, f/1.2, Canon, Tokyo, JP), and a 4096-pixel camera (AVi-iVA EM4, e2V, Chelmsford, UK). The detected spectrum is digitized at 12-bit resolution and transferred to a computer via an image acquisition board (KBN-PCE-CL4-F, Bitflow, Woburn, MA, USA). Two-directional galvo scanner GS (GVS002, Thorlabs, Newton, NJ, USA) is driven with saw-tooth pulses, which are generated by a 16-bit analog output of a data acquisition (DAQ) board (PCI-6221, National Instruments, Austin, TX, USA). Image acquisition and galvo scanner are synchronized by the external trigger from a DAQ digital output. Discrete Fourier transform is performed on each frame of 1024 axial lines (A-lines) obtained by the camera to resolve the axial depth profile of the sample.

4.2.2 Multiple aperture synthesis

To extend the DOF, the MAS element (MASE) is placed into the sample arm. The MASE is a custom-built mirror of which the front surface is coated with a dielectric film (600-1000 nm, T/R=60:40), and the back surface is coated with 100% reflective film. Multiple reflections on the front and back surfaces of MASE produce a series of illumination beams (B1-2) shown in Fig. 4.1(b). The ratio of power among B1-2 is about 40%:36%. Other higher-order illumination beams are not considered, because they are relatively weaker and only contain 24% of the total

power. Fig. 4.1(c) depicts two distinctive apertures in the objective lens (L4) pupil plane. Apertures 1-2 (A1-2) denote two apertures generated by the MASE. The transverse distance between two adjacent beams among B1-2 is determined by the geometrical thickness ($t = 0.25$ mm), the refractive index ($n = 1.5$ @ 890 nm) and the inclination angle ($\theta = 45^\circ$) of the MASE as $\Delta d_{MASE} = \sqrt{2}t \cdot \tan[\sin^{-1}(\sin\theta/n)]$, which is 0.19 mm. The optical path difference (OPD) between two adjacent beams among B1-2 is denoted as $\Delta z_{MASE} = 2nt/\cos[\sin^{-1}(\sin\theta/n)] - \Delta d$, which is 0.66 mm. If we define the small extra OPD arises due to the defocus-induced local wavefront curvature as Δz_{curve} , then the single pass OPD between two adjacent beams among B1-2 is $\Delta z = \Delta z_{MASE} + \Delta z_{curve}$. There will be three kinds of OPDs, including 0, Δz and $2\Delta z$, and three sub-images will be equally separated along the depth dimension in one cross-sectional image.

In classical theory of Fourier domain OCT, the detected interference signal $I(k)$ in k -space, including DC, AC and CC terms, was presented in Eq. (2.3). $S(k)$, DC and AC are omitted for the sake that only the CC encodes depth-resolved information for OCT imaging. Take three kinds of OPDs into consideration, Eq. (2.3) is rewritten as

$$I(k) = 2\sqrt{I_r(k)I_s(k)} \sum_{m=1}^3 \exp[i2kz + ik(m-1) \cdot \Delta z] + C.C. \quad (4.1)$$

where $C.C.$ indicates the complex conjugate, will be omitted in the following derivation. m represents the m -th sub-image. To correct OPDs

and coherently sum three sub-images located at different axial depths into one single image, we define $k = k_0 + \Delta k$, and rewrite Eq. (4.1) as

$$I(k) = 2\sqrt{I_r(k)I_s(k)} \sum_{m=1}^3 \exp[i2kz + i\alpha_m + i\beta_m] \quad (4.2)$$

where $\alpha_m = k_0(m-1) \cdot \Delta z$ and $\beta_m = \Delta k(m-1) \cdot \Delta z$. Eq. (4.2) is expressed as a constant phase α_m and an oscillation phase β_m , which can be corrected in two sequential steps.

Firstly, according to Fourier shift theorem, we correct α_m by multiplying Eq. (4.2) with a constant phase coefficient $\exp(-i\alpha_m)$, which means axially aligning two lower sub-images to the topmost sub-image. α_m gets its optimal value α_m^{op} , when $|\sum_{m=1}^3 I_m(k) \cdot \exp(-i\alpha_m)|$ gets its maximum value. Secondly, β_m can also be eliminated by multiplying an oscillation phase $\exp(-i\beta_m)$ with the Fourier spectrum of three axially aligned sub-images. $\beta_m(z)$ is depth-dependent and is strictly contained in the phase set $\{\pi/2, \pi, 3\pi/2, 2\pi\}$. $\beta_m(z)$ gets its optimal value $\beta_m^{op}(z)$, when $\max\{|\sum_{m=1}^3 I_m(k) \cdot \exp(-i\alpha_m^{op}) \cdot \exp[-i\beta_m(z)]|\}$ gets its maximum value. The resultant image with three distinctive apertures synthesized is given by

$$I_{MAS}(k) = \sum_{m=1}^3 I_m(k) \cdot \exp(-i\alpha_m^{op}) \cdot \exp[-i\beta_m^{op}(z)] \quad (4.3)$$

To correct the residual aberrations induced by turbid tissues, a guide-star algorithm is adopted by multiplying Eq. 4.3 by an additional depth-dependent phase $\gamma_m(z)$, which is contained in the phase set $\{\pi/2, \pi, 3\pi/2,$

$2\pi\}$. $\gamma_m(z)$ obtains its optimal value $\gamma_m^{op}(z)$, when $\max\{|\sum_{m=1}^3 \mathcal{F}\{I_m(k) \cdot \exp(-i\alpha_m^{op}) \cdot \exp[-i\beta_m^{op}(z)]\} \cdot \exp[-i\gamma_m(z)]|\}$ gets its maximum value. $\mathcal{F}$ represents Fourier transformation. The resultant image is given by

$$i_{MAS} = \sum_{m=1}^3 \mathcal{F}\{I_m(k) \cdot \exp(-i\alpha_m^{op}) \cdot \exp[-i\beta_m^{op}(z)]\} \cdot \exp[-i\gamma_m^{op}(z)] \quad (4.4)$$

The digitally synthesized image not only refocuses the defocused sample and preserves the diffraction limited transverse resolution over a multiple-times extended DOF, but also increases the image contrast.

4.3 Experimental results

4.3.1 DOF extension in microparticle phantom imaging

As proof-of-concept for pMAS, we investigated the capability of DOF extension using a homemade phantom of polystyrene calibration microparticles with a nominal diameter of 6 μm (No. 64090-15, nominal size 6 μm , Sigma-Aldrich, St. Louis, MO, USA), which are evenly dispersed in the agarose gel (No. PC0701-100g, Vivantis, Oceanside, CA, USA). Within the limited imaging depth of our system, samples were scanned axially using a translation stage. Five sub-images were shown in panel with standard OCT (Fig. 4.2(a)) and pMAS OCT (Fig. 4.2(b)) at five depths of 78, 233, 388, 543 and 698 μm , respectively. Each image has a size of 116 (axial) $\times$ 758 (transverse) pixels covering a range of 155 (axial) $\times$ 646 (transverse) μm . In our standard OCT system,

Figure 4.2: Cross-sectional OCT images of microparticles without (a) and with (b) pMAS processing at different depths in logarithmic scale. Transverse normalized intensity profiles of five representative microparticles were detected by standard OCT (black curve) and pMAS OCT (red curve), where FWHM enhancement ratios of five representative microparticles are shown in the margin. The scale bar is $100\ \mu\text{m}$.

the MFD (defined by the $1/e$ field) was $5.0 \pm 0.5\ \mu\text{m}$ and the focal length of collimating lens and focusing lens are both 10 mm. The theoretical diffraction-limited transverse resolution was $2.92\ \mu\text{m}$, and the corresponding DOF (defined as the confocal parameter) was $16.01\ \mu\text{m}$ ($n=1.33$, $\lambda_c = 850\ \text{nm}$). In this experiment, the narrowest full-width at half-maximum (FWHM) of microparticles captured by standard OCT at the focus was $9.23\ \mu\text{m}$, shown in second sub-image. After the micropar-

ticles size $6\ \mu\text{m}$ have been subtracted, we obtained the finest transverse resolution of $3.23\ \mu\text{m}$ and a DOF of $18.80\ \mu\text{m}$, close to the theoretical value $16.01\ \mu\text{m}$. As the Rayleigh distance is determined by the beam waist size, which is $\sqrt{2}$ -times larger than the FWHMs of microparticles at the focus. In our measured FWHM, most of the microparticles in the second and third sub-images captured by pMAS were narrower than $13.05\ \mu\text{m}$, which means that the DOF was approximately extended by a factor of 16.49 ($= 155 \times 2\ \mu\text{m} / 18.80\ \mu\text{m}$), $155\ \mu\text{m}$ here is the axial length of a sub-image. Five representative microparticles were circled in pairs, and their transverse intensity profiles were plotted for direct comparison. The FWHMs of selected microparticles are listed in Table. 4.1. Except for those close to the uppermost and lowermost boundaries, microparticles throughout a depth range of $775\ \mu\text{m}$ ($= 155 \times 5\ \mu\text{m}$) were well recovered by pMAS.

Table 4.1: Comparison of FWHMs and transverse resolution (Δx) of typical microparticles captured by standard and pMAS OCT.

Depth (μm)	FWHM (μm)		FWHM ratio	Δx (μm)		Δx ratio
	standard	pMAS		standard	pMAS	
78	68.07	19.94	3.41	62.07	13.94	4.45
233	9.23	9.15	1.01	3.23	3.15	1.03
388	16.43	9.23	1.66	10.43	3.23	2.66
543	33.17	13.61	2.44	27.17	7.61	3.56
698	48.72	18.34	2.66	42.72	12.34	3.46

4.3.2 DOF extension in biological tissue imaging

To further demonstrate the feasibility of the pMAS OCT, we performed tissue imaging experiments *ex vivo* using a fresh lemon. Additionally, the composite cross-sectional images were acquired by manual scanning, which is similar to the microparticles experiment. Four biological sub-images were imaged at four different depths at five depths of 89, 266, 443 and 620 μm , respectively. Each image had a size of 133 (axial) $\times$ 113 (transverse) pixels, covering a range of 177 (axial) $\times$ 479 (transverse) μm . Intact features of internal structures were exhibited with standard

Figure 4.3: Cross-sectional images of extracted lemon slices images of extracted lemon slices without (a) and with (b) pMAS processing at different depths in logarithmic scale. Featured structures are marked by green arrows for comparison. The scale bar is 100 μm .

OCT (Fig. 4.3(a)) and pMAS OCT (Fig. 4.3(b)). Here, the focus plane was placed in the third sub-image. For a comparison of Fig. 4.3(a) and Fig. 4.3(b), lemon structures were well digitally refocused, as indicated by sharpened cell walls and clearly reconstructed detailed features (indicated by green arrows).

4.4 Discussions and conclusions

We demonstrated a DOF extension technology employing a simple reflective mirror without sacrificing the imaging time. This design is similar to the angular compounding design and can achieve a speckle reduction performance [66, 67]. The coated mirror presented here is inexpensive and compatible with conventional OCT. For both microparticles and tissue experiments, it enables high transverse resolution cross-sectional imaging accompanied by a relatively long DOF. However, the current study has three limitations. First, 24% (-1.19 dB) of the illumination and detection powers will be wasted during aperture division. This limitation could be overcome by increasing the incident power [68]. Second, the second term in Eq. (4.1) includes two coupled situations (illumination via B1 aperture and detection via B2 aperture, and illumination via B2 aperture and detection via B1 aperture). These two situations have the same optical path difference but different wavefronts, which may result in tiny artifacts. Third, When the axial size of sub-images is larger than the optical path differences between adjacent apertures, parts of

two adjacent sub-images will superpose together, which will results artifacts. This limitation could be overcome by increasing the thickness of the coated mirror. Notably, for a better exhibition, we chose a relatively small optical path difference here that could be increased in practical application. The requirement of three times imaging depth due to three synthesized apertures could be overcome by using dual spectrometer system with spectral compounding [55]. We believe that a miniaturized portable design based on our fiber-based system, where the pMAS method is used in conjunction with the conventional OCT technology for animal models or human examination could be easily developed.

Funding: This work was supported in part by National Research Foundation Singapore (NRF-CRP13-2014-05), the National Natural Science Foundation of China (Grant No. 61705184 and 81401451), Ministry of Education Singapore (MOE2013-T2-2-107), National Medical Research Council Singapore (NMRC/CBRG/0036 /2013) and NTU-AIT-MUV program in advanced biomedical imaging (NAM/15005).

Chapter 5

Cellular-resolution *in vivo*
tomography in turbid tissue
through digital aberration
correction

Abstract

High-resolution optical coherence tomography (OCT) over a large depth range is imperative when examining cellular structures in turbid tissue. Currently, conventional spectral domain optical coherence tomography (SD-OCT) is challenged for dynamic correction of aberrations due to its point-scanning nature. Here we present a cellular resolution interferometric tomography introduced by calcite beam displacer (CBD), which stitches together each axial line (A-line) profile from several separated apertures to create an aberration correction image by adopting a guide-star algorithm. Our approach is insensitive to sample induced motion, does not require stringent control of the phase stability, and helps form images at unprecedented resolution. We demonstrated the targeted microparticles were well recovered undergo both strong tissue-induced aberration and defocus aberration. Furthermore, this novel technique enables *in vivo* red blood cells (RBCs) imaging inside the human oral mucosa at the lip. We believe that, this design is compatible with catheter-based endoscopic OCT systems, making aberration compensated endoscopy accessible to both research and clinical studies.

5.1 Introduction

Over the past decades, the development of imaging techniques including ultrasound imaging , X-ray imaging [70], magnetic resonance imaging

[71] and radar imaging [72] has historically brought great progress in the clinical practice of disease diagnosis and treatment. These imaging techniques have large penetration depth but low spatial resolution. Up to now, *in vivo* cellular resolution optical tomography in turbid tissue has become a heated topic, because it enables us to visualize imperceptible changes in cellular and sub-cellular resolution, and evaluates pathological lesion in an early period by charactering cell type, cellularity, cell polarity, cell viability and nucleus morphology [73, 74], especially for the early diagnosis of gastrointestinal cancer [75] and atherosclerosis [76].

To achieve an *in vivo* cellular resolution optical tomography in turbid tissue, the compromise between high spatial resolution and limited depth of focus has to be broke and the aberration induced by turbid tissue has to be compensated. To access valuable information inside the turbid tissues, many volumetric imaging techniques have been developed, such as confocal microscopy [77], two photon microscopy [78], second harmonics generation microscopy [79] and optical projection tomography [80]. These methods gate out light from sample areas away from the point of interest and offer excellent signal enhancement, by using nonlinear excitation, tissue clearing and expansion [81]. Such techniques also encounter several problems. First, they usually need a scanning or rotational stage, which might require physical movement and can be time consuming. Second, physics gating or thin layer of light sheet usually require a high numerical aperture (NA) lens, which provides a propor-

tionally smaller image field of view (FOV). Third, non-gating technique like optical projection tomography [80] does not compensate for tissue scattering and therefore operates theoretically under the same depth limitations as conventional microscopy. All of these techniques are thus not ideal tools for obtaining the refractive index distribution of a primarily non-transparent and non-fluorescent medium. To do so, it is useful to use OCT, i.e. coherence gating to capture the ballistic photons emerging from the sample. In SD-OCT, phase between A-lines is often uncorrelated for insufficient scanning rates or motion-induced artifact *in in vivo* situations. To restore lateral and axial resolution and suppress the aberrations relied on point-scanning images is essential.

Aberration correction also attracts great interests outside the field of OCT, and many different methods have been developed to concentrate focus energy in the high scattering media, to improve image performance for biomedical applications. For example, focusing through high scattering media usually achieved by incident light wavefront shaping [82], transmission matrix measurement [83], phase conjugation [84] and photoacoustically guided wavefront shaping [85]. In optical microscopy, adaptive optics (AO) enables diffraction-limited images after canceling tissue-induced aberrations [86–88].

Over the past decades, various techniques have been developed to mitigate either tissue induced aberration or defocus aberration for OCT. However, few method could account for them both, especially for moving

scatters in the turbid tissue. Hardware-based adaptive optics (HAO) is a straightforward approach to shape and compensate the incident wavefront using a coherence-gated reflectance signal as feedback. HAO systems remain expensive and complex. In addition, the aberration correction can only be valid for one isoplanatic patch during the imaging procedure, which extends the time of the imaging session for the application of large volumetric imaging [89–91]. Post-processing techniques, such as sub-aperture correlation [92, 93] and computational adaptive optics (CAO) [54, 94, 95] are used to prevent hardware complexity. However, they usually require heavy computation, absolute phase stability, which is challenged for *in vivo* imaging, especially in endoscopic applications. Other approaches including forward model [96, 97], inverse scattering model [97, 98], interferometric synthetic aperture microscopy (ISAM) [40, 99], have the same phase stability requirements regarding the point scanning systems; and depth-encoded synthetic aperture [41–43] may causes loss of signal in certain spatial frequency components.

Computational image formation in OCT relies upon accurate measurement of the phase of the backscattered signal, which guarantees the coherent aperture synthesis. This places restrictions on the stability of the imaging experiment beyond that of standard OCT imaging. Inspired by synthetic-aperture radar (SAR), we develop a multiple synthesis aperture (MAS) method using the linear shifted micro-cylindrical lens [60, 61]. In this study, we report a novel optical tomographic technique,

termed amplitude division aperture synthesis optical coherence tomography (ADAS-OCT), which corrects aberrations induced by turbid tissues by physical aperture synthesis and simultaneously data acquisition from sub-apertures. ADAS-OCT corrects both the sample-induced aberrations and spherical aberrations without any phase stability requirement. We show that ADAS-OCT can deliver clear images of rapidly moving blood cells by restoring the spatial resolution degraded by the aberrations caused by the human mucosa *in vivo*.

5.2 Working principle of aberration correction via ADAS-OCT

5.2.1 Concept of ADAS-OCT

For high-resolution OCT in turbid tissue, aberrations arise from both the defocused aberration introduced by high numerical aperture and unpredictable tissue-induced aberration. Although aberrations could be simulated from various physics-based models, here we instead employ intuitive aperture segmentation and synthesis method that signals from different optical paths could be constructively enhanced with the phase correction, breaks the strict requirement of phase stability (Fig. 5.1). Abnormal light rays have to deviate from its ideal path that the focus point is shifted and the phase is changed. The backward scattered wave is distorted at the intermediate image plane, so the recorded images are less

constructively, like the point spread function (PSF1) in Fig. 5.1. When optical field of each sub-aperture is optimized at the sample plane, the full width at half maximum (FWHM) of its conjugated point approaches diffraction-limited value, i.e. PSF2 in Fig. 5.1. Large number of discrete sub-apertures could lead to accurate reconstruction. In our work we found that, it is sufficient to refocus the images and cancel the tissue-induced aberration effectively even for 2 sub-apertures.

Figure 5.1: Schematic depicting the amplitude-division aperture synthesis method and interferometric wavefront distortion correction. Aperture-division detection is achieved by dividing the sample beam by the amplitude and displacing the divided beams over the plane of the objective-lens pupil. Detection of spatial-frequency division enables the distortions of the optical wavefront to be recorded as the interferometric wavefront distortions pass through the pinhole detector. The digital correction of the interferometric wavefront distortions can restore both the PSF and the signal amplitude. PSF1: PSF degraded by aberrations; PSF2: restored PSF; BS: beam splitter; OPE: optical-pathlength encoder; 1-n, represents the sub-aperture numbers; OW: optical wavefront; PD: pinhole detector; IW: interferometric wavefront; FFT: fast Fourier transform.

5.2.2 Theory of ADAS-OCT

A sample arm of a ADAS OCT is shown in Fig. 5.2(a), in which a custom-designed calcite beam displacer (CBD) and a quarter wavelength plate (QWP) are employed as optical pathlength encoder. The collimated beam splits into doubly refracted beams due to the anisotropic refractive index of CBD, one obeys Snells law called ordinary beam (*o*-beam) and the other one called extraordinary beam (*e*-beam) (Fig. 5.2(b)). Polarization of *o*-beam is perpendicular with the optical axis of CBD and polarization of *e*-beam is not perpendicular with the optical axis of CBD. Values of optical pathlength difference (OPD) Δz and the separation Δx depend on the optical parameters of our system. After the double pass through QWP, polarization direction will be shifted. *o*-beam could travel back from *e*-beam channel and vice versa, allows coupling angular diverse scattering information (Fig. 5.2(c)). As a result, there are four routes (*oo*-path, *ee*-path, *oe*-path and *eo*-path) in the illumination and detection (Fig. 5.2(d)). It should be noted that *oe*-path and *eo*-path share the same OPL and then three sub-images are coherently added up after axial alignment. Divided apertures could be scaled up by a pair of reversed calcite beam displacer is shown in Fig. 5.2(e).

Here we define the single-trip OPD between *o*-beam and *e*-beam as Δz . So the round-trip *ee*-path is $2\Delta z$ longer than *oo*-path, and the round-trip *oe*-path and *eo*-path is Δz longer than *oo*-path. Δz includes three

Figure 5.2: Schematic of cellular resolution interferometric tomography introduced by CBD. (a) Diagram of sample arm with the insertion of a single CBD and a quarter-waveplate, where the beam in red is the combination of o -beam and e -beam, the beam in green is e -beam, and the beam in blue is o -beam. (b) Principle of birefringence effect. (c) Linearly polarized light changes into circularly polarized light when passing through a quarter-waveplate. (d) Four types of round-trip optical paths provided with angular diversity by one CBD. (e) Scaled up apertures with the insertion of a pair of reversed CBDs. CBD, calcite beam displacer; SMF, single mode fiber; L1, collimating lens; QWP, quarter-waveplate; L2, focusing lens; o , ordinary beam; e , extraordinary beam; PS: principle section; OA, optical axis of CBD (in plane of paper); d , length of the CBD; Δx , the separated distance of o -beam and e -beam perpendicular to propagation direction; ϕ , the angle that are separated in the CBD; φ , the angle between the slow axis and OA of the CBD; θ , the angle between the fast axis and the OA of the CBD.

parts: Δz_1 , OPD in the CBD; Δz_2 , OPD in the QWP; Δz_3 , OPD in the focal light of the focusing lens L2; Δz_w , caused by the difference between two local wavefronts (LWFs) corresponding to two distinctive apertures occupied by *o*-beam and *e*-beam, respectively.

$$\Delta z = \Delta z_1 + \Delta z_2 + \Delta z_3 + \Delta z_w \quad (5.1)$$

where $\Delta z_1 = d \cdot [n_e / \cos(\varphi) - n_o]$, $\Delta z_2 = \lambda/4$ and $\Delta z_3 = n_a(\sqrt{\Delta x^2 + f^2} - f)$. d is the physical length of the CBD. n_o and n_e are the refractive index of CBD for *o*-beam and *e*-beam. n_a is the refractive index of air. f is the focal length of lens L2. Δx is the separated distance in the direction perpendicular to propagation direction, which is given by

$$\Delta x = d \cdot \tan \varphi \quad (5.2)$$

where $\varphi = \theta - \phi$ and $\tan \theta / \tan \phi = n_o^2 / n_e^2$.

Three different OPDs make it possible to encode the light signal from four different paths and realize path length division detection. It should be noted that *oe*-path and *eo*-path have the same OPL and then we could synthesize different apertures corresponding to three depth-separated signals.

In classical theory of Fourier domain OCT, the detected interference signal $I(k)$ in k -space, including DC, AC and CC terms, was presented in Eq. (2.3). In ADAS OCT, $S(k)$, DC and AC could be omitted for

the sake that only the CC encodes the depth-resolved information. So the m -th ($m = 1 \sim 3$, represents the aperture number) depth-encoded CC term in ADAS OCT could be expressed as

$$I_m(k) = \sqrt{I_r(k)I_s(k)}[\exp(i2kz_m) + C.C.] \quad (5.3)$$

where $C.C.$ is the abbreviation for the complex conjugate. Fourier transform ($\mathcal{F}$) of these cross-correlation terms give the standard SD-OCT images obtained through individual paths.

$$i_{SD-OCT,m}(z) = \mathcal{F}[I_m(k)] \quad (5.4)$$

Correction of aberrations in the OPD domain can be simply achieved by eliminating the phase delay α_m of m -th cross-correlation term I_m with regard to I_1 . Assuming that the aberration free condition is met when the local intensity of coherently summed image reaches its maximum, the problem is equal to finding α_m^{coh} which is the best estimate of α_m so that the local intensity $\max\{i(z)|_{z \in [z', z'']}\}$ reaches its maximum, where $i(z) = \sum_{m=1}^{2n-1} \mathcal{F}[I_m(k) \cdot \exp(i\alpha_m)]$ is the coherent summation of the Fourier transform of the cross-correlation terms denoted by Eq. (5.3), $[z', z'']$ is the local depth range with $1 \leq z' \leq z'' \leq N$, and N is the length of Fourier transformation. Then the resultant aberration corrected A-line is given by

$$i_{ADAS}(z) = \sum_{m=1}^{2n-1} \mathcal{F}[I_m(k) \cdot \exp(i\alpha_m^{coh})] \quad (5.5)$$

While the interferometric signals from the local scatterer ($z'' - z' = 10$ coherence length) are coherently summed, those from the out-of-focus scatterers are suppressed due to OPD mismatches between light paths through different sub-apertures. Thus, this digital aberration correction mechanism can restore both the spatial resolution and the peak signal intensity degraded by aberrations. To produce the aberration-uncorrected control A-line, we incoherently summed the Fourier transform of the cross-correlation terms denoted in Eq. (5.3) so that $i(z) = \sum_{m=1}^{2n-1} |\mathcal{F}[I_m(k) \cdot \exp(i\alpha_m)]|$, and then found the optimal phase value for α_m so that global intensity $\max\{|i(z)|_{z \in [1, N]}\}$ reaches its maximum value. Therefore, the aberration-uncorrected control A-line is given by,

$$i_{Control}(z) = \sum_{m=1}^{2n-1} |\mathcal{F}[I_m(k) \cdot \exp(i\alpha_m^{incoh})]| \quad (5.6)$$

The digitally synthesized image not only suppress the tissue-induced aberration and preserve the diffraction-limited transverse resolution over a multiple-times extended DOF, but also reduces the speckle and increases the signal to noise ratio (SNR).

5.2.3 Experimental setup

The schematic of ADAS-OCT system is illustrated in Fig. 5.3. The light source is a supercontinuum laser (SuperK Extreme OCT, NKT Photonics A/C, Birkerød, Denmark) providing a FWHM bandwidth of 180 nm centered at 890 nm. The light from the source is collimated

Figure 5.3: Schematic of the ADAS-OCT system. L1-6, optical lens; NPBS, non-polarizing beam splitter; CBD: calcite beam displacer; QWP, quarter wavelength plate; GS, two-dimensional galvo scanner; LP: linear polarizer; DC, dispersion compensator; NDF, neutral density filter; M: mirror; G, transmission diffraction grating; L6, camera lens; IMAQ, image acquisition; PC, personal computer; AO, analog output.

by a lens L1 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA) and then split into the reference arm and sample arm by a 50:50 beam splitter BS (BS011, Thorlabs, Newton, NJ, USA). The light in the sample arm is projected onto the galvo scanner (GVS002, Thorlabs, Newton, NJ, USA) and then focused by an objective lens L2 (M Plan Apo NIR 20 $\times$, Mitutoyo, Takatsu-ku, Kawasaki, JP). The light in the reference arm is focused by a lens L3 (M Plan Apo NIR 20 $\times$, Mitutoyo, Takatsu-ku, Kawasaki, JP) onto the reference mirror M (PF10-03-P01, Thorlabs, Newton, NJ, USA). A calcite beam displacer (CBD) is inserted in the sample arm to separate the and in the transverse direction (x-z coordinate). A quarter wavelength plate (AQWP05M-980, Thorlabs, New-

ton, NJ, USA) is inserted between the CBD and the galvo scanner to change the polarization of the back-scattered light. A linear polarizer LP (LPNIRE 100-B, Thorlabs, Newton, NJ, USA) is configured in the reference arm to rotate the polarization direction of the reference light. The light backscattered or reflected from the sample arm interferes with that from the reference arm in BS, then focused by a lens L4 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA), and guided to the spectrometer. The spectrometer consists of a 1500 lines/mm diffraction grating (PING-1500, Ibsen Photonics, Farum, Denmark), a camera lens (Canon EF 85 mm, f/1.2, Tokyo, JP), and a 4096-pixel camera (AViiVA EM4, e2V, Chelmsford, UK). The detected interference signal is digitized at 12-bit resolution and transferred to a computer via an image acquisition board (KBN-PCE-CL4-F, Bitflow, Woburn, MA, USA). Two-directional transverse scanning is performed by a two-axis galvo scanner drive with a saw-tooth pulse. These pulses are generated by a 16-bit analog output of a data acquisition (DAQ) board (PCI-6221, National Instrument, Austin, TX, USA). Image acquisition and transverse scanning are digitally synchronized by an external trigger of the DAQ board. Discrete Fourier transform is performed on each A-line obtained by the camera to resolve the axial depth profile of the tissue. In the current ADAS OCT system, -6 dB imaging depth is ~ 1.87 mm (Fig. 5.4), so we only consider three or five optical path difference in the experiments.

Figure 5.4: Measured axial PSFs on a linear scale for different delays relative to the reference arm length.

5.3 Experimental results

5.3.1 DOF extension verification using microparticle phantom

A spherical phantom of polystyrene calibration microparticles was chosen to resemble biological cells to verify that the DOF extension performance of ADAS. This phantom was constructed by mixing agarose solution (No. PC0701-100g, Vivantis, Oceanside CA, USA) with polystyrene microparticles (No. 64090-15, nominal size $6\ \mu\text{m}$, Sigma-Aldrich, St. Louis, Missouri, USA). This mixture was stored in a vial and placed in an ultrasonic bath for ten minutes to remove residual clusters. The sample (10 g) was poured into a cell culture dish, cured for 30 minutes at 100°C and then cured for 24 hours at room temperature. As ADAS is an angular multiplexed method by path length encoding, speckle will be

Figure 5.5: DOF extension verification in the phantom of microparticle microparticles. (a) Original and (b) refocused cross-sectional images of a phantom of calibration microparticles in linear scale. Insets: 4-fold magnified view of defocused microparticles indicated by the dashed boxes in (a-c). Scale bar: $100\ \mu\text{m}$.

reduced, and the SNR will increase when images from different channels are incoherently added. To demonstrate the performance of DOF extension, we compare the averaging images with both unprocessed and defocus correction under the same contrast level. Cross sectional images with a size of $535\ (\text{axial}) \times 1365\ (\text{transverse})\ \mu\text{m}$ are shown in Fig. 5.5. Except for those close to the uppermost boundaries, microparticles throughout a depth range of $201\ \mu\text{m}$ were well digitally recovered by ADAS (Fig. 5.5(b)). Most interesting, merged microparticles are transversely resolved in two sections. The theoretical diffraction-limited transverse resolution was $2.92\ \mu\text{m}$, and the corresponding DOF (defined as the confocal parameter) was $16.01\ \mu\text{m}$. Through the measurement

of the FWHM of the microparticles, the recovered depth range is 12.6 times than conventional DOF.

5.3.2 Correction of sample-induced aberrations *ex vivo*

To demonstrate the ability to correct sample-induced aberrations, we first imaged a simplified tissue phantom that consisted of a collection of polystyrene microparticles with a mean diameter of $6\ \mu\text{m}$ and that were uniformly suspended in agarose gel. We focused the sample light to the upper half of the phantom through a chicken breast tissue with thickness of $289\ \mu\text{m}$ in air (Fig. 5.6(a)), resulting in broadened transverse and axial profiles of the particles in the control image (Fig. 5.6(c, e, g)) and the SD-OCT images obtained through individual paths compared with an representative image obtained without the tissue on the phantom (Fig. 5.6(b)). In the aberration corrected image, both the transverse and axial profiles of the microparticles were significantly restored (Fig. 5.6(d, f, h)). For quantitative comparison, the FWHM values showed a mean improvement of 24.1% and 16.0% in the transverse and axial directions, respectively (Fig. 5.6(i) and Fig. 5.6(j)). In addition, a mean improvement in the peak intensity of 53.7% was obtained in favor of the signal-to-noise ratio (Fig. 5.6(k)). The image contrast and clarity of the chicken breast tissue were also significantly improved (boxes in Fig. 5.6(c, d)).

Figure 5.6: Correction of sample-induced aberrations. (a) Photograph of the tissue phantom. CB: chicken breast. (b) An image of microparticles acquired without chicken breast tissue. (c) A representative control image. This was obtained using Eq. (5.5) with $[z', z'']$ being the full depth range. The red dashed line indicates the focal plane. (d) The corresponding aberration corrected image. (e), (g) Six-fold magnified view of two insets in (c) show blurred microparticles. (f), (h) The corrected images corresponding to (e) and (g), respectively. The transverse and axial profiles are shown at the top and on the right of panels (e)-(h), respectively. The profiles in (e) and (g) are normalized to the peak values in (f) and (h), respectively. (i) and (j) The FWHM of the transverse and axial profiles are restored in the ADAS-OCT image (ADAS, green rectangle) with respect to the control image (Control, red triangle) and the SD-OCT image (SD-OCT w/o CB, blue circle). (k) Signal intensity is also restored in the ADAS-OCT image (green rectangle) with respect to the control image (red triangle). Transverse and vertical scale bars: 100 μm .

5.3.3 Swine esophagus imaging *ex vivo*

To verify the performance of aberration correction in biological tissue, we conducted a ADAS imaging experiment on the swine esophagus *ex*

vivo. Fig. 5.7 shows the resultant images of the swine esophagus tissue, including the original (Fig. 5.7(a)) and aberration compensated (Fig. 5.7(b)) cross-sectional images with a size of $1000\ \mu\text{m}$ (transverse) $\times$ $456\ \mu\text{m}$ (axial). The original image is made of three sub-images with incoherently adding, and the restored image is added up by ADAS method. Two regions beyond the focus are selected and magnified by four times for a better view of potential cell nuclei in the epithelium. Four cells in these regions are indicated by yellow arrows and numbers. Suffering from defocused and tissue induced aberrations, four cells are distorted to a great extent (Fig. 5.7(a)). In comparison, four cells restored by ADAS method turn to be sharper and brighter. This experiment demonstrates that ADAS achieves good performance of aberration correction in biological tissue *ex vivo*.

Figure 5.7: Aberration correction inside the EP layer of the swine esophagus *ex vivo*. (a) Original and (b) restored cross-sectional images of the swine esophagus in logarithmic grayscale. Four-fold magnified views of cells in the EP layer of the swine esophagus. EP: epithelium; LP: Lamina propria. Scale bar: $100\ \mu\text{m}$.

5.3.4 *In vivo* red blood cells imaging

Figure 5.8: (a) A representative control image. This was obtained using Eq. (5.5) with being the full depth range. (b) Corresponding aberration-corrected image. (c) Three-fold magnified view of the blood vessel in the control image (green dashed box in (a)). (d) Three-fold magnified view of the corresponding aberration-corrected image (blue dashed box in (b)). (f) Ratio of the peak pixel (signal) intensity of the aberration-corrected RBC image to that of the control image. Upper and lower denote the upper and lower boundaries of cells, respectively. EP: epithelium; LP: lamina propria. Transverse and vertical scale bars: 100 μm .

RBCs are ideal image phantoms due to their characteristic axial scattering profiles and spatial sparsity in blood vessels. According to our previous observations in zebra fish larvae, a typical OCT image of an RBC consists of two coherence-length limited reflection signals from the upper and lower boundaries of the cells biconcave disk. Under the aberration free condition, RBCs should be easily identified in OCT images since the platelets and white blood cells are much fewer and present very different scattering characteristics. We imaged the labial mucosa of human volunteers with the focal region aligned to the lamina propria. The optical thickness (refractive index = 1.33) was approximately 300 μm from the surface of the stratified squamous epithelium to the RBCs

in focus. In a representative aberration-corrected ADAS-OCT image (Fig. 5.8(b, d)), we could clearly identify six pairs of reflectance signals, presumably six RBCs (Fig. 5.8(e)). In contrast, in the corresponding control image (Fig. 5.8(a, c)) and SD-OCT images obtained through individual paths, RBCs numbered 4-6 could not be readily recognized due to aberrations. It is obvious that aberration correction significantly enhanced the peak signal intensity of RBCs as compared with the control image (Fig. 5.8(f)), which contributed to the visibility of RBCs. In one of the other images, we could recover signals of RBCs even when one or both reflection signals were lost due to aberrations.

5.3.5 Scalable imaging performance

The performance of the ADAS-OCT technique can be enhanced by scaling up the number of sub-apertures and increasing the displacement between them. Using the dual beam displacer setup (Fig. 5.2(e)), we obtained three equally displaced sub-apertures: oo, ee, and oe/eo. The displacement between the centers of the sub-apertures was adjusted in terms of R by rotating the second displacer ($R = 1.3$ mm is the radius of the illumination beam measured at the 1% power level). This scalability was demonstrated by correcting defocus, which is the simplest aberration but represents a general problem in microscopy and endoscopy. As before, we used the tissue phantom consisting of $6\text{-}\mu\text{m}$ -diameter microparticles suspended in agarose gel. At a sample location $250\text{ }\mu\text{m}$

Figure 5.9: Improving the transverse performance of the defocus correction by increasing the number of sub-apertures. (a-c) Control images of microparticles located 250 μm above the focal plane. The transverse resolution did not vary significantly with sub-aperture displacement in the control images. (d-f) Corresponding aberration-corrected images using two sub-apertures: oo and ee. (g-i) Corresponding aberration-corrected images using three sub-apertures: oo, ee, and oe/eo. Insets: illustrations of sub-apertures. (j) Comparison of the transverse FWHM of the microparticles on going from the control image (a) to the corrected image with two sub-apertures (d) to the corrected image with three sub-apertures (g). (k) Comparison of the transverse FWHM of the microparticles on going from the control image (b) to the corrected image with two sub-apertures (e) to the corrected image with three sub-apertures (h). (l) Comparison of the transverse FWHM of the microparticles on going from the control image (c) to the corrected image with two sub-apertures (f) to the corrected image with three sub-apertures (i). SA, sub-apertures; R, radius of the illumination beam. Transverse and vertical scale bars: 100 μm .

above the focal plane, the mean transverse FWHM of the particles was degraded to 24.1 μm , 25.6 μm , and 27.1 μm in the control images (Fig. 5.9(a-c) and red line in (j-l)). For the group of two sub-apertures (oo and ee), the transverse FWHM was found to improve by 9.1% (for displacement between sub-apertures, $= 0.2R$), 25.8% (for $= 1R$) and 57.2% (for $= 1.8R$) (Fig. 5.9(d-f) and green line in (j-l)). Larger displacement results in better correction also held true in the group of three

sub-apertures (oo, ee, and oe/eo) (Fig. 5.9(g-i) and blue line in (j-l)). In the largest displacement of $1.8R$ between the oo sub-aperture and the ee sub-aperture, the mean transverse FWHM was $9.2\ \mu\text{m}$ with three sub-apertures, which was 69.4% better than that of the control image data (Fig. 5.9(l)), while the mean transverse FWHM was $11.6\ \mu\text{m}$ with two sub-apertures. It is suggested that the number of sub-apertures is related to the improvement of the transverse FWHM.

5.4 Discussions and conclusions

ADAS-OCT offers a relatively simple route to cellular-level resolution tomography through turbid tissues *in vivo*. Our approach avoids the phase stability issue encountered by existing computational-based aberration-correction methods. Current computational formulations require full-field data acquisition, during which motion-induced phase instability is inevitable. ADAS-OCT is inherently insensitive to motion-induced phase variations or spatially variant wavefront distortions because it collects scattered signals from all sub-apertures simultaneously. Therefore, to the best of our knowledge ADAS-OCT is the first method that can correct tissue induced aberrations during a rapid cellular process, such as blood flow.

This technical advance may enable noninvasive blood count, which will potentially address many challenges in diagnosis, management, and treat-

ment of diseases. Neonatal blood sampling represents one type of clinical challenges where blood volume and accessible veins are limited for needle puncture, and capillary skin puncture may be associated with increased risk of infection, injury, pain, and maternal anxiety. While, blood count monitoring during chemotherapy represents another type of challenges where the venipuncture-based blood test may lead to complications, and delays and/or dose reductions of subsequent courses of chemotherapy. A non-invasive blood count device enabled by ADAS-OCT could potentially reduce the number of blood samples from neonatals and allow rapid tailoring the dosage and improve the life quality of patients undergoing chemotherapy.

ADAS-OCT is based on the same depth-priority point-scanning mechanism as the most of current endoscopic and intracoronary OCT systems, so that it can be potentially developed into an endoscopic technology that performs high-speed circumferential and helical scanning. In contrast, to maintain phase stability high enough to guarantee coherent aperture synthesis, existing computational-based aberration-correction methods have to rely on special scanning mechanisms such as line-field scanning or time-domain lateral priority scanning, which are not suitable for endoscopic applications in tubular organs. An ADAS-OCT endoscopic probe could be realized in practice by incorporating a beam displacer and a polarizer into a classical fiber-optic design. Using a calcite beam displacer, the two-beam separation angle could be 2.2° with

a probe diameter of 2 mm and a rigid length of 21.2 mm, based on optical simulation results using Zemax software (Zemax, LLC, Kirkland, Washington, USA). This probe diameter should be compatible with the instrument channel of a standard video endoscope or in a tethered capsule endomicroscope.

Looking towards the goal of tomographic diagnosis at the cellular level, future efforts will focus on the development of a clinically viable endomicroscopic ADAS-OCT system. The diameter and rigid length of the probe may be further reduced by using materials with larger birefringence, such as yttrium orthovanadate (YVO₄). Space constraints could be further mitigated by employing a tethered capsule that can accommodate larger-diameter optics. The ranging depth of the spectrometer could be extended through the use of spectral combination, linearization, and circular interferometric ranging.

Funding: This research is supported in part by the National Research Foundation Singapore (NRF) (NRF-CRP13-2014-05), Ministry of Education Singapore (MOE2013-T2-2-107), National Medical Research Council Singapore (NMRC/CBRG/0036 /2013) and NTU-AIT-MUV program in advanced biomedical imaging (NAM/15005).

Chapter 6

Spectral domain optical coherence
tomography with dual-balanced
detection for auto-correlation
artifacts reduction

Abstract

We developed a spectral domain optical coherence tomography (SD-OCT) to reduce auto-correlation artifacts (ACA) using dual-balanced detection (DBD). ACA was composed of the interference signals between different sample tissue depths, and shown up as artifacts in OCT images. This system employed a free-space Michelson interferometer, at the refraction plane of whose beam splitter, the light reflected experienced a $\pi/2$ phase shift with respect to the light transmitted. Then two phase-opposed interferometric spectra sharing the same spectrometer optics were obtained simultaneously using two lines of a three-line camera. This new design was of lower cost compared to the dual spectrometer design reported previously. DBD enabled this SD-OCT to achieve two-fold increase in the interested signal amplitude inherently, and obtain a SNR increase of ~ 2.9 dB experimentally. To demonstrate the feasibility and performance of this SD-OCT system with DBD, we conducted an imaging experiment using a glass plate to obtain the optimal spectral matching between dual-balanced spectrometer channels. As a result, this SD-OCT achieved ACA reduction up to about 9 dB and direct current (DC) term suppression up to about 30 dB by cancelling the identical components between dual-balanced spectrometer channels. The efficacy of ACA reduction and DC suppression was validated by imaging the polymer coating of a drug-eluting stent and fresh swine corneal tissue *ex vivo*. The quality of DBD optimized images was significantly improved

with regard to the single-channel images.

6.1 Introduction

In classical theory of FD-OCT, ACA composed of the interference signals between different sample tissue depths, shown up as fundamental artifacts in OCT images, especially for tissues with layered structures. These artifacts can be effectively suppressed by use of DBD [110–114]. DBD can be conveniently implemented in swept-source OCT with the off-the-shelf dual-balanced photo-detectors [42, 115]. However, there is few established DBD solution for spectral-domain due to unavailability of dual-balanced linear camera and spectrometer optics.

In this study, SD-OCT with DBD mainly for ACA reduction is developed. Compared with single and unbalanced detection, DBD enables SD-OCT system to achieve an SNR increase of 3 dB inherently. Most importantly, this SD-OCT with DBD achieves a good performance of ACA reduction and DC suppression [112, 114]. Compared to a dual spectrometer OCT system [112–114], two phase-opposed interferometric spectra are captured simultaneously using two lines of a three-line camera in this study, and share the identical spectrometer optics. This new design can be of lower cost and better performance compared to the dual spectrometer design.

6.2 Theory

In classical theory of Fourier domain OCT, the detected interference signal $I(k)$ in k -space, including DC, AC and CC terms, was presented in Eq. (2.3). SNR is defined as $SNR[dB] = \langle S_{OCT}^2 \rangle / \langle N_{OCT}^2 \rangle$, which can be denoted in dB as

$$SNR[dB] = 10 \cdot \log \frac{N_{ref} \cdot \sum N_{sam}}{N_{el}^2 + N_{sh}^2 + N_{RIN}^2} \quad (6.1)$$

where N_{ref} is the number of electrons in a single pixel generated by reference arm light, and $\sum N_{sam}$ is the total number of electrons over a single pixel array generated by sample arm light returning from a 100% mirror. N_{el}^2 is the electrical noise, which mainly consists of readout noise N_{read}^2 and dark noise N_{dark}^2 of the three-line camera. N_{sh}^2 and N_{RIN}^2 are the numbers of electrons generated by shot noise and relative intensity noise, respectively.

The reference and sample power can be given by

$$\begin{cases} N_{ref} = \frac{\eta\tau}{h\nu} P_{ref} \\ N_{sam} = \frac{\eta\tau}{h\nu} P_{sam} \end{cases} \quad (6.2)$$

where η is the spectrometer efficiency, including the grating diffraction efficiency and quantum efficiency, and camera lens efficiency. τ is the time consumption for interference signal detection. $h\nu$ represents the energy of a single photon. P_{ref} and P_{sam} represent the power from

reference and sample arms, respectively. Here we define f as the reciprocal of double the exposure time of the three-line camera and $\Delta\nu$ as the FWHM spectral bandwidth of the reference arm light received by a single pixel. Under the condition that $P_{sam} \ll P_{ref}$ in most application, noise components in Eq. (6.1) can be written as

$$\begin{cases} N_{el}^2 = N_{read}^2 + N_{dark}^2 \\ N_{sh}^2 = N_{ref}^2 \\ N_{RIN}^2 = (f/\Delta\nu)N_{ref}^2 \end{cases} \quad (6.3)$$

When DBD configuration is employed in SD-OCT system, then two phase-opposed interferometric spectra can be obtained simultaneously in two dual-balanced channels. The desirable signal increases by a factor of 2, and DC term and AC term can be counterbalanced between dual-balanced channels to a great extent. Under the ideal DBD condition, the relative intensity noise N_{RIN}^2 , arising from the fluctuation of light source, can be eliminated to a great extent [114]. Compared with single detection configuration, the SNR with DBD configuration can be illustrated as

$$SNR_{DBD}[dB] = 10 \cdot \log \frac{2N_{ref} \cdot \sum N_{sam}}{N_{el}^2 + N_{sh}^2} \quad (6.4)$$

6.3 Experimental methods

6.3.1 SD-OCT system configuration

The optical configuration of SD-OCT system with dual-balanced detection was depicted in Fig. 6.1, which assumed a free-space Michelson interferometer structure. Light from a super luminescent diode array (T850-HP, Superlum, County Cork, Ireland) with a center wavelength of 850 nm and a spectral full-width-half-maximum (FWHM) of 165 nm respectively was collimated by a lens L1 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA), and then split into a sample arm and a reference arm by a 50:50 non-polarizing cube beam splitter BS1 (BS011, 700-1100 nm, Thorlabs, Newton, NJ, USA). The light beam was focused by an objective lens L5 (AC127-025-B-ML, Thorlabs, Newton, NJ, USA). The numerical aperture of the objective lens was 0.052, resulting in confocal parameter of 1.3 mm and FWHM transverse resolution of $6.013 \mu\text{m}$

Figure 6.1: Optical configuration of SD-OCT system with dual-balanced detection. L1-L6, lens; BS1-BS3, beam splitter; RM, reference mirror; SMF, single mode fiber; CH1-2, dual-balanced channel; VGA, v-groove array; G, grating; CL, camera link cable; IMAQ, image acquisition; PC, personal computer; AO, analog output; GS, galvo scanner.

[116]. On the refraction plane of beam splitter BS1, the light reflected experienced a $\pi/2$ phase shift with respect to the light transmitted. The light backscattered or reflected from the sample interfered with the reference arm light in BS1. The second and third non-polarizing cube beam splitter BS2 and BS3 (BS029, Thorlabs, Newton, NJ, USA) were placed before and after the Michelson interferometer to balance the spectral power density of the light beam backscattered from the sample arm and reflected from the reference arm. As two light beams underwent a double pass through BS1, so two collimating lenses L3 and L4 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA) obtained two phase-opposed interferometric spectra concurrently, which were guided to the spectrometer through two single mode fibers of the 8 fiber v-groove array VGA (VGA-8-250, OZ OPTICS, Canada) whose v-groove spacing was $250\ \mu\text{m}$, and collimated by an achromatic lens L6 (AC127-025-B-ML, Thorlabs, Newton, NJ, USA). The spectrometer was composed of a 1765 lines/mm diffraction grating (PING-Sample-020, Ibsen Photonics, Farum, Denmark), a camera lens (85mm, f/1.8D, Nikon, Tokyo, JP), and a three-line camera (ELIIXA 3V, e2V, Chelmsford, UK). Because the spacing between adjacent two lines of the sensor was $10\ \mu\text{m}$, and the pixel size was $10\ \mu\text{m} \times 10\ \mu\text{m}$, so the vertical spacing between two channels was $20\ \mu\text{m}$. In order to obtain this tiny vertical spacing, we rotated VGA by 1.35 degrees so that two dispersed spectra distinguished with a phase difference of π were projected onto the first and second line of the three-line camera. Two fiber ends representing CH1 and CH2 were shown in

the dashed inset in Fig. 6.1 and marked as circled 1 and 2. Among 4096 pixels in a single line, 2950 pixels were occupied to detect a total spectral range of 165 nm and each sensor pixel corresponded to a spectrum band of ~ 0.06 nm. The detected signals were digitized at 12-bit resolution and transferred to personal computer via camera link cable and an image acquisition board (KBN-PCE-CL4-F, Bitflow, Woburn, MA, USA). The timing of triggering signals generated by personal computer was well controlled, and used to synchronize the camera for image capture and the galvo scanner (GVSM002/M, Thorlabs, Newton, NJ, USA), respectively. The galvanometer-mounted mirror was driven by a saw-tooth pulse to provide transverse scanning.

6.3.2 Sensitivity analysis

The light source operated at a center wavelength of 845 nm and a FWHM of 165 nm. The spectrometer efficiency, including the grating diffraction efficiency and quantum efficiency, was determined by the ratio of the power detected by two lines of the three-line camera and the power at the entrance of the spectrometer, and estimated to be 0.45. The receiver noise (including readout noise, dark noise and quantization noise) was $N_{el} = 60e^-$, and the full well capacity (FWC) was 100 Ke^- . For the exposure time of 50 μs , this SD-OCT system operated at a line rate of 10 KHz. The maximum SNR [104, 114, 117] in a single pixel was achieved on the condition that N_{RIN} was equivalent to N_{el} and shot noise dominated

both the electrical noise and RIN. With a FWHM spectral bandwidth $\Delta\nu=17.09$ GHz, we obtained $N_{ref}=7.85\times10^4e^-$, which corresponded to an average fill factor of 0.783 and a reference arm power of 0.56 nW. As a result, the shot noise was $N_{sh}=280e^-$ and dominated 70% of the total noise power. The sample power described by Eq. (6.2) was measured to be 1.98 mW, which corresponded to $N_{sam} = 1.04 \times 10^{10}e^-$. So the sensitivity was estimated to be 99.8 dB using Eq. (6.1) and 103.0 dB using Eq. (6.4) respectively. In order to characterize the sensitivity of our SD-OCT system with SD and DBD configuration, we placed a partially reflected mirror (-42.7 dB reflectivity) at the focal plane of the object lens and maintained the path-length difference between the sample and reference arm at 0.1 mm. The sensitivity was measured to be 87.7 dB with SD configuration, and calculated to be 90.6 dB with DBD configuration. The experimental results agreed well with the theoretical results. In practice, this scheme realizing DBD with two lines of a three-line camera even achieved a significant SNR enhancement comparing to that employing two independent spectrometers, because the mismatch between every corresponding pixel in two independent spectrometers including gain and noise, may be greatly balanced with DBD configuration using one spectrometer.

6.4 Experimental results

6.4.1 Optimal spectral matching

In order to match two spectra acquired from two lines of the three-line camera sharing the same spectrometer optics, we developed a spectrum matching algorithm [55, 111]. Firstly, we acquired ten sets of interference fringes and their corresponding background spectra from two channels by changing the reference delay when a partially reflecting mirror was placed at the focal plane of the objective lens. Then each of the interference fringes was subtracted by the respective background spectrum. Secondly, we manually selected parts of the spectrum whose amplitude could not be neglected, detected the zero-crossing points, and achieved their indices. Thirdly, we selected one dense interference fringe as the template, and executed a limited search by moving the other interference fringes in the same channel ranging from $-N/2$ to $N/2$ (N is the number of zero crossing points) zero-crossing points. The square of the difference of two fringes in two channels respectively got its maximum value when the optimal spectral matching was achieved. Finally, the optimal spectral matching data was verified as follows. The original interference fringes acquired from CH1 and CH2 were depicted in Fig. 6.2(a). Both spectra were subtracted by their background respectively. Then the optimal spectral matching algorithm was applied to two corresponding signals from two channels, and results were shown in Fig. 6.2(b). It was obvious that there was a phase difference between signals in CH1 and CH2. The dual-balanced signal achieved by subtracting signals in CH2 from that in

CH1 was shown in Fig. 6.2(c). The interested signal increased with two times and presented a symmetrical shape. Finally, the depth resolved signals after FFT were presented in Fig. 6.2(d). It was obvious that DBD resulted in a significant SNR increase of ~ 2.9 dB and DC suppression of ~ 30 dB comparing to single detection. So this algorithm achieved a good performance of matching two spectra acquired from two lines of the three-line camera.

Figure 6.2: Optimal spectral matching. (a) Original interference fringes from CH1 (black curve) and CH2 (red curve). (b) Original interference fringes from CH1 (black curve) and CH2 (red curve) subtracted by their background respectively. (c) Fringes achieved with DBD configuration. (d) The depth resolved signals after FFT from SD-OCT with SD (black curve) and DBD (red curve) configuration respectively.

6.4.2 Auto-correlation artifacts reduction verification

To demonstrate the performance of optimal spectral matching between two lines of the three-line camera, reduce ACA and suppress DC, we chose a glass plate (BB024050A1, Menzel-Glaser, Germany) with a thickness of about 0.2 millimeter as the multilayer sample. A simple schematic of multilayer sample was illustrated in Fig. 6.3(a). The front surface and back surface indicated by FS and BS were corresponded to Fig. 6.3(b) with arrows, which included three cross-sectional images of the glass plate obtained using SD from CH1 and CH2 respectively, and using BDB. Comparing cross-sectional image captured using DBD to that captured using SD, the results showed that ACA was nearly completely reduced, and DC term was suppressed to a great extent. The profiles of one single axial line (A-line) indicated in red line in Fig. 6.3(b) were

Figure 6.3: Auto-correlation artifacts reduction verification. (a) Schematic of multilayer sample. (b) Cross-sectional images of the glass plate obtained using SD from CH1 and CH2 respectively, and using BDB. (c) A-line profiles indicated in red line in (b). The front surface and back surface were indicated by FS and BS; DC terms and auto-correlation terms were indicated by DC and AC.

depicted in Fig. 6.3(c). Comparing A-line captured using DBD to that captured using SD, peaks that represented the front surface and back surface of the glass plate doubled, and DC term was largely suppressed up to 10.6 dB. Most importantly, the ACA reduction in CH1 and CH2 were 9.39 dB and 9.11 dB, respectively. This experiment demonstrated that DBD achieved a good performance of ACA reduction and DC suppression performance.

6.4.3 Auto-correlation artifacts reduction in stent and cornea imaging

To demonstrate the performance of ACA reduction and DC suppression, we conducted two imaging experiments: the polymer coating of a drug-eluting stent and the swine cornea *ex vivo*.

Drug-eluting stent (DES) was composed of a bare-metal stent coated with an anti-proliferative polymer, which gradually allowed drug elution into the coronary wall for weeks after stent implantation so as to inhibit cell hyperplasia that causes restenosis. By the time entire drug had been released in six to nine months, the main risk of restenosis had been minimized. However, the anti-proliferative polymer coating was clinically broken on occasion. For such a drug-eluting stent with layered structures, ACA was the dominant artifact. ACA reduction helped us obtain a better cross-sectional image of drug-eluting stent, and a better understanding of how drug-eluting stent works and how to redesign

drug-eluting stent. Partial anti-proliferative polymer of an unemployed drug-eluting stent was broke when manually expanding and flatting this stent to scan. An intuitive schematic of the drug-eluting stent was manifested as Fig. 6.4(a). The blue plane indicated the scanning direction. Cross-sectional images of the stent captured from CH1 and CH2 using SD and BDB respectively were shown in Fig. 6.4. A-line profiles indicated in red in Fig. 6.4(b) were depicted in Fig. 6.4(c). Peaks represented the surfaces of the metal and coating had doubled, and the DC term had also been eliminated to a great extent. Auto-correlation reduction in CH1 and CH2 were 5.51 dB and 6.57 dB respectively. So the performance of ACA reduction had been demonstrated when imaging a stent clinically.

Figure 6.4: Auto-correlation artifacts reduction of the drug-eluting stent. (a) An intuitive schematic of the drug-eluting stent. The blue plane indicates the scanning direction. (b) Cross-sectional images of the stent obtained using SD from CH1 and CH2 respectively, and using BDB. DC: direct current term; AC: auto-correlation term; PC: polymer coating; MA: mental alloy; MI: mirror image; PD: petri dish. (c) A-line profiles indicated in red line in (b).

In order to validate this advantage, the other experiment was conducted on a swine cornea. The results shown in Figs. 6.5(a-c) demonstrated the reduction performance of ACA between epithelium layer and endothelium layer in a swine cornea. Figs. 6.5(a-c) compared cross-sectional images acquired using SD and DBD, respectively. Figs. 6.5(a-b) showed the images processed by SD and background subtraction. Partial DC term and mass ACA existed. Fig. 6.5(c) shows the image processed by DBD, where DC term and ACA were greatly suppressed. This experiment demonstrated that DBD achieved a good performance of ACA reduction and DC suppression performance when imaging a cornea, but there still existed partial ACA due to imperfect spectral matching.

Figure 6.5: Cross-sectional images of the central area of a swine cornea captured using SD from CH1 (a) and CH2 (b) respectively, and using BDB (c). EP: epithelium layer, ST: Stroma, DM: Descemets membrane, ED: Endothelium layer. Three images consist of 674 (axial) $\times$ 472 (transverse) pixels covering 0.32 mm $\times$ 1.0 mm.

6.5 Discussions and conclusions

In summary, auto-correlation artifacts reduction performance was demonstrated experimentally using dual-balanced detection in SD-OCT. Firstly, dual-balanced detection provides a practical approach to suppress direct-current background noise and reduce the auto-correlation artifacts, which are shown up as fundamental artifacts in OCT image, especially for tissues with layered structures of highly scattering/reflection. Due to the relative large spacing between adjacent channels of VGA, noticeable mismatch still exists between two dual-balanced channels even sharing the same spectrometer optics, resulting in about 10% residual auto-correlation artifacts in images using dual-balanced detection. This issue can be solved by use of VGA of smaller spacing, for instance, 80 μm . Secondly, dual-balanced detection enables SD-OCT to achieve a SNR increase of 3 dB inherently. As a result, a shorter exposure time and higher scanning rate were achieved. However, the sensitivity of the proposed SD-OCT system with dual-balanced detection is suboptimal in that about 20% of focused light was not detected by the camera sensor because the beam spot size on the camera is larger than the pixel size. This problem can be solved by using a grating with larger area in the spectrometer. In addition, the noticeable chromatic focal shift is another reason for suboptimal sensitivity. Thirdly, dual-balanced detection employing two lines of a three-line camera can be of lower cost compared with two independent spectrometers design. Experiments on a stent and

a swine cornea have demonstrated the feasibility of dual-balanced detection in SD-OCT.

Funding: This research is supported in part by the Nanyang Technological University (Startup grant: Linbo Liu), National Research Foundation Singapore (NRF2013NRF-POC001-021 and NRF-CRP13-2014-05), National Medical Research Council Singapore (NMRC/CBRG/0036/2013), and Ministry of Education Singapore (MOE2013-T2-2-107).

Chapter 7

Single-camera full-range

high-resolution spectral domain

optical coherence tomography

Abstract

We developed a spectral domain optical coherence tomography (SD-OCT) using a dual-channel spectrometer for complex conjugate artifacts (CCA) suppression. We used a three-line camera to detect two interferometric spectra with $2\pi/3$ phase difference simultaneously. The complex interferometric signal was reconstructed by trigonometric manipulation of two real interferometric spectra, and then full-range images were obtained by use of inverse Fourier transform (iFT). Artifacts at direct current (DC) and the ghost remnant of the CCA are common issues with previously reported two-spectrometer method since slight mismatching between two spectral detection channels had strong negative effects on CCA suppression and appeared to be the limiting factor on system performance. This novel dual-channel spectrometer uses the same spectrometer optics for the two spectral detection channels, therefore, achieves a better matching between two spectral detection channels, and consequently better performance in CCA suppression as compared to the dual spectrometer solution. Full-range imaging with CCA suppression ~ 25 dB was demonstrated when imaging an attenuated reflector. The efficacy of both CCA and DC suppressions was also validated by imaging the anterior segment of a rat eye *ex vivo*. The quality of CCA-suppressed images was significantly improved with regard to those obtained with the dual-spectrometer design.

7.1 Introduction

OCT is a versatile cross-sectional and three-dimensional imaging technique, capable of producing non-invasive, high-speed and high-resolution images through highly scattering samples, such as inhomogeneous biological tissue [23]. Since firstly introduced for retinal imaging [23], it was extensively studied and widely applied in ophthalmology [34] and cardiology [35]. In aforementioned applications, it is advantageous to obtain the complex interferometric signal, which encodes amplitude and phase information of the backscattered light from the sample. Conventional Fourier domain OCT (FD-OCT) system yields only the real part of the complex interferometric signal, which results in CCA and limits full-range imaging. To achieve full-range imaging, various methods have been proposed and in-depth studied in the past decade. These methods include phase shifting implemented using piezo-transducer mounted reference mirrors [118], sinusoidal phase modulation [119, 120], electro-optic [121] or acousto-optic modulators [122], dispersive optical delay line [123], galvo scanner mirror [124–126], dual detection [127], polarization quadrature encoding [128], Hilbert transformation [129, 130], and dispersion-encoded approach [131]. However, each of these methods suffers from its own shortcomings. Phase shifting requires a precisely calibrated reference arm, and therefore is sensitive to any interferometer drifts between phase-shifted acquisitions. Polarization quadrature encoding suffers from polarization fading and requires a complicated

setup. Although Hilbert transformation requires only one axial line (A-line) per measurement location, and thus improving imaging speed, it is still a phase shifting method. Additional phase shifting element requires correct triggering, which makes the system costly and complex. The dispersion-encoded approach based on iterative algorithm is time consuming. A method was proposed to exploit the inherent phase shifts of a 3×3 fiber coupler [132–134], which enabled the instantaneous retrieval of complex interferometric signals using dual spectrometers. This design is compact and simple. However, due to mismatched optical system between two spectrometers, the performance of CCA suppression is suboptimal.

In this study, a SD-OCT employing a two-channel spectrometer for full-range imaging is developed. Compared to the dual spectrometer OCT system [132–134], two phase-shifted interferometric spectra sharing the identical spectrometer optics are captured simultaneously using two lines of a three-line camera. Such dual-channel spectrometer achieves better matching between two spectra, and guarantees that the same wavelength range is exactly sampled in each corresponding pixel of the two lines. This new design is also of lower cost and achieves better CCA suppression performance as compared to the dual spectrometer design.

7.2 Theory

In classical theory of Fourier domain OCT, the detected interference signal $I(k)$ in k -space, including DC, AC and CC terms, was presented in Eq. (2.3). DC term and AC term are omitted for simplification. CCA arises for the sake that $S(k)$ used in FD-OCT only represents the real part of the complex interferometric signal. In order to suppress CCA, the complex interferometric signal $I_{Co} = I_{Re} + j \cdot I_{Im}$ must be reconstructed, where I_{Re} and I_{Im} are the real and imaginary parts of the complex interferometric signal and are defined as

$$\begin{cases} I_{Re}(k) = 2S(k) \int_{-\infty}^{+\infty} a(z) \cos(2knz + \phi) dz \\ I_{Im}(k) = 2S(k) \int_{-\infty}^{+\infty} a(z) \sin(2knz + \phi) dz \end{cases} \quad (7.1)$$

Define the iFT of $S(k)$ as $\gamma(z)$. Making use of the Fourier transform pairs $(1/2)[\delta(z + z_0) + \delta(z - z_0)] \longleftrightarrow \cos kz$ and $j(1/2)[\delta(z + z_0) - \delta(z - z_0)] \longleftrightarrow \sin(kz)$, and the convolution property of FT $x(z) \otimes y(z) \longleftrightarrow X(k)Y(k)$, the iFT of I_{Re} , I_{Im} and I_{Co} could be calculated as

$$\begin{cases} i_{Re}(z) = \gamma(z) \otimes \sum_{n=1}^N a(z) [\delta(z + z_0) + \delta(z - z_0)] \\ i_{Im}(z) = \gamma(z) \otimes \sum_{n=1}^N ja(z) [\delta(z + z_0) + \delta(z - z_0)] \\ i_{Co}(z) = \gamma(z) \otimes \sum_{n=1}^N a(z) [2\delta(z - z_0)] \end{cases} \quad (7.2)$$

When $i_{Re}(z)$ and $i_{Im}(z)$ are summed together, CCA at depth of $z = z_0$ will be eliminated in theory. Here making use of the inherent phase shifts of a 3×3 coupler, the complex interferometric signal may be reconstructed by trigonometric manipulation of two real interferometric spectra. Assuming that the SD-OCT system is built up using a 3×3

coupler as shown in Fig.7.1, six fiber pigtails of the fiber coupler were denoted as P_x and P_y , where $x = 1 - 3$ and $y = 4 - 6$ for the fiber pigtails at the left and right sides of the fiber coupler, respectively. Two spectra are directed into fiber pigtails P_2 and P_3 , and captured simultaneously by two lines of the three-line camera. They are indicated as $I_2(k)$ and $I_3(k)$. If we assume $I_2(k)$ as the real part $I_{Re}(k)$ of the complex interferometric signal, then the imaginary part $I_{Im}(k)$ of the complex interferometric signal may be obtained by using cosine sum rule [132]. The reconstructed complex interferometric signal is quoted as

$$I_{Co}(k) = I_{Re}(k) + j \frac{I_{Im}(k) \cdot \cos(\Delta\phi_{xy}) - \beta_{xy} \cdot I_{Re}(k)}{\sin(\Delta\phi_{xy})} \quad (7.3)$$

Here $\beta_{xy} = (\alpha_{11}\alpha_{1y}\alpha_{12}\alpha_{2y}/\alpha_{11}\alpha_{1x}\alpha_{12}\alpha_{2x})^{1/2}$ is the wavelength-dependent power splitting ratio between two lines of the three-line camera. $\Delta\phi_{xy} = \phi_x - \phi_y$ is the phase difference. The coupler splitting ratio α_{xy} characterized the power transfer from the fiber pigtail P_x to the fiber pigtail P_y . For the sake that $I_{Re}(k)$ and $I_{Im}(k)$ were captured through fiber pigtails P_2 and P_3 , we take $x = 2$ and $y = 3$ into Eq. (7.3), the reconstructed $I_{Co}(k)$ is given by

$$I_{Co}(k) = I_2(k) + j \frac{I_3(k) \cdot \cos(\Delta\phi_{23}) - \beta_{23} \cdot I_2(k)}{\sin(\Delta\phi_{23})} \quad (7.4)$$

Figure 7.1: Schematic of the instantaneous complex conjugate resolved SD-OCT system employing a 3×3 truly fused-fiber coupler. SLD, super luminescent diode; OC, optical fiber coupler; P_x ($x = 1 - 3$), P_y ($y = 4 - 6$), six pigtailed of the OC; PC, polarization controller; L1-L5, lens; L6, camera lens; NDF, neutral density filter; RM, reference mirror; GS, galvo scanner; FC, fiber connector; VGA, v-groove array; F1-2, fiber ends; G, grating; IMAQ, image acquisition; WS, Workstation.

7.3 Experimental methods

The schematic of the instantaneous complex conjugate resolved SD-OCT system employing a 3×3 truly fused-fiber coupler was depicted in Fig. 7.1. A super luminescent diode array (T850-HP, Superlum, County Cork, Ireland) provided a broadband spectrum with a full-width-half-maximum (FWHM) bandwidth of 165 nm centered at 850 nm. The light source, whose total output power was measured to be 18.0 mW, was split into the sample arm and the reference arm by a 3×3 fiber coupler (AC Photonics Inc., CA, USA). The light beam in the reference arm was collimated by a lens L1 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA) to transmit through a neutral density filter (NDL-10C-4,

Thorlabs, Newton, NJ, USA), and then focused by a lens L2 (AC127-030-B-ML, Thorlabs, Newton, NJ, USA) onto the reference mirror RM (PF10-03-P01, Thorlabs, Newton, NJ, USA). L2 and RM could move together to match path length in the sample and reference arms. The light beam in the sample arm was collimated by a lens L3 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA) to project onto the galvo scanner, and then focused by an objective lens L4 (AC127-030-B-ML, Thorlabs, Newton, NJ, USA). The NA of the objective lens was 0.043, resulting in FWHM transverse resolution of $12.59\ \mu\text{m}$ and the confocal parameter of $270.42\ \mu\text{m}$ [116]. Two polarization controllers PC1 and PC2 (PLC-900, Thorlabs, Newton, NJ, USA) were placed in the sample and reference arms, respectively. The light backscattered from the sample arm and reflected from the reference arm were guided to the spectrometer through two single mode fibers of the 8-fiber v-groove array VGA (VGA-8-250, OZ OPTICS, Canada) with a v-groove spacing of $250\ \mu\text{m}$, and collimated by an achromatic lens L5 (AC127-030-B-ML, Thorlabs, Newton, NJ, USA). The spectrometer consists of a 1765 lines/mm diffraction grating (PING-Sample-020, Ibsen Photonics, Farum, Denmark), a camera lens (85mm, f/1.8G, Nikon, Tokyo, JP), and a three-line camera (ELIXA 3V, e2V, Chelmsford, UK). Because the center-to-center distance between two lines of the camera sensor was $20\ \mu\text{m}$, we rotated VGA by 1.35 degrees in order to obtain this tiny vertical spacing, and ensure that two dispersed spectra were projected onto the first and second lines of the camera simultaneously. Two fiber ends marked as F1 and

F2 were shown in Fig. 7.1. Here we termed this spectrometer as the dual-channel spectrometer. Among 4096 pixels, 3500 pixels were used to detect a total spectral range of 165 nm and each sensor pixel corresponded to a spectrum band of 0.055 nm. The A-line rate and exposure time of the camera were set to be 10K A-lines/s. The detected spectrum was digitized at 12-bit resolution and transferred to the workstation via an image acquisition board (KBN-PCE-CL4-F, Bitflow, Woburn, MA, USA) and two camera link cables. Two-directional transverse scanning was implemented by two galvanometer-mounted mirrors (GVSM002/M, Thorlabs, Newton, NJ, USA) driven with a saw-tooth pulse, which was generated by a 16-bit analog voltage driver including a data acquisition board (PCI-6221, National Instruments, Austin, TX, USA) and a shielded connector block (BNC-2110, NI). The timing of triggering spectrum acquisition was generated by the workstation and was used to synchronize the camera and the glove scanner. The discrete FT (DFT) was performed on each frame of 512 A-lines obtained by the camera to produce an axial depth profile of the sample.

7.4 Experimental results

7.4.1 Wavelength dependent coupler splitting ratio

The coupler splitting ratio α_{xy} characterized the power transfer from the fiber pigtail P_x to the fiber pigtail P_y . Here we had two reasonable as-

Figure 7.2: Wavelength dependent coupler splitting ratio. (a) Nine measured coupling coefficients α_{xy} of the 3×3 coupler. (b) The derived curves for phase shift $\Delta\phi_{xy}$. (c) The derived curves for coupler splitting coefficient β_{xy} .

sumptions: (1) perfect reciprocity (i.e., $\alpha_{14} = \alpha_{41}$); (2) lossless coupling (i.e., $\sum_{y=4}^{y=6} \alpha_{xy} = 1, x = 1 - 3$) for the 3×3 fiber coupler [132]. Given a set of measured values of α_{xy} , the interferometric phase shifts $\phi_x - \phi_y$ between coupler arms are uniquely determined. For example, if $\alpha_{xy} = 1/3$ for all fiber pigtails P_x and P_y , then the interferometric phase shift between any two output ports of a 3×3 Michelson interferometer is 120° [132]. However, splitting ratios of the fiber coupler were strongly wavelength dependent [133]. These measured coupling ratios α_{1y} , α_{2y} and α_{3y} , as shown in Figs. 7.2(a), demonstrated that the coupler splitting ratios were strongly wavelength dependent. The phase shift $\Delta\phi_{xy}$ shown in Fig. 7.2(b) and coupler splitting coefficient β_{xy} shown in Fig. 7.2(c) were derived from the measured coupling ratios according to Eq. (7.3). When two spectra were captured by the two-channel spectrometer, the

complex interferometric signal was $I_{Co} = I_{Re} + j \cdot I_{Im}$ reconstructed using the calibrated $\Delta\phi_{xy}$ and β_{xy} according to Eq. (7.4). To correct the nonlinear k -space sampling interval, each A-line was mapped to uniform frequency spacing by linear interpolation. Interpolation was composed of computing a DFT of the sampled data, zero padding, inverse DFT, and re-sampling of the resultant data at regular intervals. CCA-suppressed A-lines were obtained by inverse DFT of the resultant complex interferometric signal.

7.4.2 CCA suppression verification

To demonstrate the performance of CCA suppression, we conducted experiments to compare complex conjugate resolved A-lines with unresolved A-lines. A partially reflecting mirror (-24.5 dB reflectivity) was placed at the focal plane of the object lens. The path-length difference between the sample and reference arm was maintained at 0.05 mm. Original interference signals captured by the first and second channels were subtracted by their respective background, which was obtained by averaging over all the 512 A-lines of a frame. The resultant interferometric signals with a phase difference of 113° were illustrated in Fig. 7.3(a). Here the signal from CH1 was defined as the real part of the complex interferometric signal. The imaginary part of the complex interferometric signal was obtained using the calibrated phase shift and coupler splitting coefficient according to Eq. (7.3). Then real and imag-

Figure 7.3: Complex conjugate artifacts suppression performance. (a) Interferometric parts of original interference signals captured from the first (black) and second (red) lines of the three-line camera. (b) Real (black) and imaginary (blue) parts of the complex interferometric signal. (c-d) A-lines without and with CCA suppressed by taking inverse DFT of the real part signal and the complex interferometric signal, respectively.

inary parts of the complex interferometric signal with a phase difference of 90° were presented in Fig. 7.3(b). As can be seen, a phase shift of 90° existed between the real and imaginary parts of the complex interferometric signal. To obtain a better view of the phase shift, only a part of signals with a wavelength of 840-860 nm was shown in Figs. 7.3(a-b). When performing inverse DFT of the real part signal and the complex interferometric signal, the corresponding A-lines without and with CCA suppressed were illustrated in Fig. 7.3(c-d), respectively. Results showed

that a CCA suppression of ~ 25 dB was achieved. The residual CCA in the A-line depth profile of the single reflector in the sample arm was likely due to imperfect matching between two spectra, even sharing the same spectrometer optics. This experiment demonstrated that this SD-OCT system employing a 3×3 coupler achieved full-range A-lines with a good performance of CCA suppression and that the ranging depth has been doubled.

7.4.3 Sensitivity analysis

Figure 7.4: CCA-suppressed A-lines with different depth delays. The height of suppressed CCA kept constant within the depth delay of 0.4 mm. The peak SNR and maximum CCA suppression with a depth of 0.05 mm was 73.0 dB and 25.1 dB respectively. A -6 dB sensitivity falloff from the peak existed at a depth of 0.18 mm.

The spectrometer efficiency, including the grating diffraction efficiency and quantum efficiency, was determined by the ratio of the power detected by two lines of the camera and the power at the entrance of the

spectrometer. It was estimated to be 0.42. The electrical noise (including readout noise, dark noise and quantization noise) was $N_{el} = 60e^-$, and the full well capacity was $100 Ke^-$. For an exposure time of $70 \mu s$, this SD-OCT system operated at a line rate of 7.14 kHz. The maximum SNR in a single pixel was achieved on the condition that N_{RIN} was equivalent to N_{el} , and shot noise dominated both the electrical noise and relative intensity noise [104, 117, 135]. With a FWHM spectral bandwidth $\Delta\nu = 17.09$ GHz, we obtained $N_{ref} = 9.28 \times 10^4 e^-$, which corresponded to an average fill factor of 0.93 and a reference arm power of 0.51 nW. As a result, the shot noise $N_{sh} = 305e^-$ was and dominated 72 % of the total noise power. The sample power was measured to be 0.55 mW, which corresponded to $N_{sam} = 1.27 \times 10^{10} e^-$, and therefore the sensitivity was estimated to be 99.7 dB. In order to characterize the sensitivity of our SD-OCT system, a partially reflecting mirror was placed at the focal plane of the object lens. CCA-suppressed A-lines with different depth delays were illustrated in Fig. 7.4. The height of suppressed CCA kept constant within the depth delay of 0.4 mm. The peak SNR with a depth of 0.05 mm in Fig. 7.4 was 73.0 dB, and thus the sensitivity was measured to be 97.5 dB. The experimental results matched well with the theoretical results. The imaging range over the 6-dB sensitivity falloff is approximately 0.18 mm.

Figure 7.5: Full-range imaging of the anterior segment of a rat eye. (a) Cross-sectional image of the anterior segment of a rat eye obtained using real part of the complex interferometric signal. (b) A-line profile indicated in white dashed line in (a). (c) Cross-sectional image of the anterior segment of a rat eye obtained using the reconstructed complex interferometric signal. (d) A-line profile indicated in white dashed line in (c). EP: epithelium layer, ST: Stroma, DM: Descemet's membrane, EN: Endothelium layer, AC: anterior chamber. Two images consist of 1000 axial 500 transverse pixels covering 0.56 mm (D) 6.4 mm (W).

7.4.4 CCA suppression performance in imaging

To demonstrate the performance of CCA suppression, we conducted full-range imaging of the anterior segment of a rat eye *ex vivo*. The results were shown in Fig. 7.5. Fig. 7.5(a) was the cross-sectional image of the anterior segment of a rat eye obtained using real part of the complex interferometric signal. The CCA was manifested as overlapping mirror image, resulting in severe distortion. Fig. 7.5(c) was the cross-sectional image of the anterior segment of a rat eye obtained using the reconstructed complex interferometric signal according to Eq. (7.4). Com-

parison of Figs. 7.5(a) and (c) demonstrated a good performance of CCA suppression using the instantaneous complex conjugate resolved SD-OCT system. Furthermore, the usable imaging depth was doubled. Figs. 7.5(b) and (d) were A-line profiles indicated by white dashed line in Figs. 7.5(a) and (c) respectively. More details could be found to verify the performance of CCA suppression. Due to reflective interfaces of unknown origin within the anterior segment, some spurious reflective artifacts existed in Fig. 7.5(a) at the depth of 0.18 mm and Fig. 7.5(c) at the depth of 0.46 mm. There also existed slight residual CCA in Fig. 7.5(c) due to the imperfect spectral matching of the dual-channel spectrometer.

7.5 Discussions and conclusions

In summary, CCA suppressed full-range imaging performance was demonstrated using the instantaneous complex conjugate resolved SD-OCT system. Such a system has the following advantages. Firstly, two-channel spectrometer employing a three-line When $i_{Re}(z)$ and $i_{Im}(z)$ are summed together, CCA at depth of $z = z_0$ will be eliminated in theory. Here making use of the inherent phase shifts of a 3×3 coupler, the complex interferometric signal may be reconstructed by trigonometric manipulation of two real interferometric spectra. Assuming that the SD-OCT system is built up using a 3×3 coupler as shown in Fig.7.1, six fiber pigtails of the fiber coupler were denoted as P_x and P_y , where

$x = 1 - 3$ and $y = 4 - 6$ for the fiber pigtails at the left and right sides of the fiber coupler, respectively. Two spectra are directed into fiber pigtails P_2 and P_3 , and captured simultaneously by two lines of the three-line camera. They are indicated as $I_2(k)$ and $I_3(k)$. If we assume $I_2(k)$ as the real part $I_{Re}(k)$ of the complex interferometric signal, then the imaginary part $I_{Im}(k)$ of the complex interferometric signal may be obtained by using cosine sum rule [132]. The reconstructed complex interferometric signal is quoted as could be more compact and of lower cost compared with dual spectrometers design. Secondly, two-channel spectrometer enabled a more careful matching of two spectra to ensure that the exact same wavelength range was sampled in each corresponding pixel of the two lines. Slight mismatching of the spectrum had strong negative effects on CCA suppression and appeared to be the bottleneck of CCA suppression performance. Due to the imperfect spectral matching of the two-channel spectrometer caused by the relatively large spacing between adjacent channels of the VGA, there still existed slight residual CCA. A VGA with a smaller spacing (for instance, $80 \mu\text{m}$) may be employed to solve this issue. Thirdly, although this SD-OCT system employing a 3×3 coupler was less efficient in delivering light to sample than conventional 2×2 coupler, it was more efficient at collecting the light backscattered from the sample. Such advantage is especially significant in the exposure-limited applications such as retinal imaging. For instance, this SD-OCT system delivered 33% of the source light to the sample and collected 66% of the backscattered light from the sam-

ple. In contrary, the conventional SD-OCT system employing a 2×2 coupler delivered 50% of the source light to the sample and collected 50% of the light backscattered from the sample. In a word, full-range imaging experiment on the anterior segment of a rat eye was conducted to demonstrate the performance of CCA suppression.

Funding: This research is supported in part by the National Research Foundation Singapore (NRF-CRP13-2014-05), National Medical Research Council Singapore (NMRC/CBRG/0036/2013), Ministry of Education Singapore (MOE2013-T2-2-107) and NTU-AIT-MUV program in advanced biomedical imaging (NAM/15005).

Chapter 8

Conclusions and future developments

8.1 Contributions to the field of OCT

Multiple aperture synthesis (MAS) OCT is a new modality for cellular and sub-cellular resolution imaging over an extended depth of focus (DOF), has the great potential for in vivo endoscopic and intravascular imaging. This thesis proposes and validates novel techniques to address the fundamental issues of DOF, autocorrelation artifacts (ACA) and complex conjugate artifacts (CCA) that prevent MAS OCT to be an *in vivo* imaging tool for clinical applications. To summarize, we have

1. Invention and validation of MAS OCT imaging system using a micro cylindrical lens driven by a piezoelectric transducer. This technique can maintain a diffraction-limited transverse resolution along a DOF that is ~ 10 times larger than the Confocal parameter. Fur-

- thermore, this technique was for the first time to be applied for biological tissue imaging and the results demonstrated the feasibility and efficacy of high-resolution biological tissue imaging with a dramatic DOF extension (Chapter 3).
2. Invention and validation of a parallel MAS OCT which employs a mirror with two surfaces coated without sacrificing imaging speed. The DOF is extended without sacrificing the transverse resolution for proof-of-concept experiments. This miniaturized fiber-based OCT system makes MAS more flexible and portable without compromising features, and can be used in conjunction with the conventional OCT technology for skin or ocular application (Chapter 4).
 3. Invention and validation of an endoscopy-compatible MAS OCT (also named ADAS OCT) by employing calcite beam displacer. This OCT enables cross-sectional imaging of biological tissues with cellular and subcellular structures resolved over a depth range of $\sim 500 \mu\text{m}$. This design can be easily miniaturized and designed into a high-resolution and DOF-extended probe for endoscopic and intravascular cross-sectional imaging *in vivo* (Chapter 5).
 4. development of a novel dual-channel spectrometer by employing two lines of a three-line CCD to suppress ACA and CCA. The efficacy of ACA suppression of $\sim 9 \text{ dB}$ was validated by imaging the polymer coating of a drug-eluting stent and fresh swine corneal tissue *ex*

in vivo. The efficacy of CCA suppression of ~ 25 dB was validated by imaging the anterior segment of a rat eye *ex vivo*. This new design was of lower cost compared to the dual spectrometer design reported previously. The quality of ACA- / CCA-suppressed images was significantly improved with regard to those obtained with the dual-spectrometer design (Chapters 6&7).

As prescribed in Chapter 1, this thesis focused on the fundamental studies to demonstrate technical feasibility and develop laboratory prototype. A significant research effort is required to develop a miniaturized MAS OCT for endoscopic and intravascular applications. Feasibility studies are expected for widespread applications.

8.2 Future development of MAS OCT

8.2.1 Ranging depth of MAS OCT

As is discussed in Chapter 3, the effective spectral bandwidth was limited by the camera lens (85 mm, f/1.2, Canon Inc., Tokyo, JP) resulting in a deteriorated axial resolution of $3.0 \mu\text{m}$. A camera lens with larger aperture may be used in future to mitigate the problem. This limitation can ultimately be overcome by using two spectrometers to acquire two halves of the light spectrum and then recombining them [55]. Another newly reported technique combined a grating and a prism to achieve a linear k-space spectrometer [100], and extended the ranging depth by

~ 2 times. These solutions impart MAS with a sufficient ranging depth, a $\sim 2.0 \mu\text{m}$ axial resolution and a sufficient DOF for cellular-resolution imaging.

8.2.2 MAS OCT probe

Figure 8.1: Schematic of the polarization encoded probe. A polarization analyzer is positioned between the calcite spacer and GRIN lens. SMF: single mode fiber; BS: beam splitter; M: mirror. Blue and red rays indicate the ordinary and extra-ordinary optical paths, respectively.

The novel polarization encoded MAS OCT probe is suitable for endoscopic and intravascular imaging applications. This probe will comprise a Ferrule with single mode fiber (SMF), a calcite spacer, a polarization analyzer, a Gradient-index (GRIN) lens, a beam splitter and a mirror (8.1). The spacer will be made of birefringent calcite and the SMF material is normally non-birefringent fused silica. The interface plane between SMF and the calcite spacer will be angled with respect to the optical axis of the SMF, as shown in Figs. 8.1 and 8.2. The angle between the optical axis of SMF and the interface plane between SMF and the calcite spacer will be α which can be set to optimally 45 degrees or another acute angle. The refractive index of fused silica is around 1.46

at 800 nm. The refractive index of calcite is 1.46 and 1.65 for ordinary beam and extraordinary beam at 800 nm, respectively. The fast axis of the calcite spacer will be set to be parallel to the interface plane between SMF and the calcite spacer. The optical axis of the polarization analyzer will be 45 degrees with respect to the fast and slow axis of the calcite spacer.

Figure 8.2: Principle of polarization-based multiple aperture division and synthesis. (a) Side view of MAS OCT probe; (b) cross-sectional view of the probe showing the fast axis of calcite spacer and the optical axis of the analyzer; (c) Four round-trip light paths based on illumination paths and detection paths.

In Fig. 8.2, the light output from the SMF will undergo refraction at the interface plane between the fused silica SMF and the calcite spacer. The part of the light from SMF with the polarization direction along the fast axis will travel in the calcite space as the ordinary beam; the rest of the SMF light with the polarization direction perpendicular to the fast axis will travel in the calcite space as the extraordinary beam; The ordi-

nary and extra-ordinary beams will be separated by about five degrees in the calcite spacer. In the GRIN lens pupil plane, the apertures occupied by the ordinary and extraordinary beams will be different, forming two distinct apertures. The ordinary and extraordinary beams will go through the polarization analyzer after passing through the calcite spacer. The analyzer will rotate the polarization direction of the ordinary and extraordinary beams by 45 degrees in the illumination paths of the ordinary and extraordinary beams, so that after passing through the polarization analyzer, the polarization directions of both the ordinary and extraordinary beams will be the same as the optical axis of the polarization analyzer. The illumination light, including the ordinary and extraordinary beams, will be back-reflected or back-scattered by the sample.

In Fig. 8.2(c), the detected light will be divided into four parts by the illumination paths (ordinary and extraordinary beams) and detection paths (ordinary and extraordinary beams), including *oo* path, *oe* path, *ee* path and *eo* path. Light signals from all four light paths will be coupled back into the SMF in Figs. 8.1 and 8.2 and detected by the photo-detector (not shown). Due to the refractive index of calcite difference between the ordinary and extraordinary beams, the round trip path length (PL) of *ee* beam will be approximately $2 \times d \times (1.65 - 1.46)$ longer than that of the *oo* beam, and the round trip PL of *oe* and *eo* beam will be approximately $d \times (1.65 - 1.46)$ longer than that of the *oo* beam, where

d is the physical length of the calcite spacer. This PL difference will make it possible to encode the light signal from different paths by use of PL and realize PL division detection.

Once developed, such an MAS OCT probe may enable tomographic diagnosis of gastrointestinal abnormalities at the cellular basis in internal organs. Potential clinical benefits may include the early detection of intraepithelial neoplasia and metaplasia in the gastrointestinal tract. Such early detection can be missed by standard video endoscopy and random biopsy procedures if the lesions are focally and heterogeneously distributed over a large mucosal area, leading to a lack of morphological features at the mucosal surface. MAS OCT probe could potentially improve screening and diagnostic outcomes by capturing the cellular hallmarks of the precancerous lesions invisible to standard-of-care tools, such as goblet cells in Barratts esophagus and specialized intestinal metaplasia in the stomach.

8.2.3 Dual-channel spectrometer design

As is discussed in Chapters 5 and 6, due to the relative large spacing between adjacent channels of the 8-fiber v-groove array (VGA-8-250, OZ OPTICS, Canada), noticeable mismatch still exists between two dual channels even sharing the same spectrometer optics, resulting in noticeable residual ACA and CCA in images. This issue can be solved by use of VGA of smaller spacing (e.g. $80\ \mu\text{m}$).

Author's Publications

Journal papers

1. **E. Bo**⁺, X. Ge⁺, and Linbo Liu, "Cellular resolution tomography in turbid tissue *in vivo* through digital aberration compensation," 2018. (Submitted, co-first author)
2. **E. Bo**⁺, X. Ge⁺, X. Yu, J. Mo and Linbo Liu, "Extending axial focus of optical coherence tomography using parallel multiple aperture synthesis," *Applied Optics*, 57(13), 3556-3560 (2018). (co-first author)
3. **E. Bo**, X. Ge, L. Wang, X. Wu, Y. Luo, S. Chen, S. Chen, H. Liang, G. Ni, X. Yu and L. Liu, "Multiple aperture synthetic optical coherence tomography for biological tissue imaging," *Optics Express* 26(2), 772-780 (2018).
4. **E. Bo**, Y. Luo, S. Chen, X. Liu, N. Wang, X. Ge, X. Wang, S. Chen, S. Chen, J. Li and L. Liu, "Depth-of-focus extension in optical coherence tomography via multiple aperture synthesis," *Optica* 4(7), 701-706 (2017).
5. **E. Bo**, S. Chen, D. Cui, S. Chen, X. Yu, Y. Luo, and L. Liu, "Single-camera full-range high-resolution spectral domain optical coherence tomography," *Applied Optics* 56(3), 470-475 (2017).
6. **E. Bo**, X. Liu, S. Chen, X. Yu, X. Wang, and L. Liu, "Spectral-domain optical coherence tomography with dual-balanced detection for auto-correlation artifacts reduction," *Optics Express* 23(21), 28050-28058 (2015).

Patent application filed

1. Linbo Liu, **En Bo**, Xin Ge, Multiple Aperture Interferometric Refocusing In Optical Coherence Tomography, PAT/292/16/17/SG PRV, filed on 15 March 2016.

Conference proceedings

1. **E. Bo** and L. Liu, "Spectral domain optical coherence tomography with extended depth-of-focus by aperture synthesis," in SPIE/COS Photonics Asia, (International Society for Optics and Photonics, 2016), 1002451-1002451-1002457.
2. **E. Bo**, X. Liu, S. Chen, Y. Luo, N. Wang, X. Wang, and L. Liu, "Spectral domain optical coherence tomography with dual-balanced detection," in SPIE BiOS, (International Society for Optics and Photonics, 2016), 970013-970013-970016.
3. **E. Bo**, X. Liu, and L. Liu, "Signal-to-Noise Ratio Enhanced Spectral Domain Optical Coherence Tomography with Dual-balanced Detection," *Procedia Engineering* 140, 140-143 (2016).

Bibliography

- [1] A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, and D. Forman, “Global cancer statistics,” *CA: a cancer journal for clinicians* 61, 69-90 (2011).
- [2] S. Immigration and Checkpoints Authority, “Report on Registration of Births and Deaths 2011 by the Registry of Births and Deaths,” (2011).
- [3] W. H. F. World Health Organization, and World Stroke Organization, “Global Atlas on cardiovascular disease prevention and control,” (2011).
- [4] S. H. Itzkowitz, and D. H. Present, “Consensus conference: colorectal cancer screening and surveillance in inflammatory bowel disease,” *Inflammatory bowel diseases* 11(3), 314-321 (2005).
- [5] J. A. Eaden, and J. F. Mayberry, “Guidelines for screening and surveillance of asymptomatic colorectal cancer in patients with inflammatory bowel disease,” *Gut* 51(5), 10-12 (2002).
- [6] D. Zaak, A. Karl, R. Knchel, H. Stepp, A. Hartmann, O. Reich, and C. Stief, “Diagnosis of urothelial carcinoma of the bladder using fluorescence endoscopy,” *BJU international*, 96(2), 217-222 (2005).
- [7] M. Y. Su , C. M. Hsu, Y. P. Ho, P. C. Chen, C. J. Lin and C. T. Chiu, “Comparative study of conventional colonoscopy, chromoendoscopy, and narrow-band imaging systems in differential diagnosis of neoplastic and nonneoplastic colonic polyps,” *American Journal of Gastroenterology* 101(12), 2711 (2006).
- [8] T. Inoue, M. Murano, N. Murano, T. Kuramoto, K. Kawakami and Y. Abe, “Comparative study of conventional colonoscopy and pan-colonic narrow-band imaging system in the detection of neoplastic colonic

- polyps: a randomized, controlled trial,” *Journal of Gastroenterology* 43(1), 45-50 (2008).
- [9] H. Pohl, T. Rosch, M. Vieth, M. Koch, V. Becker and M. Anders, “Miniprobe confocal laser microscopy for the detection of invisible neoplasia in patients with Barrett’s oesophagus,” *Gut* 57(12), 1648-1653 (2008).
- [10] A. Reumkens, E. J. Rondagh, C. M. Bakker, B. Winkens, A. A. Masclee and S. Sanduleanu, “Post-colonoscopy complications: a systematic review, time trends, and meta-analysis of population-based studies,” *The American journal of gastroenterology* 111(8), (2016).
- [11] S. Kudo, Y. Tamegai, H. Yamano, Y. Imai, E. Kogure and H. Kashida, “Endoscopic mucosal resection of the colon: the Japanese technique,” *Gastrointestinal endoscopy clinics of North America* 11(3), 519-35 (2001).
- [12] R. M. Soetikno, T. Gotoda, Y. Nakanishi and N. Soehendra, “Endoscopic mucosal resection,” *Gastrointestinal Endoscopy* 57(4), 567-79 (2003).
- [13] B. E. Bouma, G. J. Tearney, C. C. Compton and N. S. Nishioka, “High-resolution imaging of the human esophagus and stomach in vivo using optical coherence tomography,” *Gastrointestinal Endoscopy* 51(4), 467-74 (2000).
- [14] B. J. Vakoc, M. Shishko, S. H. Yun, W. Y. Oh, M. J. Suter and A. E. Desjardins, “Comprehensive esophageal microscopy by using optical frequency-domain imaging (with video),” *Gastrointestinal Endoscopy* 65(6), 898-905 (2007).
- [15] M. J. Suter, B. J. Vakoc, P. S. Yachimski, M. Shishkov, G. Y. Lauwers and M. Mino-Kenudson, “Comprehensive microscopy of the esophagus in human patients with optical frequency domain imaging,” *Gastrointestinal Endoscopy* 68(4), 745-753 (2008).
- [16] W. B. Li, X. L. Zuo, C. Q. Li, F. Zuo, X. M. Gu and T. Yu, “Diagnostic value of confocal laser endomicroscopy for gastric superficial cancerous lesions,” *Gut* 60(3), 299-306 (2011).
- [17] KR. iesslich, M. Goetz, M. Vieth, P. R. Galle and M. F. Neurath, “Technology insight: confocal laser endoscopy for in vivo diagnosis

- of colorectal cancer,” *Nature Clinical Practice Oncology* 4(8), 480-490 (2007).
- [18] R. Kiesslich, L. Gossner, M. Goetz, A. Dahlmann, M. Vieth and M. Stolte, “In vivo histology of Barrett’s esophagus and associated neoplasia by confocal laser endomicroscopy,” *Clinical Gastroenterology and Hepatology* 4(8), 979-987 (2006).
- [19] G. W. Falk, T. W. Rice, J. R. Goldblum and J. E. Richter, “Jumbo biopsy forceps protocol still misses unsuspected cancer in Barrett’s esophagus with high-grade dysplasia,” *Gastrointestinal Endoscopy* 49(2), 170-176 (1999).
- [20] L. Liu, J. A. Gardecki, S. K. Nadkarni, J. D. Toussaint, Y. Yagi, B. E. Bouma, and G. J. Tearney, “Imaging the subcellular structure of human coronary atherosclerosis using micro-optical coherence tomography,” *Nature medicine* 17, 1010-1014 (2011).
- [21] M. Kashiwagi, L. Liu, J. Gardecki, and G. Tearney, “Optical Coherence Tomography in Coronary Artery Disease: Toward Sub cellular Imaging,” *Austin J Clin Cardiol* 1, 4 (2014).
- [22] Y. Luo, E. Bo, H. Liang, X. Wang, X. Yu, D. Cui, X. Ge, J. Mo and L. Liu, “Imaging cellular structures of atherosclerotic coronary arteries using circumferentially scanning micro-optical coherence tomography fiber probe *ex vivo*,” IEEE Access, 2018 (Under review).
- [23] D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, and C. A. Puliafito, “Optical coherence tomography,” *Science* 254, 1178-1181 (1991).
- [24] G. J. Tearney, M. E. Brezinski, B. E. Bouma, S. A. Boppart, C. Pitris, J. F. Southern, and J. G. Fujimoto, “*In vivo* endoscopic optical biopsy with optical coherence tomography,” *Science* 276, 2037-2039 (1997).
- [25] W. Drexler, U. Morgner, R. K. Ghanta, F. X. Krtner, J. S. Schuman, , and J. G. Fujimoto, “Ultrahigh-resolution ophthalmic optical coherence tomography,” *Nature medicine*, 7(4), 502 (2001).
- [26] R. A. Leitgeb, W. Drexler, A. Unterhuber, B. Hermann, , T. Bajraszewski, T. Le, and A. F. Fercher, “Ultrahigh resolution Fourier domain optical coherence tomography,” *Optics express*, 12(10), 2156-2165 (2004).

- [27] M. Wojtkowski, V. J. Srinivasan, T. H. Ko, J. G. Fujimoto, A. Kowalczyk, and J. S. Duker, "Ultrahigh-resolution, high-speed, Fourier domain optical coherence tomography and methods for dispersion compensation," *Optics express*, 12(11), 2404-2422 (2004).
- [28] S. H. Yun, G. J. Tearney, B. J. Vakoc, M. Shishkov, W. Y. Oh, A. E. Desjardins, M. J. Suter, R. C. Chan, J. A. Evans, I. K. Jang, N. S. Nishioka, J. F. de Boer, and B. E. Bouma, "Comprehensive volumetric optical microscopy *in vivo*," *Nature Medicine* 12, 1429-1433 (2006).
- [29] A. F. Fercher, C. K. Hitzenberger, G. Kamp, and S. Y. ElZaiat, "Measurement of intraocular distances by backscattering spectral interferometry," *Optics Communications* 117, 43-48 (1995).
- [30] B. J. Vakoc, R. M. Lanning, J. A. Tyrrell, T. P. Padera, L. A. Bartlett, T. Stylianopoulos, L. L. Munn, G. J. Tearney, D. Fukumura, R. K. Jain, and B. E. Bouma, "Three-dimensional microscopy of the tumor microenvironment *in vivo* using optical frequency domain imaging," *Nature Medicine* 15, 1219-1223 (2009).
- [31] H. Yoo, J. W. Kim, M. Shishkov, E. Namati, T. Morse, R. Shubochkin, J. R. McCarthy, V. Ntziachristos, B. E. Bouma, and F. A. Jaffer, "Intra-arterial catheter for simultaneous microstructural and molecular imaging *in vivo*," *Nature medicine* 17, 1680-1684 (2011).
- [32] M. J. Gora, J. S. Sauk, R. W. Carruth, K. A. Gallagher, M. J. Suter, N. S. Nishioka, L. E. Kava, M. Rosenberg, B. E. Bouma, and G. J. Tearney, "Tethered capsule endomicroscopy enables less invasive imaging of gastrointestinal tract microstructure," *Nature medicine* 19, 238-240 (2013).
- [33] Drexler, W., Fujimoto, J. G. Optical coherence tomography: technology and applications. Springer Science & Business Media (2008).
- [34] D. Ferrara, K. J. Mohler, N. Waheed, M. Adhi, J. J. Liu, I. Grulkowski, M. F. Kraus, C. Baumann, J. Horneegger, and J. G. Fujimoto, "En face enhanced-depth swept-source optical coherence tomography features of chronic central serous chorioretinopathy," *Ophthalmology* 121, 719-726 (2014).
- [35] H. G. Bezerra, M. A. Costa, G. Guagliumi, A. M. Rollins, and D. I. Simon, "Intracoronary optical coherence tomography: a comprehen-

- sive review: clinical and research applications,” *JACC: Cardiovascular Interventions* 2, 1035-1046 (2009).
- [36] L. Liu, C. Liu, W. C. Howe, C. Sheppard, and N. Chen, “Binary-phase spatial filter for real-time swept-source optical coherence microscopy,” *Optics letters* 32, 2375-2377 (2007).
- [37] K. S. Lee and J. P. Rolland, “Bessel beam spectral-domain high-resolution optical coherence tomography with micro-optic axicon providing extended focusing range,” *Optics letters* 33, 1696-1698 (2008)..
- [38] Z. Ding, H. Ren, Y. Zhao, J. S. Nelson, and Z. Chen, “High-resolution optical coherence tomography over a large depth range with an axicon lens,” *Optics Letters* 27, 243-245 (2002).
- [39] R. Leitgeb, M. Villiger, A. Bachmann, L. Steinmann, and T. Lasser, “Extended focus depth for Fourier domain optical coherence microscopy,” *Optics letters* 31, 2450-2452 (2006).
- [40] T. S. Ralston, D. L. Marks, P. S. Carney, and S. A. Boppart, “Interferometric synthetic aperture microscopy,” *Nature Physics* 3, 129-134 (2007).
- [41] E. Bo and L. Liu, “Spectral domain optical coherence tomography with extended depth-of-focus by aperture synthesis,” in *SPIE/COS Photonics Asia*, (International Society for Optics and Photonics, 2016), 1002451-1002457.
- [42] J. Mo, M. de Groot, and J. F. de Boer, “Focus-extension by depth-encoded synthetic aperture in Optical Coherence Tomography,” *Optics express* 21, 10048-10061 (2013).
- [43] J. Mo, M. de Groot, and J. F. de Boer, “Depth-encoded synthetic aperture optical coherence tomography of biological tissues with extended focal depth,” *Optics express* 23, 4935-4945 (2015).
- [44] C. Sheppard and T. Wilson, “Depth of field in the scanning microscope,” *Optics Letters* 3, 115-117 (1978).
- [45] C. Sheppard and X. Mao, “Three-dimensional imaging in a microscope,” *JOSA A* 6, 1260-1269 (1989).
- [46] M. Gu, X. Gan, and C. Sheppard, “Three-dimensional coherent transfer functions in fiber-optical confocal scanning microscopes,” *JOSA A* 8, 1019-1025 (1991).

- [47] M. Gu and C. Sheppard, "Three-dimensional optical transfer function in a fiber-optical confocal fluorescence microscope using annular lenses," *JOSA A* 9, 1991-1999 (1992).
- [48] C. Sheppard, D. Hamilton, and I. Cox, "Optical microscopy with extended depth of field," in *Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, (The Royal Society, 1983), 171-186.
- [49] M. Gu, C. Sheppard, and X. Gan, "Image formation in a fiber-optical confocal scanning microscope," *JOSA A* 8, 1755-1761 (1991).
- [50] M. Gu, *Principles of three-dimensional imaging in confocal microscopes* (*World Scientific*, 1996).
- [51] X. Liu, S. Chen, D. Cui, X. Yu, and L. Liu, "Spectral estimation optical coherence tomography for axial super-resolution," *Optics express* 23, 26521-26532 (2015).
- [52] S. Gabarda and G. Cristbal, "Blind image quality assessment through anisotropy," *JOSA A* 24, B42-B51 (2007).
- [53] A. Kumar, T. Kamali, R. Platzer, A. Unterhuber, W. Drexler, and R. A. Leitgeb, "Anisotropic aberration correction using region of interest based digital adaptive optics in Fourier domain OCT," *Biomedical optics express* 6, 1124-1134 (2015).
- [54] A. Ahmad, N. D. Shemonski, S. G. Adie, H.-S. Kim, W. HwuWen-Mei, P. S. Carney, and S. A. Boppart, "Real-time *in vivo* computed optical interferometric tomography," *Nat Photon* 7, 444-448 (2013).
- [55] D. Cui, X. Liu, J. Zhang, X. Yu, S. Ding, Y. Luo, J. Gu, P. Shum, and L. Liu, "Dual spectrometer system with spectral compounding for 1- μ m optical coherence tomography *in vivo*," *Optics letters* 39, 6727-6730 (2014).
- [56] J. G. Fujimoto, M. E. Brezinski, G. J. Tearney, S. A. Boppart, B. Bouma, M. R. Hee, J. F. Southern, and E. A. Swanson, "Optical biopsy and imaging using optical coherence tomography," *Nature medicine* 1, 970-972 (1995).
- [57] L. Liu, N. Chen, and C. Sheppard, "Double-reflection polygon mirror for high-speed optical coherence microscopy," *Optics letters* 32, 3528-3530 (2007).

- [58] J. Ha, M. Shishkov, M. Colice, W. Oh, H. Yoo, L. Liu, G. Tearney, and B. Bouma, "Compensation of motion artifacts in catheter-based optical frequency domain imaging," *Optics express* 18, 11418-11427 (2010).
- [59] B. A. Standish, K. K. Lee, A. Mariampillai, N. R. Munce, M. K. Leung, V. X. Yang, and I. A. Vitkin, "*In vivo* endoscopic multi-beam optical coherence tomography," *Physics in medicine and biology* 55, 615 (2010).
- [60] E. Bo, Y. Luo, S. Chen, X. Liu, N. Wang, X. Ge, X. Wang, S. Chen, S. Chen, J. Li, and L. Liu, "Depth-of-focus extension in optical coherence tomography via multiple aperture synthesis," *Optica* 4, 701-706 (2017).
- [61] E. Bo, X. Ge, L. Wang, X. Wu, Y. Luo, S. Chen, and L. Liu, "Multiple aperture synthetic optical coherence tomography for biological tissue imaging," *Optics Express* 26, 772-780 (2018).
- [62] W. Bao, Z. Ding, J. Qiu, Y. Shen, P. Li, and Z. Chen, "Quasi-needle-like focus synthesized by optical coherence tomography," *Optics Letters* 42, 1385-1388 (2017).
- [63] M. Pircher, E. Gtzinger, and C. K. Hitzenberger, "Dynamic focus in optical coherence tomography for retinal imaging," *Journal of Biomedical Optics* 11, 054013-054016 (2006).
- [64] J. Jang, J. Lim, H. Yu, H. Choi, J. Ha, J. H. Park, W. Y. Oh, W. Jang, S. Lee, and Y. Park, "Complex wavefront shaping for optimal depth-selective focusing in optical coherence tomography," *Optics Express* 21, 2890-2902 (2013).
- [65] T. S. Ralston, D. L. Marks, F. Kamalabadi, and S. A. Boppart, "Deconvolution methods for mitigation of transverse blurring in optical coherence tomography," *IEEE Transactions on Image Processing* 14, 1254-1264 (2005).
- [66] D. Cui, E. Bo, Y. Luo, X. Liu, X. Wang, S. Chen, X. Yu, S. Chen, P. Shum, and L. Liu, "Multifiber angular compounding optical coherence tomography for speckle reduction," *Optics letters* 42, 125-128 (2017).
- [67] H. Wang and A. M. Rollins, "Speckle reduction in optical coherence tomography using angular compounding by B-scan Doppler-shift encoding," *Journal of biomedical optics* 14, 030512 (2009).

- [68] American National Standards Institute. American National Standard for the Safe Use of Lasers ANSI z136.12007 (Laser Institute of America, Orlando, FL, 2007).
- [69] P. Pignoli, E. Tremoli, A. Poli, P. Oreste, and R. Paoletti, "Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging," *Circulation* 74(6), 1399-1406 (1986).
- [70] W. C. Roentgen, "On a new kind of rays," *Nature* 53, 274276 (1896).
- [71] P. C. Lauterbur, "Image formation by induced local interactions: examples employing nuclear magnetic resonance," *Nature* 242, 190191 (1973).
- [72] C. V. Jakowatz Jr, Spotlight-mode Synthetic Aperture Radar: A Signal Processing Approach (Kluwer Academic, Boston, 1996).
- [73] D. Zink, A.H. Fischer, and J.A. Nickerson, "Nuclear structure in cancer cells," *Nature reviews cancer* 4(9), 677 (2004).
- [74] B. J. Vakoc, D. Fukumura, R. K. Jain, and B. E. Bouma, "Cancer imaging by optical coherence tomography: preclinical progress and clinical potential," *Nature Reviews Cancer* 12, 363-368 (2012).
- [75] J.A. Evans, J.M. Poneros, B.E. Bouma, J. Bressner, E.F. Halpern, M. Shishkov, G.Y. Lauwers, M. MinoKenudson, N.S. Nishioka, and G.J. Tearney, "Optical coherence tomography to identify intramucosal carcinoma and high-grade dysplasia in Barretts esophagus," *Clinical Gastroenterology and Hepatology* 4(1), 38-43 (2006).
- [76] H. Yabushita, B.E. Bouma, S.L. Houser, H.T. Aretz, I.K. Jang, K.H. Schlendorf, C.R. Kauffman, M. Shishkov, D.H. Kang, E.F. Halpern, and G.J. Tearney, "Characterization of human atherosclerosis by optical coherence tomography," *Circulation* 106(13), 1640-1645 (2002).
- [77] M.J. Booth, M.A. Neil, R. Jukaitis, and T. Wilson, "Adaptive aberration correction in a confocal microscope," *Proceedings of the National Academy of Sciences* 99(9), 5788-5792 (2002).
- [78] W. Denk, J. H. Strickler, and W. W. Webb, "Two-photon laser scanning fluorescence microscopy," *Science* 248, 7376 (1990).
- [79] D.A. Dombeck, K.A. Kasischke, H.D. Vishwasrao, M. Ingelsson, B.T. Hyman, and W.W. Webb, "Uniform polarity microtubule assemblies imaged in native brain tissue by second-harmonic generation

- microscopy,” *Proceedings of the National Academy of Sciences* 100(12), 7081-7086 (2002).
- [80] J. Sharpe, U. Ahlgren, P. Perry, B. Hill, A. Ross, J. Hecksher-Sørensen, R. Baldock, and D. Davidson, “Optical projection tomography as a tool for 3D microscopy and gene expression studies,” *Science* 296, 541-545 (2002).
- [81] R. Tomer, L. Ye, B. Hsueh, and K. Deisseroth, “Advanced CLARITY for rapid and high-resolution imaging of intact tissues,” *Nature protocols* 9(7), 1682 (2014).
- [82] I. M. Vellekoop, A. Lagendijk, and A. Mosk, “Exploiting disorder for perfect focusing,” *Nat Photon* 4, 320 (2010).
- [83] S. M. Popoff, G. Lerosey, R. Carminati, M. Fink, A. C. Boccara, and S. Gigan, “Measuring the transmission matrix in optics: an approach to the study and control of light propagation in disordered media,” *Physical review letters* 104(10), 100601 (2010).
- [84] R. Horstmeyer, H. Ruan, and C. Yang, “Guidestar-assisted wavefront-shaping methods for focusing light into biological tissue,” *Nature photonics* 9, 563 (2015).
- [85] P. Lai, L. Wang, J. W. Tay, and L. V. Wang, “Photoacoustically guided wavefront shaping for enhanced optical focusing in scattering media,” *Nature photonics* 9, 126 (2015).
- [86] J. Liang, D. R. Williams, and D. T. Miller, “Supernormal vision and high-resolution retinal imaging through adaptive optics,” *JOSA A* 14, 2884-2892 (1997).
- [87] M. Rueckel, J. A. Mack-Bucher, and W. Denk, “Adaptive wavefront correction in two-photon microscopy using coherence-gated wavefront sensing,” *Proceedings of the National Academy of Sciences* 103, 17137-17142 (2006).
- [88] N. Ji, D. E. Milkie, and E. Betzig, “Adaptive optics via pupil segmentation for high-resolution imaging in biological tissues,” *Nature methods* 7, 141-147 (2010).
- [89] R. J. Zawadzki, S. M. Jones, S. S. Olivier, M. Zhao, B. A. Bower, J. A. Izatt, and J. S. Werner, “Adaptive-optics optical coherence tomog-

- raphy for high-resolution and high-speed 3D retinal *in vivo* imaging *Optics express* 13(21), 8532-8546 (2005).
- [90] J. Jang, J. Lim, H. Yu, H. Choi, J. Ha, J. H. Park, and Y. Park, "Complex wavefront shaping for optimal depth-selective focusing in optical coherence tomography," *Optics express*, 21(3), 2890-2902 (2013).
- [91] O. P. Kocaoglu, B. Cense, R. S. Jonnal, Q. Wang, S. Lee, W. Gao, and D. T. Miller, "Imaging retinal nerve fiber bundles using optical coherence tomography with adaptive optics," *Vision research* 51(16), 1835-1844 (2011).
- [92] A. Kumar, W. Drexler, and R. A. Leitgeb, "Subaperture correlation based digital adaptive optics for full field optical coherence tomography," *Optics express* 21, 10850-10866 (2013).
- [93] L. Ginner, A. Kumar, D. Fechtig, L. M. Wurster, M. Salas, M. Pircher, and R. A. Leitgeb, "Noniterative digital aberration correction for cellular resolution retinal optical coherence tomography *in vivo*," *Optica* 4, 924-931 (2017).
- [94] S. G. Adie, B. W. Graf, A. Ahmad, P. S. Carney, and S. A. Boppart, "Computational adaptive optics for broadband optical interferometric tomography of biological tissue," *Proceedings of the National Academy of Sciences* 109, 7175-7180 (2012).
- [95] N. D. Shemonski, F. A. South, Y. Z. Liu, S. G. Adie, P. S. Carney, and S. A. Boppart, "Computational high-resolution optical imaging of the living human retina," *Nature photonics* 9(7), 440 (2015).
- [96] Y. Yasuno, J. I. Sugisaka, Y. Sando, Y. Nakamura, S. Makita, M. Itoh, and T. Yatagai, "Non-iterative numerical method for laterally superresolving Fourier domain optical coherence tomography," *Optics express* 14, 1006-1020 (2006).
- [97] A. Kumar, W. Drexler, and R. A. Leitgeb, "Numerical focusing methods for full field OCT: a comparison based on a common signal model," *Optics express* 22, 16061-16078 (2014).
- [98] D. L. Marks, T. S. Ralston, S. A. Boppart, and P. S. Carney, "Inverse scattering for frequency-scanned full-field optical coherence tomography," *JOSA A* 24, 1034-1041 (2007).

- [99] T. S. Ralston, D. L. Marks, P. S. Carney, and S. A. Boppart, "Real-time interferometric synthetic aperture microscopy," *Optics Express* 16, 2555-2569 (2008).
- [100] G. Lan, and G. Li, "Design of a k-space spectrometer for ultra-broad waveband spectral domain optical coherence tomography," *Scientific Reports* 7, 42353 (2017).
- [101] M. Choma, M. Sarunic, C. Yang, and J. Izatt, "Sensitivity advantage of swept source and Fourier domain optical coherence tomography," *Optics express* 11, 2183-2189 (2003).
- [102] J. F. De Boer, B. Cense, B. H. Park, M. C. Pierce, G. J. Tearney, and B. E. Bouma, "Improved signal-to-noise ratio in spectral-domain compared with time-domain optical coherence tomography," *Optics letters* 28, 2067-2069 (2003).
- [103] R. Leitgeb, C. Hitzenberger, and A. Fercher, "Performance of fourier domain vs. time domain optical coherence tomography," *Optics Express* 11, 889-894 (2003).
- [104] N. Nassif, B. Cense, B. Hyle Park, S. H. Yun, T. C. Chen, B. E. Bouma, G. J. Tearney, and J. F. d. Boer, "*In vivo* human retinal imaging by ultrahigh-speed spectral domain optical coherence tomography," *Optics letters* 29, 480-482 (2004).
- [105] M. J. Marques, A. Bradu, and A. G. Podoleanu, "Optical Coherence Tomography and Scanning Laser Ophthalmoscopy: approaches to dual-channel retinal tissue imaging," in *Frontiers in Optics*, (Optical Society of America, 2014), FTu2F. 3.
- [106] B. Cense, N. Nassif, T. Chen, M. Pierce, S. H. Yun, B. Park, B. Bouma, G. Tearney, and J. de Boer, "Ultrahigh-resolution high-speed retinal imaging using spectral-domain optical coherence tomography," *Optics Express* 12, 2435-2447 (2004).
- [107] W. Shi, X. Liu, C. Wei, Z. J. Xu, S. S. W. Sim, L. Liu, and C. Xu, "Micro-optical coherence tomography tracking of magnetic gene transfection via Au-Fe₃O₄ dumbbell nanoparticles," *Nanoscale* (2015).
- [108] R. Leitgeb, L. Schmetterer, W. Drexler, A. Fercher, R. Zawadzki, and T. Bajraszewski, "Real-time assessment of retinal blood flow with ultrafast acquisition by color Doppler Fourier domain optical coherence tomography," *Optics Express* 11, 3116-3121 (2003).

- [109] M. A. Choma, A. K. Ellerbee, S. Yazdanfar, and J. A. Izatt, "Doppler flow imaging of cytoplasmic streaming using spectral domain phase microscopy," *Journal of biomedical optics* 11, 024014-024018 (2006).
- [110] A. G. Podoleanu, "Unbalanced versus balanced operation in an optical coherence tomography system," *Applied Optics* 39, 173-182 (2000).
- [111] M. Mujat, B. H. Park, B. Cense, T. C. Chen, and J. F. de Boer, "Autocalibration of spectral-domain optical coherence tomography spectrometers for *in vivo* quantitative retinal nerve fiber layer birefringence determination," *Journal of biomedical optics* 12, 041205-041206 (2007).
- [112] W. C. Kuo, Y. S. Lai, C. M. Lai, and Y. S. Huang, "Balanced detection spectral domain optical coherence tomography with a multiline single camera for signal-to-noise ratio enhancement," *Applied optics* 51, 5936-5940 (2012).
- [113] A. Bradu and A. G. Podoleanu, "Fourier domain optical coherence tomography system with balance detection," *Optics express* 20, 17522-17538 (2012).
- [114] W. C. Kuo, C. M. Lai, Y. S. Huang, C. Y. Chang, and Y. M. Kuo, "Balanced detection for spectral domain optical coherence tomography," *Optics express* 21, 19280-19291 (2013).
- [115] M. A. Choma, K. Hsu, and J. A. Izatt, "Swept source optical coherence tomography using an all-fiber 1300nm ring laser source," *Journal of biomedical optics* 10, 044006-044009 (2005).
- [116] M. Wojtkowski, V. Srinivasan, T. Ko, J. Fujimoto, A. Kowalczyk, and J. Duker, "Ultrahigh-resolution, high-speed, Fourier domain optical coherence tomography and methods for dispersion compensation," *Optics express* 12, 2404-2422 (2004).
- [117] S. Yun, G. Tearney, B. Bouma, B. Park, and J. de Boer, "High-speed spectral-domain optical coherence tomography at 1.3 μm wavelength," *Optics Express* 11, 3598-3604 (2003).
- [118] M. Wojtkowski, A. Kowalczyk, R. Leitgeb, and A. Fercher, "Full range complex spectral optical coherence tomography technique in eye imaging," *Optics letters* 27, 1415-1417 (2002).

- [119] Y. K. Tao, M. Zhao, and J. A. Izatt, "High-speed complex conjugate resolved retinal spectral domain optical coherence tomography using sinusoidal phase modulation," *Optics letters* 32, 2918-2920 (2007).
- [120] E. Bo, F. Duan, C. Lv, F. Zhang, and F. Feng, "Sinusoidal phase modulating interferometry system for 3D profile measurement," *Optics and Laser Technology* 59, 137-142 (2014).
- [121] E. Gtzinger, M. Pircher, R. Leitgeb, and C. Hitzenberger, "High speed full range complex spectral domain optical coherence tomography," *Optics express* 13, 583-594 (2005).
- [122] A. Bachmann, R. Leitgeb, and T. Lasser, "Heterodyne Fourier domain optical coherence tomography for full range probing with high axial resolution," *Optics express* 14, 1487-1496 (2006).
- [123] A. H. Dhalla and J. A. Izatt, "Complete complex conjugate resolved heterodyne swept-source optical coherence tomography using a dispersive optical delay line," *Biomedical optics express* 2, 1218-1232 (2011).
- [124] B. Baumann, M. Pircher, E. Gtzinger, and C. K. Hitzenberger, "Full range complex spectral domain optical coherence tomography without additional phase shifters," *Optics express* 15, 13375-13387 (2007).
- [125] S. Makita, T. Fabritius, and Y. Yasuno, "Full-range, high-speed, high-resolution 1-m spectral-domain optical coherence tomography using BM-scan for volumetric imaging of the human posterior eye," *Optics express* 16, 8406-8420 (2008).
- [126] C. T. Wu, T. T. Chi, C. K. Lee, Y. W. Kiang, C. C. Yang, and C. P. Chiang, "Method for suppressing the mirror image in Fourier-domain optical coherence tomography," *Optics letters* 36, 2889-2891 (2011).
- [127] K. S. Lee, P. Meemon, W. Dallas, K. Hsu, and J. P. Rolland, "Dual detection full range frequency domain optical coherence tomography," *Optics letters* 35, 1058-1060 (2010).
- [128] B. Vakoc, S. Yun, G. Tearney, and B. Bouma, "Elimination of depth degeneracy in optical frequency-domain imaging through polarization-based optical demodulation," *Optics letters* 31, 362-364 (2006).

- [129] R. K. Wang, “*In vivo* full range complex Fourier domain optical coherence tomography,” *Applied Physics Letters* 90, 054103 (2007).
- [130] R. K. Wang, “Fourier domain optical coherence tomography achieves full range complex imaging *in vivo* by introducing a carrier frequency during scanning,” *Physics in medicine and biology* 52, 5897 (2007).
- [131] B. Hofer, B. Povazay, B. Hermann, A. Unterhuber, G. Matz, and W. Drexler, “Dispersion encoded full range frequency domain optical coherence tomography,” *Optics express* 17, 7-24 (2009).
- [132] M. A. Choma, C. Yang, and J. A. Izatt, “Instantaneous quadrature low-coherence interferometry with 3×3 fiber-optic couplers,” *Optics Letters* 28, 2162-2164 (2003).
- [133] M. Sarunic, M. A. Choma, C. Yang, and J. A. Izatt, “Instantaneous complex conjugate resolved spectral domain and swept-source OCT using 3x3 fiber couplers,” *Optics Express* 13, 957-967 (2005).
- [134] M. V. Sarunic, B. E. Applegate, and J. A. Izatt, “Real-time quadrature projection complex conjugate resolved Fourier domain optical coherence tomography,” *Optics letters* 31, 2426-2428 (2006).
- [135] E. Bo, X. Liu, S. Chen, X. Yu, X. Wang, and L. Liu, “Spectral-domain optical coherence tomography with dual-balanced detection for auto-correlation artifacts reduction,” *Optics express* 23, 28050-28058 (2015).

Appendices

Download links for Matlab codes of multiple aperture synthesis

1. <https://researchdata.ntu.edu.sg/dataset.xhtml?persistentId=doi:10.21979/N9/LJHRKF>
2. <https://github.com/boen123/Depth-of-focus-extension-in-optical-coherence-tomography-via-multiple-aperture-synthesis>
3. <https://www.mathworks.com/matlabcentral/fileexchange/65880-boen123-depth-of-focus-extension-in-optical-coherence-tomography-via-multiple-aperture-synthesis>