Low-cost and digital refocusing approaches to high-resolution spectral-domain optical coherence tomography

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**LOW-COST AND DIGITAL REFOCUSING
APPROACHES TO HIGH-RESOLUTION
SPECTRAL-DOMAIN OPTICAL
COHERENCE TOMOGRAPHY**

WANG LULU

SCHOOL OF ELECTRICAL & ELECTRONIC ENGINEERING

2020

LOW-COST AND DIGITAL REFOCUSING APPROACHES TO HIGH-RESOLUTION SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY

WANG LULU

School of Electrical & Electronic Engineering

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2020

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

.... 24/Jan/2020.

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.... *Wang Lulu*

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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.

...24/Jan/2020....

Date

....

Liu Linbo

Authorship Attribution Statement

This thesis contains material from 2 papers published in the following peer-reviewed journals where I was the first author.

Chapter 3 is published as Lulu Wang, Xiaojun Yu, Ge Xin, Xuan Wu, Xianghong Wang, En Bo, Nanshuo Wang, Xinyu Liu, Guangming Ni, and Linbo Liu, “Design and optimization of a spectrometer for high-resolution SD-OCT.” *Laser Physics Letters*, 2019, 16(4): 045603.

The contributions of the co-authors are as follows:

- Assoc. Prof. Linbo Liu provided the initial project direction and edited the manuscript drafts.
- Dr. Xiaojun Yu assisted in the system building and manuscript revision.
- All authors read and edited the manuscript.

Chapter 4 is published as Lulu Wang, Qiaozhou Xiong, Xin Ge, En Bo, Jun Xie, Xinyu Liu, Xiaojun Yu, Ge Xin, Xianghong Wang, Nanshuo Wang, Si Chen, Xuan Wu, Xinyu Liu, and Linbo Liu, “Cellular resolution corneal imaging with extended imaging range.” *Optics express*, 2019, 27(2): 1298-1309.

The contributions of the co-authors are as follows:

- Assoc. Prof. Linbo Liu provided the initial project direction and edited the manuscript drafts.
- Mr. Qiaozhou Xiong, Dr. Xin Ge, Mr. Jun Xie, Dr. Xinyu Liu, Mr. Xianghong Wang, all of them guided Matlab programming.
- Mr. Qiaozhou Xiong, Dr. Xinyu Liu, Dr. Nanshuo Wang, Mr. Xianghong Wang, all of them guided Labview programming.
- Miss Shu Fen and Miss Si Chen prepared the experimental tissues.
- All authors read and edited the manuscript.

.... 24/Jan/2020

Date

..... Wang Lulu

WANG LULU

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I believe I can always do better!

Summary

Imaging pathophysiological processes noninvasively at the cellular and subcellular level are critical for understanding and diagnosis of human diseases. High-resolution optical coherence tomography (OCT), including but not limited to ultrahigh-resolution OCT (UHR OCT) and micro-OCT (μ OCT), are particularly suitable for this purpose owing to their superb spatial resolutions. However, technical difficulties have to be overcome for the translation of these technologies for clinical use. First of all, the penetration depth of the current high-resolution OCT, co-determined by the focal depth, ranging depth, and the sensitivity, is insufficient. Secondly, there is a compelling need to lower the cost of devices so that the tests are affordable to more people.

The sensitivity limitation of the current μ OCT is partly due to the low transmission efficiency of the refractive spectrometer optics. I developed a low-cost, high transmission efficiency all-reflective-optics-based spectrometer, which provides 27% better transmission efficiency without any compromise in the ranging depth and the axial resolution. In addition, the cost of the camera lens is reduced by 2/3. The cost of the spectrometer is reduced by 50% with respect to a commercial spectrometer with similar performances.

To extend ranging depth, I developed a dual-spectrometer system. To combine the spectra acquired at the two line scan cameras, I developed a fully automatic algorithm to search for the overlapping region in the spectral interferograms. A total ranging depth of 3.5 mm and a 6-dB roll-off range of 1.44 mm were demonstrated experimentally, which is 94% and 78% larger than a single spectrometer system, respectively. In combination with a forward-model based digital refocusing method, I demonstrate that this system is capable of visualizing the full thickness of the pig cornea over the depth of 2.2 mm ($n = 1.375$) and visualize corneal endothelial cells over a depth range of 120 μ m *ex vivo*.

To mitigate the focal depth requirement, I developed a new digital refocusing method, where I use digital phase pupil filters to improve the transverse resolution. The method is based on the multiple aperture synthesis OCT setup, and digital phase masks were selected using the guide star approach. We experimentally observed 24% improvement

in the mainlobe width, and the sidelobe problem is suppressed by 1.1 dB through averaging results from multiple distinct phase masks.

In conclusion, I have developed and validated three optical imaging methods to address the penetration limitation of high-resolution OCT. My first two efforts in improving the spectrometer design mainly restore the signal strength by reducing the transmission loss and the path length-dependent signal “roll-off”, respectively. These two methods can be combined to an optimal dual-spectrometer design, where a reflective parabolic collimator and low-cost refractive camera lens are used in each spectrometer. My efforts in digital refocusing OCT can be potentially used to mitigate the focal range limitation of high-resolution OCT. My future work will be focused on the development of an advanced μ OCT system featured with transverse super-resolution and high-efficiency, low-cost spectrometers.

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Symbols and Acronyms

Symbols

k	Variable in wavenumber domain
z	Variable in spatial domain
ω	Angular frequency
ν	Frequency
λ_c	Center wavelength
$\Delta\lambda$	Wavelength bandwidth of the light spectrum
Δk	Wavenumber bandwidth of the light spectrum
l_c	Axial resolution
δx	Transverse resolution
z_{RL}	Rayleigh range
z_{FR}	Focal range/depth of focus
z_{max}	Maximum ranging depth
$\delta_s k$	Optical spectral resolution
$\delta_g k$	Grating spectral resolution
R_k	Reciprocal linear dispersion
Δx	Pixel width
Δy	Pixel high
a	Spot size at full width at half maximum (FWHM)
r_0	Fiber mode spot size
r_{co}	Fiber core radius
N	Covered pixel number of CCD

Acronyms

AO	Adaptive Optics
AR	Angle Recess
AS-OCT	Anterior Segment Optical Coherence Tomography
BS	Beam Splitter
CAO	Computer-Adaptive Optics
CBD	Calcite Beam Displacers
CCD	Charge-Coupled Device
CL	Camera Link cable
DALK	Deep Anterior Lamellar Keratoplasty
DAO	Digital Adaptive Optics
DAQ	Data Acquisition card
DM	Descemet Membrane
DOF	Depth of Focus
Ed	Endothelial layer
EPT	Epithelium
FD-OCT	Fourier Domain Optical Coherence Tomography
FM	Forward Model
FOV	Field of View
FT	Fourier Transforms
FWHM	Full Width at Half Maximum
GV	Galvo Scanner
HAO	Hardware Adaptive Optics
IMAQ	Image Acquisition board
ISAM	Interferometric Synthetic Aperture Microscopy
MAS	Multiple Aperture Synthesis

MFD	Mode Field Diameter
MI	Maximum Intensity Criteria
NA	Numerical Aperture
OCM	Optical Coherence Microscopy
OCT	Optical Coherence Tomography
O-FWHM	Optimized FWHMs criteria
OPD	Optical Path Differences
PC	Polarizer Controller
PSF	Point-Spread Function
QWP	Quarter-Wave Plate
RC	Reflective Collimator
RMS	Root Mean Square Radius
SD-OCT	Spectral Domain Optical Coherence Tomography
SL	Schwalbe's Line
SMF	Single Mode Fiber
SNR	Signal-to-Noise Ratio
SS-OCT	Swept-Source Optical Coherence Tomography
TD-OCT	Time Domain Optical Coherence Tomography
UHR OCT	Ultrahigh-Resolution Optical Coherence Tomography
μ OCT	Micro Optical Coherence Tomography

Chapter 1

1. Introduction

1.1 Motivation

OCT is a non-invasive, high-resolution modality [1]. Thanks to the advanced technologies development, high-resolution OCT, including ultrahigh-resolution OCT (UHR OCT) and micro-OCT (μ OCT), have been demonstrated with 1~2 μ m spatial resolution which could provide significant value for optical biopsy, image-guided surgery, and tumorigenesis study. However, technical difficulties have to be overcome for the translation of these technologies for clinical use.

First of all, there is a compelling need to lower the device's cost so that the tests are affordable to more people. To solve this issue, efforts have been made in recent years. Lu *et al.* demonstrated a handheld high-speed swept-source (SS) OCT and reduced the cost by employing a MEMS mirror instead of typical galvanometer mirrors [2]. In addition, Wang *et al.* developed a miniaturized, fully packaged silicon photonic integrated, low-cost SS-OCT [3]. However, the axial resolution of both systems is limited to 13 μ m in air. Furthermore, Pande *et al.* indicated a low-cost hand-held probe for linear OCT which could be built employing only standard off-the-shelf, inexpensive components, but at a significant loss of SNR and imaging depth [4]. Very recently, Kim *et al.* demonstrated a low-cost spectral-domain OCT (SD-OCT) with the total cost of \$7200, earning ~5-fold cost reduction compared to the commercial system with comparable performances [5]. Efforts also have been made in the spectrometer which is a critical sub-system for the SD-OCT system. Lee. *et al.* demonstrated a low-cost spectrometer based on Czerny Turner configuration by using two spherical mirrors as collimation and focusing optics, but with the roll-off severe loss [6]. This thesis tries to cut expensive components from spectrometers. With proper optical design and optimization, we achieve a much lower cost spectrometer while maintaining a cellular resolution and other performances equivalent to the commercial spectrometers.

Secondly, a large penetration depth, co-determined by the ranging depth, sensitivity roll-off, and focal depth for high-resolution SD-OCT systems, are required for many clinical applications. The 6 dB sensitivity roll-off usually is influenced by grating spectral resolution, spot size, pixel size, and the non-linear sampling frequency in the wavenumber space. Due to the advanced techniques, this issue could be solved effectively. For example, Bajraszewski *et al.* demonstrated an OCT system to extend the roll-off by using an optical frequency comb to strongly reduce the influence of the inter-pixel crosstalk and the limited spot size, but with six-fold time expense [7]. In addition, linear-in-wavenumber spectrometers by employing the customized prisms were demonstrated, which could effectively improve the sensitivity in deep imaging regions [8, 9]. This thesis tries to extend the ranging depth and reduce the depth-dependent drop of sensitivity by increasing the spectral-sampling frequency and grating spectral resolution, respectively.

In addition to the hurdles mentioned above, the focal range is limited in a high transverse resolution OCT system. To solve this issue, various approaches based on hardware adaptive optics [10, 11] and digital processing [12-14] have been demonstrated. Using adaptive optics is associated with high cost and system complexity [15]. Therefore, this thesis tries to employ the digital processing method to extend the focal depth.

1.2 Objectives

The main objective of this research is to develop novel techniques and apparatus to overcome the penetration limitation and cost issues to facilitate the clinical application of high-resolution SD-OCT. The specific aims include:

- (1) Developing an all-reflective-optics-based spectrometer to reduce the cost.
- (2) Developing a dual-spectrometer system to extend penetration depth.
- (3) Developing a digital refocusing technique to mitigate the focal depth requirement.

1.3 Major contributions

The main contributions of this thesis are to make the OCT system possible for a high-resolution OCT system with low cost and large ranging depth as a practical imaging tool for clinical applications. The specific achievements of the thesis include:

- (1) The sensitivity limit of the current high-resolution OCT system is partly due to the low transmission efficiency of the refractive spectrometer optics, which was overcome by using the proposed all-reflective-optics-based spectrometer. The OCT system could be more cost-effective than existing technologies in clinical translation and commercialization.
- (2) For a high-resolution OCT system with $1\sim3\ \mu\text{m}$, the required broadband light source limits the ranging depth to $1\sim2\ \text{mm}$. The developed dual-spectrometer OCT system doubled the ranging depth, which provided the potential for disease diagnosis, especially for large-area scanning.
- (3) There is a trade-off between the depth of focus and lateral resolution. The developed digital refocusing method based on the multiple aperture synthesis (MAS) OCT setup combined with the digital pupil filters could overcome this problem.

1.4 Thesis organization

The following chapters of this thesis are organized as:

Chapter 2 consists of four parts. In the first part, an OCT background is introduced. We focus on the Fourier-domain (FD-OCT) system and discuss its improvement from the original invention. In addition, the cellular resolution, endoscopic, and intravascular OCT imaging technology are introduced. In the second part, the foundations of the SD-OCT is induced. In the third part, the basic knowledge of the spectrometer and its main performance parameters, including ranging depth and 6 dB sensitivity roll-off, are discussed. In the fourth part, confocal reflectance microscopy and forward model theorem are discussed. In the last part, the main performance parameters of SD-OCT, including the axial resolution, transverse resolution and focal range, are introduced.

Chapter 3 proposes an all-reflective-optics-based spectrometer which improves transmission efficiency, reduces the cost, and eliminate chromatic aberration. Its cost is only about half of the price of a commercial spectrometer with similar performances.

Chapter 4 proposes a dual-spectrometer system for mitigating the penetration problem. Thanks to the improved spectral-sampling frequency and grating spectral resolution, the developed dual-spectrometer OCT system doubled the total ranging depth and the increased the 6 dB sensitivity roll-off range by 78%. Furthermore, the Rayleigh range was extended by ~8-fold by using the forward model digital refocusing method.

Chapter 5 illustrates a novel digital refocusing method to mitigate the focal depth requirement. The method is based on the multiple aperture synthesis optics and guide star principle. The transverse resolution was improved by 24%. In addition, the sidelobe artifacts of the super-resolving images were effectively reduced with respect to the images without averaging.

Chapter 6 summarizes the work presented in this thesis and future work.

Chapter 2

Optical Coherence Tomography Theory

2.1 OCT background

Medical imaging technologies can improve the understanding and diagnosis of human diseases. Tomographic imaging techniques such as computed tomography [16], magnetic resonance imaging [17], and ultrasound imaging [18] have had a significant impact on both research and clinical practice. However, their resolutions are limited to tens or hundreds of micrometers, which is not enough for many diseases diagnosis with the requirement of the cellular-level resolution.

Developed by Fujimoto's group in 1991, OCT is proposed as a non-invasive, high-resolution modality [1]. It can achieve a spatial resolution of 10 μm or less, which is about ten times as high as ultrasound. OCT was initially introduced in its time-domain (TD) configuration, where the depth profile of the sample in the axial dimension was obtained by the linear scanning of the reference reflector [1, 19]. An evolution of OCT is from TD-OCT to Fourier domain OCT (FD-OCT), including SD-OCT and swept-source OCT (SS-OCT), both with a 20-30 dB sensitivity improvement and tremendous imaging speed-boosting [20]. The improved sensitivity given by SD-OCT due to how it essentially measures all of the echoes' light simultaneously can be parlayed into a higher OCT scan acquisition rate and greater penetration depth. SS-OCT is advantageous in that it does not require a spectrometer and line scan camera [21]. Therefore, it can achieve faster imaging speeds than SD-OCT which is limited by the camera speed. The primary challenge in SS-OCT is that it requires a high-speed, swept narrow linewidth light source.

FD-OCT is promising for ultrahigh-resolution imaging because it overcomes the image speed limitation of TD-OCT. Due to the dramatic technological advances in optics and light source technology, FD-OCT can achieve down to sub-micrometer resolution, named as ultra-high resolution OCT (UHR-OCT) and micro-OCT (μOCT), by applying state of the art broad bandwidth light source technology and specially designed objectives [22-25]. Recently developed μOCT is able to achieve $\sim 2 \times 2 \times 1 \mu\text{m}$ spatial resolution and enables imaging the cellular and subcellular structures of the cornea, human atherosclerotic plaques, human airway epithelia, etc [25, 26]. The developed

μ OCT or UHR-OCT have the potential to provide real-time, *in situ* visualizations of tissue microstructure without being time intensive as in biopsy which needs to excisionally remove the tissue and process it into a specimen [27].

With flexible probes, endoscopic [28, 29] and intravascular OCT [30] imaging technologies enable imaging internal hollow organs. Among them, intravascular imaging becomes the second most developed clinical OCT application. For a typical probe design, the light is directed to the distal end of the probe through a single-mode fiber, then focused by a grin lens and reflected by a prism radially away from the probe. After that, the three-dimensional information containing volumetric structural details of the hollow organs is recorded [31]. The OCT probe has the potential to be made sufficiently small and pliable to operate with gastrointestinal track and major blood vessels.

OCT has matured into an critical clinical imaging modality due to the numerous advantages that it offers to clinicians, including the fast speed and flexible probes, etc. OCT has been established and adopted as a gold standard in ophthalmology. The application of OCT to image other parts of the human body have been demonstrated, including monitoring skin cancer and endoscopic gastrointestinal tract imaging for tumor detection, monitoring and risk assessing of vulnerable lipid plaques in the vascular system, monitoring intracoronary stenting, and tracking structural weakness associated with tooth decay [32].

2.2 Working principle of SD-OCT

A standard SD-OCT system can be described by the generalized schematic shown in Figure 2. 1a. Light from a broadband light source is split into two arms of the Michelson interferometer using a beam splitter or a 50/50 fiber coupler. The light incident on the tissue undergoes partial backscattering due to the refractive index inhomogeneity at different depths within the tissue. Then, the backscattered light from the tissue and the reference arm are recombined, and the interference signal is recorded at the spectrometer. After Fourier transforms (FT), the axial structural information of the tissue, which commonly referred to as an A-scan (Figure 2. 1b), can be obtained. Lateral scanning the beam incident on the tissue, then a series of A-scans are collected to

generate the cross-sectional image (Figure 2. 1b). Similarly, multiple cross-sectional images are corrected and arrayed to generate three-dimensional volume data sets (Figure 2. 1b).

Figure 2.1 Working Principle of OCT. (a) Schematic of a general SD-OCT system. (b) OCT axial-scan generation (left), cross-sectional image (middle) and three-dimensional volumetric data set (right) [32].

Let us consider the simplified Michelson interferometer performed using low-coherence interferometry, as shown in Figure 2. 2 [32]. The light source is a polychromatic plane wave and its electrical field can be expressed as, $E_i = s(k, w) \cdot \exp[i(kz - wt)]$, where

$s(k, \omega)$ is the amplitude of the electrical field. It is a function of the wavenumber $k = 2\pi/\lambda$ and angular frequency $\omega = 2\pi\nu$.

Figure 2.2 Schematic of a Michelson interferometer used in OCT.

The electrical field reflected from the reference reflector is assumed to r_R (power reflectivity $R_R = |r_R|^2$). The distance from the beam splitter to the reference reflector is z_R in air.

For simplicity, we assume the sample composed of a series of discrete reflecting surfaces located at different axial positions along the sample beam axis $r_s(z_s)$, each with an amplitude reflectivity of $r_{sn} (n = 1, 2, \dots, N)$ (power reflectivity $R_{sn} = |r_{sn}|^2$). z_s is the variable distance in the sample arm measured from the beam splitter. Therefore, the amplitude reflectivity from the sample can be assumed as the summation of a series of axially spaced data functions: $r_s(z_s) = \sum_{n=1}^N r_{sn} \sigma(z - z_{sn})$. Therefore, the electrical field from the sample is denoted as, $E_s = (E_i/\sqrt{2})[r_s(z_s) \otimes e^{i2kz_s}]$, which is the sample field reflectivity function convolution with the sample reflectivity profile. The returning field interfere at the square-law detector, which can be denoted as

$$\begin{aligned}
 I_D(k) &= \frac{\rho}{2} \langle (E_R + E_s)(E_R + E_s)^* \rangle \\
 &= \frac{\rho}{2} \langle \left| \frac{S(K)}{\sqrt{2}} r_R e^{i(2kz_R)} + \frac{S(K)}{\sqrt{2}} \sum_{n=1}^N r_{sn} r_R e^{i2kz_{sn}} \right|^2 \rangle \\
 &= \frac{\rho S(K)}{4} [R_R + \sum_{n=1}^N R_{sn}]
 \end{aligned}$$

$$\begin{aligned}
& + \frac{\rho S(k)}{2} \sum_{n=1}^N [2\sqrt{R_R R_{sn}} \cos(2k(z_R - z_{sn}))] \\
& + \frac{\rho S(k)}{4} \sum_{n=1}^N \sum_{m \neq n}^N [2\sqrt{R_{sn} R_{sm}} \cos(2k(z_{sn} - z_{sm}))]
\end{aligned} \tag{2.1}$$

Here, $S(k) = \langle |s(k)|^2 \rangle$ is the spectral power dependence of the light source. It composed of three components: (1) a path length-independent DC terms; (2) cross-correlation terms which are the interference signals dependence on the path length difference between the reference arm and sample reflectors; (3) autocorrelation terms which are interference signals occurring between the different sample reflectors appearing as artifacts.

In practice, the autocorrelation terms can be negligible as they are small compared to the DC terms and cross-correlation terms. The DC terms can be eliminated by subtracting the reference background signal. Therefore, the cross-correlation terms used for OCT imaging generation are left and shown as follows:

$$I_D(k) \propto \frac{\rho S(k)}{2} \sum_{n=1}^N [2\sqrt{R_R R_{sn}} \cos(2k(z_R - z_{sn}))] \tag{2.2}$$

After carrying out inverse FT on (2.2), the signal in wavenumber domain k changes to spatial domain z . Based on the convolution theorem of FT, $x(z) \otimes y(z) \xleftrightarrow{F} X(k)Y(k)$, and Fourier transform pair, $\cos(kz_0) \xleftrightarrow{F} \frac{1}{2} [\delta(z + z_0) + \delta(z - z_0)]$, the inverse FT of (2.2) can be calculated as follows:

$$I_D(z) = \frac{\rho}{4} [\gamma(z) \otimes \sum_{n=1}^N \sqrt{R_R R_{sn}} (\delta(z \pm 2(z_R - z_{sn})))] \tag{2.3}$$

Here, a Gaussian-shaped light source spectrum is used. The normalized Gaussian function $S(k)$ and its inverse Fourier transform $\gamma(z)$ are given by:

$$\gamma(z) = e^{-z^2 \Delta k^2} \xleftrightarrow{F} S(k) = \frac{1}{\Delta k \sqrt{\pi}} e^{-\left[\frac{(k-k_0)}{\Delta k}\right]^2} \tag{2.4}$$

where k_0 is the central wavenumber, and Δk is the wavenumber bandwidth. After carrying out the convolutions and then utilizing the shifting properties of the delta function, the interferometric measurement results referred to as the A-scan can be obtained as follows:

$$I_D(z) = \frac{\rho}{4} \sum_{n=1}^N \sqrt{R_R R_{sn}} [\gamma[2(z_R - z_{sn})] + \gamma[-2(z_R - z_{sn})]] \quad (2.5)$$

Figure 2.3 Fourier-domain low-coherence interferometry A-scan illustration. (a) All A-scan terms including DC term, auto-correlation term, cross-correlation term, and mirror-image artifacts. (b) Cross-correlation term generation process.

The results in (2.3) and (2.5) for a Gaussian-shaped light source spectra and discrete sample reflectors are plotted in Figure 2. 3. Three main modifications of the reproduced cross-correlation terms are shown in this Figure. Firstly, the zeros position is at the position of the reference reflector z_R . Secondly, the displacement of each sample reflector from the reference position is doubled. This is because the interferometer measures the round-trip distance to each reflector. Thirdly, each reflector signal broadened to a width of the coherence length by convolution the function $\gamma(z)$ whose full width at half maximum (FWHM) is denoted as the axial resolution of OCT imaging.

From the Equation (2.3), (2.5), and Figure 2. 3, we can see that there exists a mirror image of the depth profile appearing on the opposite side of zero path length; this is termed complex conjugate artifacts. This artifact is not severe if the sample arm is kept

entirely to one side of the zero path length as it displays only the positive or negative distance.

2.3 Theory of spectrometer

The spectrometer is a critical sub-system of SD-OCT. A typical refractive-optics-based spectrometer mainly includes a collimator, diffraction grating, focusing camera lens, and linear detector array, as shown in Figure 2. 4 [32]. Figure 2. 5 shows a more detailed

Figure 2.4 Zemax model of a typically used spectrometer for SD-OCT. It includes an achromatic collimator, a grating, an achromatic objective/camera lens, and a line scan camera [32].

Figure 2.5 Ray diagram of the light propagation to one line array detector. It demonstrates the mapping between wavelength and pixel number. θ_i is the incident

angle on the grating. θ_c is the transmitted angle for wavelength λ_c which transmits through the center of the camera lens. F is the focal length of the camera. x_c is the distance from the first pixel to the position of λ_c on the line scan detector array. w is the pixel width of the detector. (The figure is reproduced from reference [33]).

schematic of the part of the spectrometer [33]. Based on the grating Equation and geometrical optics, the wavelength λ_j incident on a given pixel of the camera is given by:

$$\lambda_j = g \left(\sin \theta_i + \sin \left(\sin^{-1} \left(\frac{\lambda_c}{d} - \sin \theta_i \right) + \tan^{-1} \left(\frac{\omega \cdot j - x_c}{F} \right) \right) \right) \quad (2.6)$$

where θ_i is the incident angle on the grating with spacing g , λ_c is the wavelength of light transmitted through the center of the camera lens with a focal length of F , x_c is the distance from the first pixel to the position of λ_c on the line scan detector which has the pixel width of w . The spectrally encoded output from the line scan camera contains the information to obtain a phase-resolved depth profile. In such a system, there are three main parameters, including the frequency resolution, spectral response, and spot size of the beam, that influence the spectrometer's quality. Specifically, the first two parameters determine the roll-off properties and the third one determines the axial resolution of the image."

Figure 2.6 Sensitivity roll-off as a function of imaging depth. The pixel sampling interval determines the maximum ranging depth which was limited by aliasing.

Frequency resolution: A spectrometer using a linear detector array samples the illumination spectrum and results in a discrete signal. According to the sampling theorem, the spectral sampling interval in frequency (the distance between each pixel) determines the un-aliased imaging range (the maximum ranging depth), as shown in Figure 2. 6.

Based on the theorem, the sampling frequency of the photodiode array has to be twice as large as the highest occurring frequency in the spectrum [34]. For $z = z_{max}$, the period of the cosine fringe/spectral resolution is : $\delta_s k = \pi/(nz_{max})$. Therefore, the spectrometer has to resolve at least $\delta_s k/2$. With $|\delta_s k| = 2\pi\delta_s\lambda/\lambda_0^2$, the maximum ranging depth is given by:

$$z_{max} = \frac{1}{4n} \frac{\lambda_0^2}{\delta_s \lambda} \quad (2.7)$$

It can be observed that the imaging range of the SD-OCT system is inverse proportional to the frequency resolution. As the pixel number of a detector array is finite, the trade-off between the axial resolution and ranging depth should be taken into consideration in spectrometer design.

Spectral response: Spectral response is also an essential parameter for imaging quality. The interfering signals pass through the spectrometer optics before they are collected by the detector array. If a spectral response reduces the signal efficiency or filters the signal non-uniformly, the sensitivity, axial resolution, and the image contrast could be reduced.

Sensitivity roll-off: The penetration depth of the SD-OCT system is also limited by the sensitivity roll-off which is primarily determined by the grating spectral resolution, the spot size of a single wavelength component of the beam illuminated on the array, and the pixel width of the detector. The smaller the grating spectral resolution or spot size or the pixel size, the better the roll-off performance.

The grating disperses optical wavelengths or frequencies along with the detector array. The focusing optics combined with the collimation lens determine the focused spot profile (point spread function (PSF)) of each wavelength illuminated on the detector. The PSF can be modeled as a 2D Gaussian distribution, whose tails cover all of the

pixels. The geometry of the model is shown in Figure 2. 7. The signal intensity $I(x_j)$ for the j -th pixel of the detector located at coordinate x_j is as follows [9]:

$$I(x_j) = \int_0^\infty \int_0^s h(x, y, k) \left[\frac{1}{2} p_{ref}(k) + \frac{1}{2} p_{sam}(k) + \sqrt{p_{ref}(k)p_{sam}(k)} \cos(\Delta\phi) \right] ds dk \quad (2.8)$$

Where p_{ref} and p_{sam} are the power spectral densities from the reference arm and sample arm. $\Delta\phi$ is the phase difference between the two optical fields obtained from the two arms. The function of $h(x, y, k)$ is a normalized Gaussian distributed 2D PSF. An arbitrary wavenumber k_i is represented by the PSF_i (center location of x_i). Replacing k_i by x_i in PSF_i , the weight of the contribution of k_i to the investigated point (x, y) in pixel j can be expressed as a normalized 2D Gaussian distribution as follows:

$$h(x, y, x_i) = \frac{4\ln 2}{\pi a^2} e^{-(4\ln 2/a^2)((x-x_i)^2+y^2)} \quad (2.9)$$

The differential variable ds is defined by $ds=dx dy$, and s is the area for specific pixel j . Then the signal amplitude $I(x_j)$ can be expressed as:

$$\begin{aligned} I(x_j) = & \frac{1}{4} \operatorname{Erf} \left(\frac{\Delta y \sqrt{\ln 2}}{a} \right) \\ & \cdot \int_0^\infty \left[\operatorname{Erf} \left(\frac{(\Delta x - 2x(k) + 2x_j) \sqrt{\ln 2}}{a} \right) + \operatorname{Erf} \left(\frac{(\Delta x + 2x(k) - 2x_j) \sqrt{\ln 2}}{a} \right) \right] \\ & \cdot \left[p_{ref}(k) + p_{sam}(k) + 2 \sqrt{p_{ref}(k)p_{sam}(k)} \cos(2k\Delta\phi) \right] dk \end{aligned} \quad (2.10)$$

where $x(k)$ is a coordinate function and varies with wavenumber k , and it is defined by the spectrometer design. Erf , Δy , Δx and x_j are the error function, pixel height, pixel width, and the center position of pixel j , respectively. The spot diameter a is defined as the full width at half maximum (FWHM) of the focused spot. S_{ref} and S_{sam} are spectral densities of the reference and sample arm, respectively. The integral over k is the convolution of the effective pixel function in the first bracket with the ideal signal in the second bracket.

Evaluating the Equation with the assumption that the spectrum is distributed on the detector as a linear function of wavenumber k and the light source spectrum is a Gaussian-shape. After carrying out FT of the Equation (2.10), the simplified result is shown in (2.11). The FT of the first bracket factor under the integral represents the roll-off, and the FT of the second bracket factor represents the A-scan signal.

$$A(z_i) \propto \exp\left(-\frac{(aR_k z_i)^2}{4 \ln 2}\right) \cdot \frac{\sin(\Delta x R_k z_i)}{\Delta x R_k z_i} \quad (2.11)$$

Figure 2.7 Schematic of the detector array illustrates the illumination by the spectrum and by the PSF of an individual wavelength. Single wavelength PSF_i of wavenumber k_i covers multiple pixels; each rectangular pixel measures width Δx and height Δy . Illuminating wavelength i is centered on pixel i . Pixel j is the pixel under investigation. PSF: point spread function.

It can be observed from Equation. (2.11) that there are three primary parameters which affect the roll-off properties, including the spot size a , the pixel width Δx , and the reciprocal linear dispersion R_k of the spectrometer.

The spot size a defined by the FWHM of the wavelength-dependent spot diameter and can be expressed as follows [35, 36]:

$$a = 1.22 \cdot \frac{\lambda_0 \cdot F_{cam}}{D} \quad (2.12)$$

Where λ_0 is the center wavelength, F_{cam} is the focal length of a camera, $D=2 \cdot F_{col} \cdot NA$ is the exit pupil diameter. F_{col} is the focal length of the collimator and NA is the numerical aperture of the fiber. The reciprocal linear dispersion, R_k , in Equation 2. 11, indicates

the width of the spectrum that is the spectrum in wavenumber spreads over 1 μm at the focal plane. It is determined by the groove number of the grating and the focal length of the camera in the spectrometer [35].

$$R_k = \frac{dk}{dx} = \left(\frac{2\pi}{\lambda_0^2} \cdot R_\lambda \right) = \left(\frac{2\pi}{\lambda_0^2} \cdot \frac{d_{grating} \cdot \cos\theta_f}{F_{cam}} \right) \quad (2.13)$$

where $d_{grating}$ is the grating spacing, θ_f is angle of the dispersed light in the first order. $R_\lambda = d\lambda/dx$ is the reciprocal linear dispersion in λ -space. Therefore, based on the definition mentioned above of the spectral resolution of the grating, $a \cdot R_k$ in the Equation (2.11) represents the grating spectral resolution, $\delta_g k$ ($\delta_g k = (2\pi/\lambda_0^2) \cdot \delta_g \lambda$). $\delta_g \lambda$ can be estimated by Equation (2.14) as follows [37]:

$$\delta_g \lambda = \frac{\lambda_0 \cdot d_{grating}}{D} \quad (2.14)$$

Therefore, the Equation (2.11) can be expressed as follows:

$$F(z_i) = \exp\left(-\frac{(1.22 \cdot \delta_g k \cdot \cos\theta_f)^2}{4 \ln 2} \cdot z_i^2\right) \cdot \frac{\sin(\Delta x R_k z_i)}{\Delta x R_k z_i} \quad (2.15)$$

In which the spectral resolution of the grating, $\delta_g k$, was included in Equation (2.15). The values of $1.22 \cdot \cos\theta_f$ can be approximated as 1 for the center wavelength. The grating spectral resolution has the most influence on the sensitivity roll-off.

The spectrometer resolution or grating spectral resolution (exponential function), together with spectral range integrated by each pixel (*sinc* function) determine the sensitivity roll-off performance. The spectral sampling interval (spectral spacing between pixels) determines the unambiguous imaging range or aliasing. Therefore, for a spectrometer design, the detector array resolution, optical resolution, chromatic aberration should be taken into consideration. Chromatic aberration could be eliminated through all-reflective-optics. The optical resolution or the spot profile is more challenging to control, which usually needs specific optics design and optimization.

2.4 Image formation in SD-OCT

2.4.1 Fiber-based SD-OCT system

As is illustrated in Figure 2. 8, the sample arm in a fiber-based SD-OCT system mainly includes a collimation lens, an objective lens, and a galvo scanner (GV) which is used for transverse scanning. The sample is placed near the back focal plane of the objective lens, and its backscattered light interferes with that reflected from the reference mirror.

Figure 2.8 Schematic diagram of a typical fiber-based SD-OCT system. The blue dotted square shows the sample arm optics. GV: galvo scanner; DAQ: data acquisition card; RC: reflective collimator; CCD: charge-coupled detector; IMAQ: image acquisition board; SMF: single-mode fiber.

The OCT sample arm is the same as that of the confocal microscopy (Figure 2. 9). Let $U_1(x_0)$ be the amplitude distribution of the electric field on the output end of fiber F_1 . After transmitting through the imaging system, based on the imaging theory, the field amplitude at a point x_2 on the input end of fiber F_2 is given by [38]:

$$U_2(x_s, x_2) = \iint_{-\infty}^{\infty} U_1(x_0) h_1(x_0 + \frac{x_1}{M_1}) r_f(x_s - x_1) h_2(x_1 + \frac{x_2}{M_2}) dx_0 dx_1 \quad (2.16)$$

where x_s is the scan position at the objective lens, h_1 and h_2 are the amplitude PSF of the objective O_1 and collector L_2 , corresponding to the Fourier transform of the pupilfunction P_1 and P_2 , respectively. $M_1 = f/d$ and $M_2 = d/f$ are the lens

magnification. f and d denote the focal length of the objective and the distance between the lens and the fiber tip. r_f is the amplitude reflectivity of an object.

Figure 2.9 Geometry of a reflective-mode fiber-optical confocal imaging system. F_1 and F_2 are optical fibers. L_1 and L_2 are collimation lenses and focusing lens, respectively. O_1 are the objective lens. d is the distance between the lens and the tip of the optical fibers. $U_1(x_0)$ is the amplitude distribution of the electric field on the output end of fiber F_1 . $U_2(x_s, x_2)$ is the field amplitude at a point x_2 on the input end of fiber F_2 . $U_3(x_s, x_3)$ is the field amplitude output from the fiber F_2 at a point x_3 .

An arbitrary electric field $U(x, z)$ can be expanded as a superposition of a complete set of orthonormal modes in an optical fiber-based on optical waveguide theory [39]. For single-mode-fiber (SMF), it can be expressed as follows:

$$U(x, z) = a_1 f_1(x) \exp(i\beta_1 z) \quad (2.17)$$

where $a_1 = \frac{\int_{-\infty}^{\infty} f_1^*(x) U(x, z=0) dx}{\int_{-\infty}^{\infty} |f_1(x)|^2 dx}$ is the modal amplitude. $f_1(x)$ is the profile of fiber modes on the plane perpendicular to the z axis which is the propagation direction along the fiber. β_1 is the propagation constant.

Therefore, the field amplitude $U_3(x_s, x_3)$ output from the fiber F_2 at a point x_3 can be given by:

$$U_3(x_s, x_3) = \frac{f_1(x_3) \exp(i\beta_1 z_0) \int_{-\infty}^{\infty} f_1^*(x_2) U_2(x_s, x_2) dx_2}{\int_{-\infty}^{\infty} |f_1(x)|^2 dx} \quad (2.18)$$

where z_0 is the length of the optical fiber F_2 . Hence, the intensity measured by the detector with area D has a uniform intensity sensitivity, is

$$I(x_s) = \int_{-\infty}^{\infty} |U_3(x_s, x_3)|^2 dx_3 \quad (2.19)$$

Substituting Equation. 2.18 to Equation 2.19 and using Equation 2.16, and considering the condition that $\int_{-\infty}^{\infty} |f_1(x_3)|^2 dx_3$ is constant due to the profile of $f_1(x_3)$ is fixed for a particular fiber. Then, we have:

$$\begin{aligned} I(x_s) &= \iint_{-\infty}^{\infty} g_1(x_1) g_1^*(x'_1) r_f(x_s - x_1) r_f^*(x_s - x'_1) dx_1 dx'_1 \\ &= |g_1(x_s) \otimes r_f(x_s)|^2 \end{aligned} \quad (2.20)$$

The Equation. 2.20 shows that the OCT image formation behaves as a coherent microscope with an effective PSF given by:

$$\begin{aligned} g_1(x_1) &= \iint_{-\infty}^{\infty} U_1(x_0) h_1\left(x_0 + \frac{x_1}{M_1}\right) f_1^*(x_2) h_2\left(x + \frac{x_2}{M_2}\right) dx_0 dx_2 \\ &= \left[U_1\left(\frac{x}{M_1}\right) \otimes h_1\left(\frac{x}{M_1}\right) \right] \left[f_1^*\left(\frac{x}{M_1}\right) \otimes h_2(x) \right] \end{aligned} \quad (2.21)$$

Therefore, the introduction of the SMF in the imaging system does not change its coherence.

The periodic components of the object are given by its spectrum, which can be derived by the Fourier transform of the object reflectivity r_f :

$$R_f(m) = \int_{-\infty}^{\infty} r_f(x) \exp(-2\pi i x m) dx \quad (2.22)$$

hence,

$$r_f(x_s - x_1) = \int_{-\infty}^{\infty} R_f(m) \exp[2\pi i(x_s - x_1)m] dm \quad (2.23a)$$

$$r_f^*(x_s - x'_1) = \int_{-\infty}^{\infty} R_f^*(p) \exp[-2\pi i(x_s - x'_1)p] dp \quad (2.23b)$$

therefore, Equation 2.20 can be rewritten as follows:

$$I(x_s) = \iint_{-\infty}^{\infty} C(m, p) R_f(m) R_f^*(p) \exp [2\pi i(m - p)x_s] dm dp \quad (2.24)$$

$$C(m, p) = \iint_{-\infty}^{\infty} g_1(x_1) g_1^*(x'_1) \exp [-2\pi i m x_1 + 2\pi i p x'_1] dx_1 dx'_1 \quad (2.25)$$

where m and p are spatial frequencies in the transverse direction. $C(m, p)$ is called the transfer function for the given system and describe the strength of a periodic component in the image. Due to the system is a coherent system, $C(m, p) = C(m) * C(p)$.

$$C(m) = \int_{-\infty}^{\infty} g_1(x_1) \exp(-2\pi i m x_1) dx_1 \quad (2.26a)$$

$$C(p) = \int_{-\infty}^{\infty} g_1^*(x'_1) \exp(2\pi i p x'_1) dx'_1 \quad (2.26b)$$

they are the Fourier transform of the effective point spread function $g_1(x_1)$ and $g_1^*(x'_1)$ respectively. Substituting Equation 2.21 to 2.26, and employing a combination of the shift and similarity theorems, the following Equation can be obtained:

$$C(m) = [\tilde{U}_1(M_1 m) P_1(\lambda f m)] \otimes \tilde{f}_1(M_1 m) P_2(\lambda f m) \quad (2.27a)$$

here $\tilde{U}_1(m)$ and $\tilde{f}_1(m)$ are the Fourier transform of $U_1(x)$ and $f_1(x)$. For a cylindrically symmetric two-dimensional system:

$$C(l) = [\tilde{U}_1(M_1 l) P_1(\lambda f l)] \otimes \otimes [\tilde{f}_1(M_1 l) P_2(\lambda f l)] \quad (2.27b)$$

here $\otimes \otimes$ denotes the 2-D convolution operation. $l = (m^2 + n^2)^{1/2}$ is the radial spatial frequency in the transverse direction. The Fourier transform of the amplitude profile of the single-mode-fiber (SMF) $\tilde{f}_1(l)$ can be expressed as following based on Gaussian approximation:

$$\tilde{f}_1(l) = 2\pi r_0^2 \exp \left[-\frac{1}{2} (2\pi l r_0)^2 \right] \quad (2.28)$$

where r_0 is the fiber mode spot size, and given by

$$r_0 = \frac{r_{co}}{\sqrt{2 \ln V_f}} \quad (2.29)$$

here r_{co} is the fiber core radius, and fiber parameter $V_f = (2\pi/\lambda) r_{co} NA_f$. NA_f is the NA of the fiber. Equation 2.28 is also available for the defocused 2-D coherence transfer function once including the defocusing effect into the following pupil function:

$$P(\lambda fl) = \exp \left[\left(\frac{i}{2} \right) \left(\frac{2\pi}{\lambda} \right) z (\sin \alpha)^2 \left(\frac{\lambda fl}{a_0} \right)^2 \right] \quad (l < \frac{a_0}{\lambda f}) \quad (2.30a)$$

$$P(\lambda fl) = 0 \quad (l > \frac{a_0}{\lambda f}) \quad (2.30b)$$

a_0 is the pupil radius, $\sin \alpha$ is the semi angular aperture, z is the axial distance from the focal plane.

2.4.2 Digital refocus: Forward Model theorem

Figure 2.10 Unfolded schematic of the telescopic imaging system in the sample arm of a SD-OCT system. L_1 and L_2 are lenses; Sample represents the sample. (ξ, η) represents the first surface of the sample in the focus plane. (x, y) represents the camera/detector plane. f_1 and f_2 are the focal length of the lens of L_1 and L_2 , respectively. M is the magnification of the telescopic imaging system. z is the depth in the sample. Δz_S is the distance between the first surface of the sample and the relative reference mirror position (RMP). $z \cdot n_0 + \Delta z_S$ is the optical path length difference (OPD) between the reference light and sample light. z_R and z_S is the transmitted path length of the reference light and sample light, respectively.

A telescopic imaging system forms the image of the object field onto the 2D camera with magnification $M = f_2 / f_1$, as shown in Figure 2. 10. Assuming that the first surface

of the sample indicated by plane (ξ, η) is in focus and imaged onto the camera plane (x, y) , and the sample lies entirely on one side relative to the reference mirror plane.

As was discussed in chapter 2.2 (Working principle of SD-OCT), the interference signal from the reference and sample arm is collected by the camera and the signal we interested is the cross-correlation terms which is given by [40]:

$$E(x, y, k) = E_s(x, y, k)E_R^*(k) \quad (2.31)$$

The image of the object field E_s is given by

$$E_s(x, y, k) = \frac{\exp[ik(\Delta z_s + z_R)]}{M} \iint E_0(\xi, \eta, k) P\left(\frac{x}{M} - \xi, \frac{y}{M} - \eta\right) d\xi d\eta \quad (2.32)$$

where E_0 is the object field convolved with the PSF (P) of the telescopic imaging system with magnification M . Here, we suppose that the field of view is isoplanatic, and P does not vary with k . The phase factor $\exp[ik(\Delta z_s + z_R)]$ is due to the light propagation from (ξ, η) plane to (x, y) plane. The object field E_0 is the summation of all the fields from different layers in the sample within depth range Z , and it is expressed by:

$$E_0(\xi, \eta, k) = \int FT^{-1} \left\{ \exp[ik(\Delta z_s + zn_0) + i\varphi] \tilde{E}_{o,z}(f_\xi, f_\eta, k) \times \exp[i2\pi z \sqrt{(kn_0/2\pi)^2 - f_\xi^2 - f_\eta^2}] \right\} dz \quad (2.33)$$

where $FT^{-1}\{\cdot\} = \iint \{\cdot\} \exp[2\pi i(f_\xi \xi + f_\eta \eta)] df_\xi df_\eta$ is the 2D inverse Fourier transform, $\tilde{E}_{o,z}$ is the 2D spatial Fourier transform of the back-scattered field $E_{o,z}$ which is propagated from depth z to (ξ, η) plane. (f_ξ, f_η) is the coordinate in the spatial Fourier domain corresponding to time-domain (ξ, η) . The term $\exp[ik(\Delta z_s + zn_0) + i\varphi]$ is the phase accumulation caused by the planar illumination beam. φ is the initial reference phase and n_0 is the refractive index of the sample.

The optical field reflected from the reference arm can be expressed by:

$$E_R(k) = \kappa S(k) \exp(ikz_R + i\varphi) \quad (2.34)$$

where $S(k)$ is the power spectral density function of the source at a frequency k , κ is the attenuation caused by the reference arm and beam splitter. Then, the Equation (2.32) can write as follows:

$$\begin{aligned}
E(x, y, k) = & \frac{\kappa S(k)}{M} \iint \int FT^{-1} \left\{ \exp[ik(2\Delta z_S + zn_0)] \tilde{E}_{o,z}(f_\xi, f_\eta, k) \right. \\
& \times \exp \left[i2\pi z \sqrt{(kn_0/2\pi)^2 - f_\xi^2 - f_\eta^2} \right] \left. P\left(\frac{x}{M} - \xi, \frac{y}{M} - \eta\right) d\xi d\eta dz \right\}
\end{aligned} \quad (2.35)$$

Taking the 2D spatial Fourier transform of the signal and employing the convolution theorem, then the Equation can be obtained as follows:

$$\begin{aligned}
\tilde{E}(k_x, k_y, k) = & \kappa MS(k) \tilde{P}(Mk_x, Mk_y) \int \exp[ik(2\Delta z_S + zn_0)] \tilde{E}_{o,z}(Mk_x, Mk_y, k) \times \\
& \exp \left[iz \sqrt{(kn_0)^2 - (Mk_x)^2 - (Mk_y)^2} \right] dz
\end{aligned} \quad (2.36)$$

here $k_x = 2\pi f_x$, $k_y = 2\pi f_y$, $\tilde{P}$ is 2D Fourier transform of P .

For convenience, Equation (2.36) can be rewritten as

$$\tilde{E}(k_x, k_y, k) = \kappa MS(k) \tilde{P}(Mk_x, Mk_y) \int \exp[ik(2z_R + zn_0)] \tilde{E}'_{o,z}(Mk_x, Mk_y, k) dz \quad (2.37)$$

here

$$\tilde{E}'_{o,z}(Mk_x, Mk_y, k) = \tilde{E}_{o,z}(Mk_x, Mk_y, k) \times \exp \left[iz \sqrt{(kn_0)^2 - (Mk_x)^2 - (Mk_y)^2} \right] \quad (2.38)$$

In OCT system, the NA of the imaging system usually is low. Hence, the Equation (2.38) can be approximated to

$$\tilde{E}'_{o,z}(Mk_x, Mk_y, k) = \tilde{E}_{o,z}(Mk_x, Mk_y, k) \times \exp \left[-i \frac{\lambda_0 z M^2}{4\pi n_0} (k_x^2 + k_y^2) \right] \quad (2.39)$$

Taking 1D FT of Equation (2.36) along the axial dimension

$$E(k_x, k_y, z') = \kappa M \tilde{P}(Mk_x, Mk_y) \left[\tilde{S}(z') \otimes \int \tilde{E}'_{o,z}(Mk_x, Mk_y, z) \delta(z - z') dz \right] \quad (2.40)$$

$$\tilde{E}'_{o,z}(Mk_x, Mk_y, z) = \tilde{E}_{o,z}(Mk_x, Mk_y, z) \times \exp \left[-i \frac{\lambda_0 z M^2}{4\pi n_0} (k_x^2 + k_y^2) \right] \quad (2.41)$$

To remove the defocus error, a correction term of γ needs to be multiplied:

$$\gamma(Mk_x, Mk_y, z) = \exp \left[i \frac{\lambda_0 z M^2}{4\pi n_0} (k_x^2 + k_y^2) \right] \quad (2.42)$$

The distance z of a given layer in the image volume from the focus plane, the first surface of the sample, can be calculated by:

$$z = \frac{N_z \lambda_0^2}{2n_0 \Delta \lambda} \quad (2.43)$$

Therefore, multiplying of the Equation of (2.40) with the correction term and then calculate the 2-D Fourier transform to obtain the defocus corrected image volume with depth invariant transverse resolution. This is the main principle of forward model digital refocus approach.

2.5 Main parameters of SD-OCT system

2.5.1 Axial resolution

Figure 2.11 Fourier transform relationship between the Gaussian-shaped coherence function $\gamma(z)$ and the light source spectrum $S(k)$. l_c defines the coherence length at FWHM; k_0 is the central wavenumber; Δk is the wavenumber bandwidth at half-width at 1/e of its maximum. (This figure is reproduced from reference [32]).

The axial resolution, also known as the depth resolution or the coherence gate, is the coherence length of the light source. A Gaussian-shape light source spectrum is illustrated here as an example. The normalized Gaussian function $S(k)$ and its inverse Fourier transform $\gamma(z)$ is shown in Figure 2. 11 and is given in Equation 2.44.

$$\gamma(z) = e^{-z^2 \Delta k^2} \stackrel{F}{\leftrightarrow} S(k) = \frac{1}{\Delta k \sqrt{\pi}} e^{-\left[\frac{(k-k_0)}{\Delta k}\right]^2} \quad (2.44)$$

where k_0 is the central wavenumber of the light source spectrum, and Δk is its wavenumber bandwidth which corresponds to the half-width of the spectrum at $1/e$ of its maximum. The inverse Fourier transform of the source spectrum $\gamma(z)$, also called the coherence function, determined the axial PSF in OCT imaging systems. Typically, the PSF is characterized by its FWHM value and is the coherence length of the light source, l_c , which is a function of the light source bandwidth and is shown as follows:

$$l_c = \frac{2\sqrt{\ln(2)}}{\Delta k} = \frac{2\ln(2)}{\pi} \frac{\lambda_0^2}{\Delta \lambda} \quad (2.45)$$

where λ_0 is the center wavelength of the light source ($\lambda_0 = 2\pi/k_0$) and $\Delta \lambda$ is the wavelength bandwidth of the light source, defined as the FWHM of the light source spectrum (therefore, $\Delta k = \frac{\pi}{\sqrt{\ln 2}} \frac{\Delta \lambda}{\lambda_0^2}$).

2.5.2 Transverse resolution, focal range, and lateral FOV

Transverse resolution: The axial and transverse resolution are independent in the OCT system. The transverse resolution is the same in confocal microscopy and determined by the diffraction-limited spot size of the focused optical beam. Based on the diffraction theorem, the lateral PSF is given by [32]:

$$I(v) = \left(\frac{2J_1(v)}{v}\right)^4 \quad (2.46)$$

where $J_1(v)$ is a first-order Bessel function and $v = \frac{2\pi x \sin(\alpha)}{\lambda_0}$ is the normalized lateral range parameter. x is the lateral distance from the optical axis, and α is half angular optical aperture subtended by the objective. The numerical aperture of the objective lens is given by $NA = \sin(\alpha)$. Then, the lateral resolution δx as its full width at half maximum power or as its $1/e^2$ power can be respectively calculated to:

$$\delta x = 0.37 \frac{\lambda_0}{\sin(\alpha)} = 0.37 \frac{\lambda_0}{NA} \quad (2.47)$$

Figure 2.12 Schematic of generic OCT sample optics. f and NA is the focal length and numerical aperture of the objective lens respectively. The lateral resolution, δx , is measured at FWHM power of the lateral point spread function. z_{FR} is the focal range. The axial resolution, l_c , is determined by the light source and is the coherence length measured at the FWHM power of the axial PSF for each reflector. Lateral field of view FOV is determined by the lateral scanning range of imaging system. λ_0 is center wavelength of the incident light source, and $\Delta\lambda$ is its bandwidth.

The spot size is proportional to center wavelength and inversely proportional to the numerical aperture which also can be given by $NA = d/2f$. d is the diameter of the incident beam on an objective lens, and f is the focal length of the objective lens.

The irradiance/PSF profile does not stay constant as the beam propagates through space. Due to diffraction, a Gaussian beam will converge and diverge from an area called the beam waist which is where the beam diameter reaches a minimum value. The focal range z_{FR} is governed by the transverse resolution and is two times the Rayleigh range (z_{RL}) which is defined as the value of z where the cross-section area of the beam is doubled. The focal range can be expressed as follows:

$$z_{FR} = 2z_{RL} = 2 \frac{\pi(\delta x)^2}{\lambda_0} \quad (2.48)$$

Thus, there is a trade-off between the transverse resolution and the focal range, as is illustrated in Figure 2. 13. Employing a large numerical aperture system can improve transverse resolution, but it also will decrease the focal range.

Figure 2.13 Schematic of OCT focusing beam in sample arm. The low and high NA optics geometries show a trade-off between transverse resolution and focal range. NA is the numerical aperture of the objective lens; δx is lateral resolution; z_{RL} is Rayleigh range which is the half value of the focal range z_{FR} ; l_c is axial resolution.

Lateral FOV: The lateral field of view for an OCT system is determined by the employed lateral scanning system. A beam scanner, such as a galvo scanner, steers the sample beam to a maximum one-side scan angle θ_{max} . Hence, the lateral field of view of the OCT system can be expressed as $FOV = 2f\theta_{max}$.

Chapter 3

Design and optimization of a spectrometer for high-resolution SD-OCT

We report the design of a low cost, high-efficiency spectrometer for high-resolution spectral-domain optical coherence tomography (SD-OCT). The advantages of the proposed spectrometer are three-fold: first, the cost of the spectrometer is only about half of the price of a commercial spectrometer with similar performances; second, we use a concave mirror instead of a camera lens (as typically used) as the focusing optics to both improve the transmission efficiency and eliminate chromatic aberrations; third, the efficiency of the proposed spectrometer is approximately 1.23 times that of a refractive optics-based spectrometer for an SD-OCT system, with comparable ranging depth and axial resolution. Both theoretical analyses and imaging experiments were conducted to characterize and validate the performance of the proposed spectrometer.

3.1 Introduction

Spectral-domain optical coherence tomography (SD-OCT) has been established as a diagnostic imaging tool over the past few decades due to its advantages of high sensitivity and speed [41-47]. With the development of broadband light source technologies, studies have pushed the axial resolution to 1-2 μm [45, 48, 49], which has enabled visualization of cellular and subcellular structures. However, the increased spectral bandwidth presents new challenges for spectrometer design. In addition, a compelling demand has to be met to lower the cost of devices, which is a major roadblock towards the successful clinical application of ultrahigh-resolution OCT (UHR OCT) and Micro-OCT [50-53]. Currently, spectrometers typically utilize refractive optical components, i.e., achromatic lenses, to collimate the interference signal from the detection fiber to a diffraction grating followed by focusing of the angularly dispersed spectrum onto a line-scan camera using a refractive camera lens [32]. A customized Cooke triplet lens [54], multi-element scan lens, camera, and commercially available photography lenses have all been used for the camera lens [55, 56]. The currently available refractive optics may be associated with the following issues to support the ultrahigh resolution and low-cost applications. First, the use of an achromatic lens as the collimation lens does not usually eliminate all of the chromatic aberrations and is also costly compared to reflective lenses. Secondly, most of the commercial camera lenses employed in spectrometer designs suffer significant losses in the near-infrared (NIR) wavelength range as they were specifically designed for visible light [56, 57]. It

was proposed that replacing the refractive camera lens with a reflective one could help eliminate the chromatic aberrations [21, 58]. The Czerny-Turner type spectrometer is a commonly used instrument relying only on two spherical mirrors and a plane grating [59, 60]. Recently, Gabor-domain optical coherence microscopy (GD-OCM) was proposed in [61] by using astigmatism corrected Czerny–Turner spectrometer [62]. To the best of our knowledge, however, few studies have evaluated the reflective optics-based spectrometers for ultrahigh-resolution OCT, especially the influences of camera lens focal length on the system parameters such as spectral resolution, ranging depth and spectrometer efficiency.

We report an all-reflective-optics based spectrometer design to both to improve transmission efficiency and eliminate chromatic aberrations. In this study, we firstly used Zemax software to design reflective optics according to a typical ultrahigh-resolution design input. We secondly fabricated an all-reflective-optics based spectrometer and conducted experiments to characterize the performances of the proposed design, and the results were compared with those obtained using a typical refractive-optics based spectrometer.

3.2 Method

Item description	Surface#	Radius (mm)	Thickness (mm)	Semi-diameter (mm)	Tilt angles (°)	
					x axis	y axis
Fiber (NA = 0.13)	0	Infinity	50.8			
Reflective Collimator (RC12APC-P01, Thorlabs Inc.)	1	11	-120	11	90	
Grating: 960 lines/mm	2	Infinity	-35	width: 12.7 length: 12.7	23.9	
Fold mirror (silver coated)	3	Infinity	85	width: 6.5 length: 15	-5	
Concave mirror (silver coated)	4	-256	-128	25.4	-24	2.1
Linear CCD image plane	5			14.8		

Table 3. 1 Design parameters for a reflective optics-based spectrometer

The design input of the proposed all-reflective-optics spectrometer has a center wavelength of 845 nm and 170 nm spectral bandwidth (3dB). The input fiber was Nufern 780-HP with a nominal numerical aperture (NA) of 0.13 at 1% intensity, and the linear camera AViiVA EM4, e2V, Chelmsford, UK) had a pixel size of $14 \times 28 \mu\text{m}$ (width $\times$ height) and array size of 2048. The objective of the design was to achieve improved spectrometer efficiency, ranging depth, and axial resolution with respect to the corresponding all-refractive-optics spectrometer. We evaluated the spectrometer efficiency defined by the transmission efficiency of the optics (collimator, grating, and camera lens) multiply the ratio of -6 dB roll-off length to the maximal ranging depth.

The axial resolution of an SD-OCT system is depended on the center wavelength and the spectral bandwidth, which is usually measured experimentally as the axial point-spread function (PSF) in response to a partial mirror input (sample). The axial profile is reconstructed by the inverse Fourier transform of the interference fringe following dispersion correction using a previously reported method [57]. The coherence length of the light source was $2.7 \mu\text{m}$ in the air, as specified in the datasheet by the manufacturer.

Both the optical resolution of the spectrometer optics and detector array resolution influence the spectral resolution and, consequently the axial imaging range of an SD-OCT system. The maximum axial imaging range of an SD-OCT system is given by:

$$Z_{max} = \frac{\lambda_0^2}{4 \cdot \frac{\Delta\lambda}{N}} \quad (3.1)$$

where N is the total number of illuminated pixels at the line-scan detector. λ_0 and $\Delta\lambda$ are the center wavelength and the bandwidth at full width at half maximum (FWHM) of the light source, respectively. The effective spectrometer bandwidth $\delta_s\lambda = (\Delta\lambda/N)$ represents the spectral range integrated over a single pixel [32, 63]. Considering an ideal case of linear sampling in wavenumber space, for a given spectral bandwidth of the input light, covering all the pixels of the linear camera can maximize the ranging depth. However, practical ranging depth is limited by the depth dependent sensitivity roll-off, which can be calculated using Equation (3.2) [9, 44, 64, 65]:

$$F(Z_i) = 10 \log_{10} \left[\frac{\sin(pR_k Z_i)}{pR_k Z_i} \cdot \exp \left(-\frac{a^2 R_k^2 Z_i^2}{4 \ln 2} \right) \right] \quad (3.2)$$

where Z_i is the imaging depth within the range of $0 \sim Z_{max}$, and p is the pixel size that affects the interferometric signal. R_k is the reciprocal linear dispersion and indicates the width of the spectrum that is the spectrum in wavenumber spreads over 1 μm at the focal plane and can be given by $R_k = (2\pi/\lambda_0^2) \cdot (d_{grating} \cdot \cos\theta_f / F_{cam})$ [35]. $d_{grating}$ is the grating spacing. θ_f is the angle of the dispersed light in the first order. F_{cam} is the focal length of a camera. a is the full width at half maximum (FWHM) wavelength-dependent spot diameter and is defined as $a = 1.22 \cdot (\lambda_0 \cdot F_{cam}) / D$ [35, 36]. D is the exit pupil diameter and is determined by the fiber and the collimator. As the spectral resolution of the grating is defined as the ability to distinguish between two wavelengths separated by a small line width $\delta_g \lambda$ and can be estimated by $\delta_g \lambda = \lambda_0 \cdot d_{grating} / D$ [66]. Therefore, $a \cdot R_k = 1.22 \cdot \delta_g k \cdot \cos\theta_f$ ($\delta_g k = (2\pi/\lambda_0^2) \cdot \delta_g \lambda$) represents the grating spectral resolution in k-space. The values of $1.22 \cdot \cos\theta_f$ can be approximated as 1 for the center wavelength. Therefore, the sensitivity roll-off is mainly determined by the grating spectral resolution or spectrometer spectral resolution ($\delta_r k = a \cdot R_k$) and the pixel width of the detector.

3.3 Spectrometer design

According to the above-mentioned design inputs, we propose to replace the refractive optics of the reference spectrometer: achromatic collimator (AC127-030-B, Thorlabs, USA) and camera lens (85 mm, f/1.8D, Nikon, USA) with reflective concave mirrors (Table 3. 1). In the all-reflective optics spectrometer, the light exiting from the optical fiber was first collimated by a 90° off-axis reflective parabolic mirror, with a reflective focal length of 50.8 mm (RC12APC-P01, Thorlabs, Newton, New Jersey, USA) before dispersed by a grating. We used a planar mirror to fold the output of the grating and, subsequently, a concave mirror to focus the beam onto the linear camera (Figure 3. 1). In addition, considering different types of the light source (approximately Gaussian-shaped or rectangular-shaped light source) and the limited detector size, a proper diffraction grating with 960 lines/mm was used in the designed spectrometer system.

Figure 3.1 Spectrometer optical design and properties of PSF and effective spectrometer bandwidth. (a) Optical design for reflective optics-based spectrometer: Ray tracing of the designed spectrometer obtained using Zemax software. (b) The point-spread function (PSF) in the full width at half maximum (FWHM) and the effective spectrometer bandwidth per pixel for the spectrometer designs with different focal lengths.

There are multiple factors to consider during the design, but we found the most significant two are the tilt angle and the focal length of the concave mirror after the grating. We designed a tilt angle of the concave mirror with respect to the center beam from the grating to avoid blocking the beam from the concave mirror to the detector by the folding mirror. However, this tilt angle gave rise to aberrations, and consequently, it will broaden the spot size. Although we could increase the focal length to reduce the tilt angle and the corresponding aberration, it also resulted in increased spot sizes. In

addition, with larger focal length (F_{cam}), the effective spectrometer bandwidth per pixel ($\delta_s \lambda$) is decreased, and therefore the imaging range is improved. Therefore, we had to make a compromise between focal length and the tilt angle to get the optimal sensitivity roll-off. For the concave mirror with focal lengths of 80, 90, 100, 110, 120, 130, 140 and 150 mm, the minimized tilt angles evaluated using Zemax software are 5° , 3.8° , 3.3° , 2.6° , 2.3° , 2° , 1.8° , and 1.6° , respectively. We simulated the spot sizes (a) and the effective spectrometer bandwidth (Figure 3. 1b) using Zemax, and the sensitivity roll-off curves based on the spot sizes in Figure 3. 1b using Equation 3.2 (Figure 3. 2a). The pixel size (p) considered here was $14 \mu\text{m}$, as the pixel height of $28 \mu\text{m}$ only affects the total intensity of the signal [9]. According to our simulation results, there was a trade-off between the effective spectrometer bandwidth per pixel and the spot size. For our spectrometer design, we chose the best compromise: a focal length of 128 mm and tilt angle of 2.1° , to obtain the optimal 6-dB roll-off range up to 1.6 mm. The optimized design parameters are listed in Table 3. 1.

The detailed performance of the proposed design was characterized using spot diagrams and Huygens PSF cross-sections (Figure 3. 2b&c). The FWHM of Huygens PSF increases from 13 to $16 \mu\text{m}$ as the wavelength increases (Figure 3. 2b), which is similar in both X and Y directions (Figure 3. 2c). Root mean square (RMS) radii of the spot diagrams are well within the Airy disk, all of which demonstrate good focus quality. While it was not possible to obtain theoretical parameters for the sensitivity roll-off for a commonly used refractive optics-based spectrometer equipped with a commercial camera lens, as the optical configuration of the commercial camera lens is not available.

Figure 3.2 Simulation results analysis of roll-off, PSF, RMS. (a) The sensitivity roll-off for the spectrometer designs with different focal lengths and optimal tilt angles. (b) The variation of the PSF FWHM (red, rectangle) and root mean square (RMS) radius (blue, triangle) with wavelength. (c) Spot diagram (first and third line), and the cross-sectional PSF profile (second and fourth line) for wavelengths of $\lambda_1 \sim \lambda_9$ (760-920 nm, 20 nm space) obtained using Zemax software. Black rectangles in the top Figure demonstrates the pixel pitch ($14 \mu\text{m} \times 28 \mu\text{m}$). Red and blue lines show cross-section in the X and Y directions, respectively.

3.4 Experimental verification

We constructed a high-resolution SD-OCT system and compared the performance of the proposed spectrometer with that of a typical refractive optics-based spectrometer (Figure 3. 3). A superluminescent diode (SLD) source with the output power of 8.8 mW was directed into a broadband 50:50 fiber coupler (TW850R5A2, Thorlabs, Newton, New Jersey, USA) which split the input light to the reference arm and the sample arm. Light backscattered from the sample interfered with light back-reflected from the

reference arm, with half of the interference signal detected by the spectrometer. The refractive components in the reference and sample arms were identical. The reference arm included a polarization controller (PC), an achromatic collimation lens (L1) (AC080-010-B-ML, Thorlabs, Newton, New Jersey, USA), a focusing lens (L2) (AC127-020-B-ML, Thorlabs, Newton, New Jersey, USA), and a mirror, while in the sample arm, a pair of galvo scanners (GVSM001/M, Thorlabs, Newton, New Jersey, USA) were used to scan the beam.

Figure 3.3 Experimental configuration for SD-OCT imaging systems equipped with spectrometers based on typical refractive optics and reflective optics. PC: polarizer controller; L1-L4: achromatic lens; RM: reference mirror; AO: analog output; DAQ: data acquisition card; RC: reflective collimator; CL: camera link cable. IMAQ: image acquisition board.

The refractive optics-based spectrometer (Figure 3. 3) consisted of an achromatic lens (AC127-030-B-ML, Thorlabs, Newton, New Jersey, USA), a transmission diffraction grating (1200 l/mm@840 nm, Wasatch Photonics, USA), a camera lens (85 mm, f/1.8D, Nikon, USA), and the same line-scan detector. For reflective-optics based spectrometer, the diffraction grating was 960 lines/mm (Wasatch Photonics Inc.). The diffraction efficiency of the gratings of both spectrometers was similar. The interference signal was digitized with 12-bit resolution and then transferred to a computer via camera link cables and an image acquisition board (KBN-PCE-CL4-F, Bitflow, Woburn, Massachusetts,

USA). The galvo scanners and the camera with maximum exposure time were synchronized by a triggering signal generated by the computer.

The performances of the reflective-optics based spectrometer were characterized by efficiency, axial ranging depth (6 dB roll-off), and axial resolution. The experimentally measured transmission efficiency for the refractive setup and reflective setup are about 65% and 82%, respectively. They were obtained by comparing the light power at the spectrometer entrance and the power after focusing optics output using monochromatic light of 780 nm (S1FC780, Thorlabs, Newton, New Jersey, USA). For the refractive setup, the ratio of -6dB roll-off length (~ 0.84 mm) to the maximal ranging depth (~ 1.7 mm) is about 0.49. While for the reflective setup, the ratio is about 0.47 (~ 0.94 mm/2 mm). Therefore, the spectrometer efficiency for refractive setup and reflective setup are about 0.3 and 0.37, respectively. Therefore, the efficiency of the reflective spectrometer is approximately 1.23 times that of a refractive optics-based spectrometer for an SD-OCT system.

Figure 3. 5 shows that the -6 dB sensitivity roll-off ranges were ~ 842 μm and ~ 943 μm for the refractive and reflective optics-based spectrometers, respectively. The improvement of the imaging depth for the reflective setup should attribute to the lesser grating spectral resolution, 0.067 nm, which is about 0.02 nm smaller than that of the refraction setup. One of the reasons for the difference between the calculated (~ 1.6 mm) and measured (~ 943 μm) roll-off values is nonlinearity in k -space. The spectral bands integrated by the pixel of the detector is unequal, which would increase the roll-off loss [9, 65]. In addition, the used imperfect digital remapping process based on [57] led to the degradation of the effective 6-dB roll-off range to some extent.

The axial resolutions of the refractive and reflective optics-based spectrometers were respectively measured to be approximately 2.70 μm and 2.64 μm at an optical path difference (OPD) of ~ 50.8 μm . The slightly improved axial resolution may be due to the elimination of chromatic aberrations. Therefore, the system performance shows improved efficiency when using the reflective optics-based spectrometer, with comparable ranging depth and axial resolution.

Figure 3.4 Experimental results of spectral intensity. (a) Intensity (arb. units) versus pixel index (arb. units) and (b) intensity (arb. units) versus wavelength with an input power of 2.9 μW. Reflective optics (red) and refractive optics (blue)-based spectrometer systems (Figure 3. 4).

Figure 3.5 Experimental results of depth profile of one imaged prism. (a) Refractive optics, and (b) reflective optics-based spectrometer for an SD-OCT imaging system.

3.5 Discussions

Figure 3.6 Refractive-optics-based, reflective-optics-based, commercial spectrometer sensitivity roll-off comparison.

	Total cost (USD)	Optical Transmission	Chromatic aberration	6 dB sensitivity roll-off (μm)	Ranging depth (mm)	note
Commercial spectrometer (Wasatch Photonics.)	7150	70%	Data not available	1000	2	Sale price
Refractive-optics-based Spectrometer	1037	65%	YES	856	2	Bill of materials
Reflective-optics-based Spectrometer	876	82%	NO	943	2	Bill of materials
Better performance than Refractive	16%, lower	26%, improved		87 μm , better		
Better performance than Commercial		17%, improved		97 μm , smaller		

Table 3. 2 Commercial, refractive-optics-based, reflective-optics-based spectrometer performance comparison.

Compared to a typical used refractive-optics-based spectrometer, the cost and the optical transmission of the proposed spectrometer is reduced by 16% and improved by 26%, respectively. Compared to a commercial spectrometer (Wasatch Photonics.), the optical transmission of the proposed spectrometer is improved by 17%.

Figure 3. 6 and Table 3. 2 respectively demonstrate the comparison of sensitivity roll-off and primary performance parameters for the commercial spectrometer (Wasatch Photonics), all-refractive-optics-based spectrometer, and the proposed all-reflective-optics-based spectrometer. Compared to a commercial spectrometer, the cost and the optical transmission of the proposed spectrometer is reduced by 88% and improved by 17%, respectively, while containing a comparable ranging depth. Compared to a typical used refractive-optics-based spectrometer, the cost and the optical transmission of the proposed spectrometer is reduced by 16% and improved by 26%, respectively. In addition, the optical transmission of the proposed spectrometer is improved by 17% compared to a commercial spectrometer (Wasatch Photonics.). Besides, this study provides a simple design that enables researchers to customize their own spectrometer by themselves.

Although the 6 dB roll-off of the proposed spectrometer is 97 μm smaller than that of the commercial spectrometer, the much lower cost makes it a good choice for practical applications. To give more details, in the proposed reflective spectrometer, we used a collimator module (RC12APC-P01, Thorlabs, Newton, New Jersey, USA)) that contained a 90° off-axis parabolic mirror (MPD129-P01, Thorlabs, Newton, New Jersey, USA). The retail price of the mirror is \$197.88 which is much lower than the collimator module. Therefore, it is possible to save cost to include the collimator lens mount in the spectrometer design. In terms of the cost of optics, the total cost of the reflective spectrometer is \$876 (collimator \$198, grating \$495, mirror \$83, and concave mirror \$100), and the total cost of the refractive spectrometer optics is \$1037 (collimator \$58, grating \$495, and camera lens \$484)).

Although a comparable axial resolution was obtained in the current experiment, many clinical applications require cellular or sub-cellular resolution. Therefore, a broadband light source should be taken into consideration. For an SD-OCT system with a Gaussian-shaped light source with 3dB wavelength range of 760-930 nm while the full range of 720-980 nm, cellular level imaging can be achieved successfully by using the proposed concave mirror-based spectrometer with a focal length of 100 mm.

Chapter 4

Cellular resolution corneal imaging with extended imaging range

Current optical coherence tomography (OCT) technology for imaging the anterior segment of the eye has been established as a clinical gold standard for the diagnosis of corneal diseases. However, the cellular resolution level information that is critical for many clinical applications is still not available. The major technical challenges toward cellular resolution OCT imaging are the limited ranging depth and depth of focus (DOF). In this work, we present a novel ultrahigh-resolution OCT system that achieves an isotropic spatial resolution of $<2\ \mu\text{m}$ in tissue. The proposed system could approximately double the ranging depth and extend the DOF using the dual-spectrometer design and the forward-model based digital refocusing method, respectively. We demonstrate that the novel system is capable of visualizing the full thickness of the pig cornea over the ranging depth of 3.5 mm and the border of the corneal endothelial cells 8 times Rayleigh range away from the focal plane. This technology has the potential to realize cellular resolution corneal imaging *in vivo*.

4.1 Introduction

Corneal disease is the second most common cause of blindness or visual impairment worldwide, as corneal homeostasis can be perturbed by lots of pathological conditions [67, 68]. Corneal transplantation remains the major and most effective method for visual restoration once corneal clarity is affected [69]. It has been found that, in developed countries, up to 50% of all corneal transplantations are used to treat endothelial dystrophy [70]. Nowadays, surgical techniques have enabled surgeons to perform selective replacement of layer during transplantation to treat endothelial decompensation, which helps to improve corneal graft survival and surgical outcomes [69]. Particularly, selective replacement of the endothelial layer, Descemet's membrane (DM) layer, and stroma layer may help to recover visual rapidly and decrease the risk of complications, compared to replacing the entire cornea. Therefore, accurate delineation of the fine posterior layers in real-time and *in situ* carries important implications in pre-, intra- and post- operative assessment of patients requiring corneal transplantation. Also, accurate depiction of stroma, DM, and endothelial layers would also assist with deep anterior lamellar keratoplasty (DALK) that is currently hindered by the technical difficulty in separating the stroma from the DM [69, 71]. In addition, accurate evaluation of the quality of endothelial cells would be of great significance in pre- and post- operative clinical treatment of patients undergoing surgery, which

determines corneal graft survival in corneal transplantation [72]. Thus, efficient visualization of cellular and extracellular components *in situ* would provide valuable information for the assessment of eye corneas. Furthermore, a large scanning area of the cornea is necessary because it can potentially provide an improvement in the precise location of corneal lesions and the planning of medical and surgical treatment [73].

Current imaging techniques such as confocal microscopy [74, 75] and high-frequency ultrasound have limitations, such as a narrow field of view or limited resolution, respectively [76]. Optical coherence tomography (OCT) [77, 78] and optical coherence microscopy (OCM) [79] have been proposed to provide high-resolution cross-sectional and *en face* imaging of the cornea, respectively. Current anterior segment OCT (AS-OCT) systems obtain a cross-section of the cornea with 5–20 μm axial resolution, which is insufficient to delineate the DM and endothelial layers [80, 81]. Ultrahigh-resolution OCT (UHR-OCT) has enabled a significant axial resolution improvement to $\sim 3\text{--}5\ \mu\text{m}$ [51, 82, 83], which demonstrated the ability of OCT to measure the thickness of precorneal tear film [84], visualize anterior, stromal, posterior corneal layers [85], and assess of keratocyte cells density [86]. Recently, Bizheva et al. developed a UHR-OCT with axial resolutions of $1\ \mu\text{m}$ to delineate all three layers of the posterior cornea successfully [87]. However, the lateral resolution of these systems is $5\text{--}21\ \mu\text{m}$, which is not sufficient to resolve endothelial cells. Most recently, a UHR-OCT with $0.95 \times 1.8\ \mu\text{m}$ (axial $\times$ lateral) ($n=1.38$) resolution was reported using similar approaches published in [87], but a different microscope objective lens with a higher power of magnification [88]. However, the depth of focus (DOF) caused by high lateral resolution makes it difficult to keep the area of interest within the DOF owing to the axial motion of the eyes. Also, the relatively small scanning area of such UHR-OCT systems hinders their application in clinical practice for large area anterior eye imaging. Therefore, the requirements of large scanning imaging area, high axial resolution and high lateral resolution with extended DOF are necessary to provide a possibility to improve diagnostics and visualization of eye corneas.

To achieve a high lateral resolution over an extended DOF, various approaches have been proposed, including Bessel beam illumination [89, 90], adaptive optics (AO) controlled third-order spherical aberration [15], and multiple aperture synthesis [91, 92].

However, the Bessel beam illumination is at the expense of signal loss and sidelobe artifacts; the AO method is associated with the high cost and system complexity; multiple aperture synthesis is time-consuming. In addition, all the methods above require modifications of the optical system with non-standard optical components, which makes the system complex and costly. These drawbacks can be avoided by using numerical focusing methods, including interferometric synthetic aperture microscopy (ISAM) [93], digital adaptive optics (DAO) [94], and forward model (FM) [40, 95, 96]. All these numerical methods are suitable for cellular resolution anterior segment imaging. FM, IS, and DAO can improve the depth of focus (DOF) by a factor of 7x, 7x, and 11x respectively, as was shown in Figure 6 and Figure 7 in the reference paper [40]. Besides, the standard method of C-mode imaging, i.e., zone focus OCT imaging, can enhance the DOF by acquiring multiple images with a different focal position of the objective lens and then fuse these images to create a single cross-sectional image as was shown in Figure 12 in paper [97]. However, this technique requires rapid scanning of a lens and setting the focal depth coincident with the interferometer zero delay. Therefore, this technique is time-consuming which makes it difficult for clinical applications. In this paper, we chose FM due to its simplicity and lower computational cost. One of the other problems is the limited ranging depth with regard to the axial range of the cornea. As for a spectral-domain (SD) OCT system, the maximum depth range is directly linked to spectral sampling density or spectral resolution [32]. Given that the spot size on the detector is not a limiting factor, therefore, increasing the number of pixels of the detector can raise the ranging depth [73, 98].

In this thesis, we present a novel SD-OCT imaging system, which achieves cellular-level spatial (axial and lateral) resolutions over an extended ranging depth and depth of focus. We used a previously reported dual-spectrometer design to extend the ranging depth so that it could cover the full thickness of the cornea of large animals. In order to address the issue of limited depth of focus, we employed the forward-model based digital refocusing method [40, 95] to maintain a high lateral resolution over an extended depth of focus. The endothelial cell borders, even at the out-of-focus regions, can be visualized thanks to this method.

4.2 Experimental setup

The output of the super-luminescent diode (SLD) light source with a bandwidth of 760-930 nm (3-dB spectral range) is split by a broadband 50:50 fiber coupler (TW850R5A2, Thorlabs, Newton, New Jersey, USA) into the reference light and the sample light. The mode field diameter (MFD) of the fiber is $5.0 \pm 0.5 \mu\text{m}$ (defined by the $1/e^2$ field) at 850 nm. The sample arm consists of an achromatic collimation lens (L3) (AC050-015-B, Thorlabs, Newton, New Jersey, USA), a galvo scanner (GVS002, Thorlabs, Newton,

Figure 4.1 Schematic of the dual-spectrometer high-resolution SD-OCT imaging system. PC: polarizer controller; L1-L4: achromatic lens; RM: reference mirror; AO: analog output; DAQ: data acquisition card; CL: camera link cable; IMAQ: image acquisition board.

NJ, USA), an objective lens (L4) (M Plan Apo NIR 20 $\times$, NA=0.45, Mitutoyo, Takatsuku, Kawasaki, JP). The reference arm uses identical transmission optics to balance dispersion. In the detection arm, the interference light is first collimated by an achromatic lens (AC127-030-B, Thorlabs, Newton, New Jersey, USA), before it is split into two beams by spectral range (745-870 nm and 835-960 nm) using a dichroic filter (850nm, Edmund Optics, Barrington, Illinois, USA). Each of the two beams are detected by a spectrometer comprised of a grating with 1,765 lines/mm (PING-Sample-020,

Ibsen Photonics, Farum, Denmark), a camera lens (85 mm, f/1.8D, Nikon, Melville, New York, USA), and a linear CCD detector (AViiVA EM4, e2V, Chelmsford, UK). The setup of the system is shown in Figure 4. 1. The total number of pixels covered by the two spectra are 1837 and 1624, respectively. There is a ~35 nm overlap between the spectral ranges of two beams, which allowed us to combine their spectral interference signals coherently (Figure 4. 2b). We used an image acquisition board (KBN-PCE-CL4-F, Bitflow, Woburn, MA, USA) to acquire spectral interference signals from each spectrometer at 12-bit resolution. For comparison purposes, we also constructed another high-resolution SD-OCT imaging system with identical configurations except it has a single spectrometer with a transmission grating (1200l/mm@840 nm, Wasatch Photonics Inc, Logan, Utah, USA).

4.3 Coherent spectral combination and ranging depth

The spectral combination method was developed based on a method previously published by our group [99]. The previous method requires a manual search for the overlapping region in the spectral interferograms, which in our method has been improved to be fully automatic.

Firstly, we obtained 10 sets of interference fringes from both spectrometers at evenly spaced optical path differences (OPD) from 25.4 to 254 μm and subtracted the reference spectrum from them. For simplicity, the fringes obtained from the spectrometer 1 and spectrometer 2 were named *fringe1* and *fringe2*, respectively (Figure 4. 2b).

Secondly, we used the interference fringes at the OPD of 177.8 μm for spectral combination because it contains a large number (153) of zero-crossing points while it does not significantly suffer from discrete sampling errors. We obtained the pixel indices ($k_1(i)$ and $k_2(j)$) corresponding to the indices of the zero-crossing points (i and j) in the *fringe1* and *fringe2* using linear interpolation. As there might be differences in spectral linewidth and offset for each pixel between *fringe1* and *fringe2*, the relation between them was modelled using the following quadratic interpolation function:

$$k_2(z, j) = a \times k_1^2(z, i) + b \times k_1(z, i) + c. \quad (4.1)$$

Here i and j are range from 1 to N and 1 to P , respectively. N and P correspond to the total numbers of the zero-crossing points in *fringe1* and *fringe2*, respectively ($P < N$ in this work). z corresponds to the OPD. Both k_1 and k_2 are ranged from 1 to 2048.

Thirdly, in order to search for the overlapping region in both fringes, we conducted an exhaustive search for the best match between zero-crossing points of each fringe. Specifically, we considered $N-1$ candidate regions in each of two fringes: In *fringe1*, the m -th ($m = 1, 2, \dots, N-1$) candidate region started from the pixel number of the m -th zero crossing point (α) and ended at the last zero-crossing point. The corresponding m -th candidate region in *fringe2* started from the pixel number of the first zero-crossing point and ended at the m -th zero crossing point.

Fourthly, we fitted the pixel indices k_2 in the m -th candidate region in *fringe2* with those (k_1) in the corresponding m -th candidate region in *fringe1* using Eq. (1), in order to obtain the value of a , b , and c .

Fifthly, we fitted *fringe2* using *fringe1* based on the obtained quadratic interpolation function (Equation (1)), and the result of fitting is *fringe2'*. Then, we combined the *fringe1* and *fringe2'* for all 10 sets of the fringes and calculated the sum of the magnitude of the combined fringe, i.e., $S = \text{sum}(\text{abs}(\text{fringe1} + \text{fringe2}'))$.

Lastly, since the correct combination could result in maximal constructive interference over the entire overlapping region for the fringes acquired at all OPDs, the sum of the magnitude of combined fringe should decrease monotonically as OPD increases. Meanwhile, those of the rest combination could fluctuate as OPD increases. Based on this criteria, we found three starting pixel number (α) 1078, 1091 and 1104 of the overlapping region in *fringe1* (Figure 4. 2a). It turned out that at $\alpha = 1091$, the two fringes was combined constructively while in the other two cases there were destructive interferences.

Therefore, the combined interference fringe covered 3161 pixels (Figure 4. 2b), which is about 1535 pixels more than the single spectrometer system. Theoretically, this could improve sensitivity by 1.4 dB. After dispersion calibration using a previously reported method [43, 57], we obtained the k-space linearized spectrum (Figure 4. 2c). This spectrometer provided a maximum ranging depth of about 3.5 mm and a 6-dB roll-off

range of about 1.44 mm (Figure 4. 2d), which is respectively, 94% and 78% larger than the single spectrometer system.

The full-width-at-half-maximum (FWHM) axial resolution that resulted from the combined spectrum at OPD of 177.8 μm was tested to be 2.56 μm in air, which corresponds to 1.86 μm in tissue ($n = 1.375$ [100]) (Figure 4. 3a). The axial resolution is 0.11 μm better than that of the single spectrometer of 2.67 μm obtained with the same OPD, which could be caused by the change of spectral shape. The axial resolution degraded over the depth to 3.54 μm at a depth of 1.44 mm (Figure 4. 3b). With a sample power of $\sim 850 \mu\text{W}$ and the OPD of 177.8 μm at the working A-line rate of 20 kHz, the measured sensitivity of the dual spectrometer SD-OCT system was 100 dB, about 2.4 dB higher than that of the single spectrometer system. The actual improvement was higher than the expected (1.4 dB), which was possible because the noise was suppressed by the coherent combination relative to the signal in the 35 nm overlap region [42, 99].

Figure 4.2 Coherent spectral combination. (a) The sum of the magnitude of the combined spectra as a function of the pixel number (α) of the first overlapping zero-crossing point in fringe1. Inset: the sum of magnitude is monotonically decreased as OPD increases when $\alpha = 1091$. (b) The best matched and combined interference spectra, $z = 177.8 \mu\text{m}$. (c) Combined interference spectrum after dispersion calibration of (b);

red curves: envelopes of spectrometer output. (d) Measured axial point-spread functions (PSFs) on a linear scale for different delays relative to the reference arm length.

Figure 4.3 Measured axial resolution profile and variation with depth. (a) Measured axial point-spread function (PSF) of the dual spectrometer system and the typical used single spectrometer system. (b) Axial resolution on a linear scale for different delays relative to the reference arm length.

4.4 Focal depth extension

According to the fiber mode field diameter (MFD) of $5.0 \pm 0.5 \mu\text{m}$, the theoretical transverse resolution (FWHM) in focus was estimated to be $1.96 \mu\text{m}$ and the corresponding DOF was $28 \mu\text{m}$. Obviously, it is difficult to keep the area of interest within the DOF *in vivo*. To solve this limitation, we employed the digital refocusing method of the forward model (FM) [40, 95, 96] as was discussed in chapter 2.4.2 to restore the lateral resolution in out-of-focus regions digitally. The effective interference signal from the reference and sample arm collected by the camera is given as follows (same with equation 2.35):

$$E(x, y, k) = E_s(x, y, k)E_R^*(k) = \frac{\kappa S(k)}{M} \iint \int FT^{-1} \left\{ \exp[ik(2z_R + zn_0)] \tilde{E}_{o,z}(f_\xi, f_\eta, k) \times \exp[i2\pi z \sqrt{(kn_0/2\pi)^2 - f_\xi^2 - f_\eta^2}] \right\} P\left(\frac{x}{M} - \xi, \frac{y}{M} - \eta\right) d\xi d\eta dz \quad (4.1)$$

Taking the 2D spatial Fourier transform of the signal and employing the convolution theorem, then the equation can be given as follows (same with equation 2.35):

$$\tilde{E}(k_x, k_y, k) = \kappa MS(k) \tilde{P}(Mk_x, Mk_y) \int \exp[ik(2z_R + zn_0)] \tilde{E}_{o,z}(Mk_x, Mk_y, k) \times \exp[iz\sqrt{(kn_0)^2 - (Mk_x)^2 - (Mk_y)^2}] dz \quad (4.2)$$

Taking 1D FT along the axial dimension, then the equation is as follows (same with equation 2.40 and 2.42):

$$E(k_x, k_y, z') = \kappa M \tilde{P}(Mk_x, Mk_y) [\tilde{S}(z') \otimes \int \tilde{E}'_{o,z}(Mk_x, Mk_y, z) \delta(z - z') dz] \quad (4.3)$$

$$\tilde{E}'_{o,z}(Mk_x, Mk_y, z) = \tilde{E}_{o,z}(Mk_x, Mk_y, z) \times \exp[-i \frac{\lambda_0 z M^2}{4\pi n_0} (k_x^2 + k_y^2)] \quad (4.4)$$

For removing the defocus error, the aberrated system was corrected by multiplication with a phase-only filter, γ , in the 2-D spatial frequency domain [40, 95, 96]. As it is difficult to maintain phase stability sufficient for three-dimensional aberration correction, in this work, the correction factor only was multiplied in each B-scans. Consequently, the correction factor used in this paper was expressed as follows:

$$\gamma(Mk_x, z) = \exp(+i \frac{\lambda_0 z M^2}{4\pi n_0} \times k_x^2) \quad (4.5)$$

$$z = \frac{N_z \lambda_0^2}{2n_0 \Delta\lambda} \quad (4.6)$$

where z is the distance of a given layer in the image volume from the focus position, N_z is the axial distance in terms of axial pixels, λ_0 is the center wavelength, $\Delta\lambda$ is the bandwidth of the light source, n_0 is the average background refractive index of the sample and was set to be 1.4 in this work, M is a magnification factor, $k_x = 2\pi f_x$, f_x is the coordinate in the spatial Fourier domain. As a total of 1024 lines per frame are acquired in our image acquisition process, the coordinate of the 512th A-line was set to be the origin.

4.5 Corneal imaging

Figure 4.4 High-resolution SD-OCT images of pig cornea *ex vivo*. (a) A representative cross-sectional image of pig cornea in full-thickness. Inset: zoomed-in view of the rectangular area, yellow arrows indicate endothelial cells (Ed). (b) Corresponding H&E stained histology. (c) The *en face* view of the apical side of the endothelial cells presented regularly arranged polygonal cells *ex vivo*. EPT: epithelium; S: stroma; DM: Descemet's membrane; Ed: Endothelial layer. Scale bars: 25 μm .

Figure 4.5 Numerical defocus correction at endothelial layer *ex vivo*. (a) A representative cross-sectional image of the pig cornea; the distance between the endothelial (Ed) layer and the focal point was about 80 μm ; yellow arrow indicates the Ed cells layer. (b) Un-corrected *en face* view of one Ed layer. (d) The zoomed view of the rectangular area of (b). (c) and (e) Forward model (FM) correction results of Ed cells. (f) and (g) Cross-sectional imaging of Ed layer of the original (Org) and after FM correction, respectively. Scale bar: 25 μm .

Figure 4.6 Numerical defocus correction at endothelial layer *ex vivo*. (a) A representative cross-sectional image of the pig cornea; the distance between the endothelial (Ed) layer and the focal point was about $123\ \mu\text{m}$; yellow arrow indicates the Ed cells layer. (b) Un-corrected *en face* view of one Ed layer. (d) The zoomed view of the rectangular area of (b). (c) and (e) Forward model (FM) correction results of Ed cells. (f) and (g) Cross-sectional imaging of Ed layer of the original (Org) and after FM correction, respectively. Scale bar: $25\ \mu\text{m}$.

The extended ranging depth enabled imaging the pig cornea with full-thickness *ex vivo* (Figure 4. 4a), which was not possible with the single spectrometer system due to insufficient ranging depth. With the corneal endothelium in focus, we could clearly delineate the posterior layers as previously published by our group [101], including the

endothelium (yellow arrow) and the Descemet's membrane (DM, red arrows). The *en face* view of the endothelial layer from a 3D reconstruction of SD-OCT data was shown in Figure 4. 4c, in which endothelial cell borders as well as their hexagonal shape were clearly delineated.

When the endothelium was $\sim 80\ \mu\text{m}$ away from the focal plane (Figure 4. 5a), the lateral resolution degraded so that the cell borders and the hexagonal shape were not distinguishable in the *en face* view (Figure 4. 5b&d). These critical cellular features were restored in the FM corrected images (Figure 4. 5c&e). In addition, in the cross-sectional images, FM also restored the continuities of the apical endothelial surface (Figure 4. 5f&g). All these improvements in cellular details and imaging quality demonstrate the DOF was extended effectively. When the endothelium layer was $\sim 123\ \mu\text{m}$ or more than 8 times Rayleigh range away from the focal plane (Figure 4. 6a), the cellular structures in the *en face* view of the endothelium were totally lost (Figure 4. 6b&d). We were still able to use FM to partially recover cell borders and the shape information (Figure 4. 6c&e), and improve the continuities of the apical surface of the endothelium in the cross-sectional view (Figure 4. 6f&g). We found that the magnification factor in the FM model $M = 610\text{-}640$ provided the best visibility of the cell border, which was determined by exhaustive search in the range from 1 to 1000.

Due to the curvature of the cornea, the extended ranging depth is also required for large area scanning. For example, we can achieve a lateral field of view (FOV) $7.6\ \text{mm} \times 7.6\ \text{mm}$ by replacing the objective lens with $f = 50\ \text{mm}$ (AC254-050-B-ML, Thorlabs Inc., Newton, New Jersey, USA). Over this FOV, the whole corneal depth range of $2.2\ \text{mm}$ ($n=1.375$) was captured without any mirror effect and was shown in Figure 4. 7. Although the lateral resolution was not high enough to resolve cellular structures, we could still clearly delineate the endothelium in a significant part of the displayed FOV. In addition, with the same objective lens of $f = 50\ \text{mm}$, the termination of the endothelium and Descemet's membrane (Schwalbe's line, SL) as well as angle recess (AR) area could be visualized clearly thanks to the high sensitivity and resolution of the proposed dual-spectrometer system (Figure 4. 8). The application of the dual-spectrometer high-resolution SD-OCT system to the cornea imaging can potentially provide substantial information over a large area of the cornea. This information may

be especially useful for disease diagnosis, precise localization of lesions, and the planning of medical and surgical treatments [102].

Figure 4.7 High-resolution SD-OCT cross-sectional image of pig cornea *ex vivo* over 7.6×2.2 mm ($n=1,375$). Inset is the zoomed-in view of the rectangular area; Arrows indicate endothelial cells layers. Scale bar: 50 μ m.

Figure 4.8 High-resolution SD-OCT cross-sectional image of anterior chamber angle of the pig eye *ex vivo*. Ed: endothelial layer; SL: Schwalbe's line; AR: angle recess. Scale bar: 200 μ m.

4.6 Discussion and conclusion

Imaging the cellular structures of the posterior cornea, particularly the endothelial cells, is critical for the evaluation of the corneal grafts during corneal transplantation and diagnosis of corneal diseases. Although the current anterior segment OCT has been established as a clinical gold standard, it is not capable of providing cellular information. In this study, we developed an SD-OCT imaging system and image processing methods that address the two main technical hurdles towards cellular resolution OCT corneal imaging *in situ* and *in vivo*. Specifically, we provided solutions to the limited DOF and ranging depth and validated the feasibility of our approach in intact pig eyes *ex vivo*. Further development of an imaging system suitable for *in vivo* use will significantly extend the capability of current OCT and benefit a number of clinical applications.

In addition to the corneal imaging, the proposed dual-spectrometer system has provided the possibility for imaging the angle recess area owing to the high sensitivity and resolution. The identification of Schwalbe's line (SL) may help clinicians to better assess the anterior chamber angle and then manage narrow-angle glaucoma [103]. Although the current study has approximately doubled the ranging depth to 3.5 mm, which covers the full thickness of the pig cornea over a lateral field of view 7.6 mm. However, larger ranging depth of at least 8 mm is needed to cover the whole anterior segment, especially the anterior chamber angle [104, 105]. To do so, we could use a linear camera with larger pixel number to extend the imaging range. However, currently, there is no commercially available larger format camera with equivalent full well capacity and noise performance so that the sensitivity of the imaging system might be compromised by doing so. Additionally, we could use a linear- k spectrometer design, which can improve the sensitivity roll-off by more than twice [65, 106]. Future research will focus on developing a linear- k dual-spectrometer system using 4096-pixels cameras. This spectrometer could improve the ranging depth up to 8.8 mm by extended spectral range covering 8000 pixels while keeping acceptable system sensitivity roll-off. In addition, though the ranging depth has been extended by approximately an order of magnitude, the improved DOF using the forward model method may still not be sufficient enough for *in vivo* use. The forward model is based on simple geometric optics modeling to determine the defocus phase [40, 95]. Therefore, it cannot compensate for

any aberration other than defocus, for example, chromatic dispersion, astigmatism, coma, and those caused by the turbidity of the tissue caused under pathological conditions. Aperture synthesis (AS) based method and digital adaptive optics method could complement the forward model in compensating these aberrations [91, 92, 94]. In addition, the AS method can extend the DOF by ~ 10 -fold, and it corrects optical aberrations in the optical path difference domain without using full-field data so that it can be combined with the FM-based method to extend the DOF further.

One of the potential limitations for our system for the future study of the cornea *in vivo* would be motion artifacts. The quality of the *en face* images of corneal endothelium would be significantly compromised by eye motion, and therefore, the basolateral cellular structure could not be clearly identified. Motion artifacts will break the phase stability condition under which the forward model is valid. The straightforward solution to the phase stability issue is to improve the imaging speed. A promising solution is to adopt the line-field SD-OCT design, which enables more than 3,500 B-scans per second [107, 108]. In addition, the motion issue may also be mitigated by alleviating the resolution requirement dependent on clinical needs. For the purpose of evaluating the endothelial loss under the pathological conditions or after corneal transplantations, it may not be necessary to visualize individual endothelial cells so that it is possible to scan the whole cornea with a shorter time. For the purpose of evaluating endothelial cell density, cell hexagonality, and coefficient of variation (COV), a high lateral resolution is required. In this case, the motion artifacts have to be dealt with by improving the imaging speed. Another potential limitation is the insufficient image acquisition speed A-line rate of 70 kHz. Therefore, improving the image acquisition speed by using a camera with higher acquisition speed will mitigate the problem.

In conclusion, we developed a novel SD-OCT imaging system that addresses two standing problems in cellular resolution imaging of the cornea. We used a dual spectrometer design and the forward-model based digital refocusing method to significantly extend the ranging depth and depth of focus, respectively. The results of this study demonstrated that cellular resolution imaging of the cornea *in vivo* is potentially feasible.

Chapter 5

Improving transverse resolution of multiple aperture synthesis optical coherence tomography using digital phase pupil filter

High-resolution optical coherence tomography (OCT) imaging provides valuable information for disease diagnosis. However, such an approach is usually hampered by the trade-off between the resolution and the focal range. We report a modified aperture synthesis method, which can significantly improve the transverse resolution through the whole transverse field-of-view concerning the standard OCT. The method is based on multiple aperture synthesis OCT optics. However, instead of maximizing the intensity, we search for digital pupil filters, using a guide star, that provide the best resolution. With these filters, we digitally obtain multiple images from a single B-frame, which are averaged to obtain an improved image. We experimentally observed a 24% improvement in the main lobe width and the side lobe problem was suppressed by 1.1dB through averaging results from multiple distinct filters.

5.1 Introduction

Optical coherence tomography (OCT) is a powerful imaging technology with the capability to perform non-contact, noninvasive, high-speed, and high-resolution cross-sectional imaging in biological tissue [1, 44, 109]. The ability of OCT to image microstructures is limited by its spatial resolutions. The axial resolution may be improved by the use of a light source of broad bandwidth [32, 110], but improving the transverse resolution, which is proportional to the numerical aperture (NA), is challenging. One may use high NA optics to achieve cellular resolution in the transverse directions [101, 111], but with a significant reduction of the depth of field (DOF). Therefore, a trade-off should be considered for a high-resolution OCT system. Optical aberrations and diffraction effects degrade resolution. OCT imaging can be implemented at diffraction limit through aberration correction in coordination with hardware adaptive optics (HAO) [10] or computer-adaptive optics (CAO) [12, 13], but system complexity and a high phase stability requirement limit its applications. Multiple aperture synthesis (MAS) is simple and does not require phase stability [91, 92], but the best transverse resolution achieved so far is no better than the diffraction-limited resolution of a single sub-aperture. In fact, the transverse resolution of the current MAS technique is lower than that of the standard OCT with the same objective pupil diameter making it challenging to implement MAS in space-limited or aperture-limited scenarios, such as endoscopy.

In this report, we propose a novel method, termed as modified aperture synthesis, to improve the transverse resolution of the OCT system. In this method, we split the objective lens pupil into multiple physical sub-apertures in a way similar to MAS-OCT reported previously by our group ([112], Chapter 5). The aperture synthesis is carried out by modulating interferograms acquired through all the sub-apertures with a phase mask before summation. Through exhaustive search, we can obtain digital pupil filters that produce a transverse resolution 24% higher than that of the standard OCT. Since there exist multiple such phase filters, by averaging the synthesized images obtained using those filters, we can suppress the side lobe by 1.1 dB.

5.2 Imaging system and method

In this work, we used MAS optics and a dual-spectrometer [113] for the OCT system design (Figure 5. 1a). This system assumes the basic set-up using in SD-OCT as shown in Figure 4. 1 (Chapter 4), with broadband light delivered to the input port of a Michelson interferometer and the spectral interference signals detected using the dual-spectrometer. The supercontinuum light source (SuperK Extreme OCT, NKT Photonics A/C, Birkerød, Denmark) has a bandwidth of 180 nm centered at 800 nm and is split by a broadband 50:50 fiber coupler (TW850R5A2, Thorlabs, Newton, New Jersey, USA) into the reference light and the sample light. The light in the sample arm was guided by a galvo scanner (GVS002, Thorlabs, Newton, NJ, USA) to an objective lens L4 (M Plan Apo NIR 10 $\times$, NA=0.26, Mitutoyo, Takatsu-ku, Kawasaki, JP). The objective lens used has an effective focal length of 20 mm, resulting in a transverse resolution of 3.92 μm (diffraction-limited spot size) and a corresponding depth of focus (DOF) of 114 μm . The reference arm uses identical transmission optics to balance dispersion. The light backscattered from both arms was coupled to the dual-spectrometers [113].

In the sample arm, two custom-designed calcite beam displacers (CBDs) (Table 5. 1), together with a quarter-wave plate (QWP) (AQWP05M-980, Thorlabs, Newton, NJ, USA) served as the amplitude beam divider and the optical path encoder (Figure 5. 1a-d). In the CBD shown in Figure 5. 1b, the separation angle between the o- and e-beams is $\Phi = 5.8^\circ$ according to the design parameters given in Table 5. 1. The corresponding beam displacement Δx is given by

$$\Delta x = d \cdot \tan \Phi \quad (5.1)$$

where $\Phi = \theta - \varphi$, $\tan \theta / \tan \varphi = n_o^2 / n_e^2$. $n_o = 1.65$ and $n_e = 1.49$ are the refractive index of CBD for the o - and e -beams according to the refractive-index sphere. The thickness of the CBD was $d = 5$ mm, therefore, the displacement between the centers of the sub-apertures Δx was adjusted as $0.27R$ (R is equal to 1.95 mm here and represents the radius of the illumination beam measured at 1% power level). When the polarization axis of the QWP was 45° relative to the linear polarization direction of the o - and e -beams (Figure 5. 1c), three equally displaced sub-apertures in the pupil plane of the objective lens L4 and five cross-correlation terms in the optical path difference (OPD) domain were obtained (Figure 5. 1d). The displacement between the centers of the sub-apertures Δx was adjusted as $0.27R$ ($R = 1.95$ mm was the radius of the illumination beam measured at 1% power level). The illumination beam on the CBDs was split into two parts including the ordinary beam (o -beam) and the extraordinary beam (e -beam) own to the anisotropic refractive index of the CBDs (Figure 5. 1b and c). The optical paths of the illumination light consisting of two ordinary-paths and two extraordinary-paths are termed the oo -path and ee -path, respectively. The path consisting of a combination of the ordinary-path and extraordinary-path is called the oe/eo -path (Figure 5. 1c). A linear polarizer LP (LPNIRE 100-B, Thorlabs, Newton, NJ, USA) was placed in the reference arm for polarization management.

In SD-OCT, recording of the OPD is achieved by interference between the sample light with the reference light, governed by the following simplified formulation:

$$I(k) = 2S(k) \int_{-\infty}^{\infty} \sqrt{I_r(k)I_s(k)} \cos(2kz) dz \quad (5. 1)$$

where $I(k)$ is the spectral interference signal detected by the spectrometer, $s(k)$ is the spectral distribution of the light source, $I_r(k)$ and $I_s(k)$ are the electric field reflectivity from the reference arm and the sample arm at a depth of z , respectively. In the sample arm, one can synthesize the aperture of the objective lens by dividing the illumination beam by its amplitude and displacing the beamlets evenly along the radial direction (Figure 5. 1). In the pupil plane, each beamlet creates a sub-aperture with a distinct center spatial frequency, which defines both an illumination light path and a detection

Figure 5.1 Schematic of the high-resolution MAS SD-OCT imaging system and theory of sample arm. (a) Schematic of the MAS SD-OCT imaging system. L1-L4: achromatic lens; LP: linear polarizer; DC: dispersion compensator; NDF: neutral density filter; RM: reference mirror; CBD: calcite beam displacer; QWP: quarter-wave plate; PC: polarizer controller; AO: analog output; DAQ: data acquisition card; CL: camera link cable; IMAQ: image acquisition board. (b) The theory of birefringent beam displacement – CBD. d : length of the CBD; Δx : separated distance of the o- and e-beams perpendicular to the propagation direction; OA: optical axis of the CBD (in plane of paper); Φ : separation angle of the e-beam in the CBD; θ : angle between the slow

axis and OA of the CBD; θ : angle between the fast axis and OA of the CBD; $E_{//}$ and $E_{\perp}$, transverse and vertical electric field, respectively. (c) Each of the displaced beams was circularly polarized on the transmission path to maximize the coupling back to the fiber pinhole. (d) The sub-aperture numbers and optical paths by using the two CBDs. The yellow beam is the combination of the oo- and ee-beams. (The figure 5. 1b-d are reproduced from [112]- Chapter 5).

Dimensions	8 mm $\times$ 8 mm
Thickness d	5 mm
Optical axis angle φ	48°
Anti-reflection (AR) coating	AR / AR @700-1000 nm
Wavefront distortion	$< 1/2 \lambda$
Coating surface 1	R < 0.5% @700-1000 nm
Coating surface 2	R < 0.5% @700-1000 nm
Surface quality	20-10 scratch-dig
Parallelism	7 arc seconds

Table 5. 1 Design parameters of the CBDs shown in Figure 5. 1a.

light path. The OPD of each separate round-trip path can be shifted by a fixed-length using an optical path encoder (Figure 5. 1) so that the corresponding interferometric signals do not overlap with each other in the OPD domain. In such a MAS-OCT system, there are $2n$ round-path trip signals, and 5 OPD numbers which is represented by m ($m=1, 2, \dots, 5$). The depth-encoded cross-correlation term is given by the expression:

$$I_m(k) = \sqrt{I_r(k)I_s(k)}[\exp(i2kz_m) + C.C.] \quad (5.2)$$

where $C.C.$ stands for complex conjugate. Fourier transform (F) of these cross-correlation terms gives the standard SD-OCT images obtained through individual paths

$$i_{SD-OCT,m}(z) = F[I_m(k)] \quad (5.3)$$

The resolution improvement in the OPD domain can be achieved by adding high-frequency phase terms $\exp(-i\varphi_m)$ in each cross-correlation term I_m . Assuming that the optimal resolution is obtained when the full width at half maximum (FWHM) of the microparticles reaches its minimum, the problem is equal to finding the best phase assembly, $\gamma_j^{coh} = \exp(-i\varphi_m)$, so that the FWHM of the microparticles reaches its minimum, where $i(z) = \sum_{m=1}^5 [I_m(z) \cdot \gamma_j^{coh}]$ is the coherent summation of the Fourier transform of the cross-correlation terms denoted by Equation 5. 2, $z \in [z', z'']$ is the local depth range with $1 \leq z' \leq z'' \leq N$ and N is the length of Fourier transformation. Then the resultant A-line signal multiplied with the random phase masks is given by

$$i_{MAS}(z) = \sum_{m=1}^5 [I_m(z) \cdot \gamma_j^{coh}] \quad (5.4)$$

To achieve the optimized phase masks, we generated a large number of phase masks with randomly generated phase values with a step size of 7 in the range of 0 to 2π . Then we selected those that render a super-resolving main lobe, according to the criteria in which the FWHM of the main lobe was almost narrowed to its minimum. Each phase mask includes five-phase components corresponding to the five sub-images (1, 2, ...5) in the OPD domain (Figure 5. 2). Considering one optimal phase mask with high-frequency signal compensation could be effective to improve the transverse resolution in the whole image, one arbitrary microparticle was chosen as the guide star. Then, the optimized phase masks assemblies, γ_j , were inverted to obtain the best estimation of the phase masks assembly of γ_j^{coh} . We chose the phase masks that produced the almost optimal main lobe width of the transverse PSF. In this work, several almost-optimal phase masks were achieved and the resulting images were averaged to suppress the side lobe. The steps are listed below:

- 1) Remove the optical path differences among images acquired through all 5 paths by depth decoding.
- 2) Calculate 1-D FFT from the received signal $I(x, k)$ to $I(x, z)$.
- 3) Generate a large number of phase masks with random phase values with a step size of 7 in the range 0 to 2π . Multiply random phase assembly, $\gamma_j^{coh} = \exp(-i\varphi_m)$ ($j=1, 2, \dots$), with the resulting five signals separately, then sum them

coherently. Transverse resolution of one B-scan image is evaluated from the FWHM (two digits after the decimal point) of one selected microparticle, where α is the minimum of the FWHM and β is the nearest integer greater than or equal to α . By running the algorithm with different phase assembly, the optimal phase assembly γ_h ($h=1, 2, \dots, q$; q is the total number of the obtained optimized phase masks) is picked out.

- 4) Average the selected images of n numbers to achieve the final B-scan image.
- 5) Apply the acquired optimized phase masks from one B-scan to the others, and repeat step (4).

Figure 5.2 Overview of the data processing to obtain the digital phase masks for a B-scan OCT image.

5.3 Results

To demonstrate the ability of the proposed method to improve transverse resolution at each depth, we first imaged polystyrene microparticles (LB8-2ML, Sigma-Aldrich, Diameter 0.8 μm) in water. Sub-images (1, 2, ...5) obtained from the depth-encoded

cross-correlation terms and the reconstructed images by the MAS method with the maximum intensity criteria (MAS MI) [112] and with the optimized FWHM criteria (O-FWHM) are shown in Figure 5. 3a over a depth range of 90 μm . The transverse resolution improvement can also be intuitively observed in the transverse profiles and zoomed images (Figure 5. 3b) of three microparticles (dotted square in Figure 5. 3a).

We investigated the FWHM of microparticles at depths from 15 μm to 105 μm , before and after digital processing. The comparison results shown in Figure 5. 3c indicate that the FWHM improved obviously after digital processing. We only discuss the first sub-image in the following content as it has the best signal to noise ratios (SNRs). The mean of the FWHM of microparticles in the first sub-image is 6.08 μm . Using the MAS method with the MAS MI criteria and the O-FWHM criteria, the FWHMs were improved by 16% and 24%, respectively. The improvement was restricted by the limited displacement between sub-apertures ($\Delta x = 0.27R$). Further analysis results of the variance on the FWHMs before and after digital processing showed a significant difference with P values of $2e^{-5}$ (MI) and $3e^{-10}$ (O-FWHM), respectively. By comparing the range and distribution of the transverse FWHM (Figure 5. 3d and Table 5. 2), we can observe that the proposed method provided a better resolution improvement, including better median value, better first quartile value, etc. Among all of the 62 microparticle scatterings, at least 25% of them broke the diffraction limit (theoretical resolution = 3.92 μm) after using the proposed method. The SNRs of the images ($\text{SNR} = 10\log_{10}[\max(F^2)/\sigma^2]$, F and σ^2 are the maximum intensity and noise variance of the image, respectively) were measured to be 27.3 dB (Org), 32 dB (MI), and 29 dB (O-FWHM), respectively, indicating a 3 dB reduction for the proposed method compared with the MAS method with MI criteria.

Figure 5.3 Microparticles images and analysis. (a) One B-scan image. $830\ \mu\text{m} \times 106\ \mu\text{m}$ ($1950\ \text{lateral pixels} \times 120\ \text{axial pixels}$, $n = 1.33$) microparticles images (1,2,...5) from 5 OPD, and after digital processing based on MAS with MI criteria and with optimized FWHM criteria. (b) The comparison of the normalized transverse intensity profiles of three microparticle samples from 5 OPDs before and after digital processing. Inset figure is the zoomed image of the microparticles shown in the dotted square in

Figure 5. 3a. (c) The transverse FWHM distribution of microparticles scatterings along with depth for the B-scan image. Black circle: from the first path single channel; blue diamond and red star: after digital processing based on MAS MI and O-FWHM, respectively. (d) The notched rectangles and bars indicate 25/75 and 0/100 percentiles. *, **, *** indicate P-value: $2e-5$, $3e-3$, and $3e-10$, respectively. OPD: optical path difference. MAS: multiple aperture synthesis. MI: maximum intensity criteria. O-FWHM: optimized FWHM criteria.

	Median (μm)	First quartile (μm)	Third quartile (μm)	Lower adjacent (μm)	Upper adjacent (μm)
Org	5.68	5.25	6.61	4.5	8.56
MAS MI	4.88	4.5	5.98	3.48	7.71
MAS O-FWHM	4.61	3.83	5.19	3.19	7.16

Table 5. 2 The statistic values corresponding to the boxplot shown in Figure 5. 3d.

Figure 5. 4 shows the microparticle images before (Org) and after digital processing, by using multiple optimized phase masks and one optimal phase mask for this B-scan image. Compared with the images obtained after digital processing using the optimal phase mask (the FWHMs of the microparticles were narrowed to the minimum after employing the optimal phase mask), the side lobe artifacts were suppressed by 1.1 dB after averaging the results obtained by applying the several obtained distinct, optimized phase masks.

Figure 5.4 The B-scan images of microparticle scatterings. $102\mu\text{m}\times 103\mu\text{m}$ (240 lateral pixels $\times$ 140 axial pixels, $n = 1.33$) microparticle images before (Org) and after digital processing by using one optimal phase mask and multiple optimized phase masks.

Figure 5.5 The transverse FWHM distribution of microparticle scatterings along with depth for the other two B-scan images. Black circle: original image obtained from single aperture channel; red star: after digital processing based on MAS with the optimized FWHM criteria.

By applying the obtained optimized phase masks to the other B-scan images, the transverse resolution of the microparticles was also dramatically improved, demonstrating that the proposed method is feasible and reliable for optical system resolution improvement. Figure 5. 5 shows the comparisons of the transverse FWHM of the microparticles for the other two B-scan images before and after digital processing based on the proposed method. These two B-scan images were obtained with a ~ 2.57 mm equidistant distance from the B-scan image shown in Figure 5. 6a. The means of the FWHMs for the two B-scan images before and after digital processing were $6.23\mu\text{m}$ and $6.14\mu\text{m}$, respectively. They narrowed to $4.79\mu\text{m}$ and $4.90\mu\text{m}$ after applying the

Figure 5.6 Tissue phantom images and analysis. (a) $695\ \mu\text{m} \times 168\ \mu\text{m}$ ($1630\ \text{lateral pixels} \times 190\ \text{axial pixels}$, $n=1.33$) phantom tissue images before and after digital processing based on the optimized FWHM criteria. (b) The comparison of the normalized transverse intensity profiles of three microparticle samples before (black, dot line) and after digital processing based on the proposed method. Inset figure is the zoomed image of the microparticles shown in the dotted square in Figure 5. 6a. (c) The comparison of the normalized transverse intensity profiles of microparticle scatterings along with depth before (Org, black circle) and after digital processing based on the proposed method.

optimized phase masks, which indicated that the means of the transverse resolution improved by 23% and 20%, respectively.

We also imaged a simplified chicken breast tissue with the same polystyrene microparticle and water solution to show the ability of the proposed method to improve the transverse resolution of the microparticles with sample-induced aberrations. We focused the sample light to the microparticles through the tissue with a thickness of about 120 μm in air, resulting in broadened transverse profiles of the microparticles (Figure 5. 6a). After utilizing the MAS with an optimized FWHM criteria-based digital processing method, the transverse resolution improvement can be intuitively observed in the transverse profiles and zoomed images (Figure 5. 6b) of three microparticles chosen in different locations (dotted square in Figure 5. 6a). The mean of the FWHMs of the microparticles was measured to be 5.23 μm before digital processing (Figure 5. 6c). It reduced to 4.07 μm after digital processing, thus improving by 23%.

5.4 Discussion and conclusion

In this work, we demonstrated a modified aperture synthesis method for transverse resolution improvement. In this method, the phase of the interference signal from sub-apertures is modulated, which may produce superresolving main lobes similar to those produced by physical phase masks [114, 115]. A brief theoretical analysis is given below. The OCT sample arm is the same as that of the confocal microscopy. The intensity measured by the detector behaves as a coherent microscope with an effective PSF (g_1) as follows (equations below are same the equations 2.20, 2.24 and 2.25 respectively):

$$\begin{aligned} I(x_s) &= \iint_{-\infty}^{\infty} g_1(x_1) g_1^*(x'_1) r_f(x_s - x_1) r_f^*(x_s - x'_1) dx_1 dx'_1 \\ &= |g_1(x_s) \otimes r_f(x_s)|^2 \end{aligned} \quad (5.5)$$

$$I(x_s) = \iint_{-\infty}^{\infty} C(m, p) R_f(m) R_f^*(p) \exp [2\pi i(m - p)x_s] dm dp \quad (5.6)$$

$$C(m, p) = \iint_{-\infty}^{\infty} g_1(x_1) g_1^*(x'_1) \exp [-2\pi i m x_1 + 2\pi i p x'_1] dx_1 dx'_1 \quad (5.7)$$

Where $R_f(m)$ (same with equation 2.22) is the Fourier transform of the object reflectivity r_f , m and p are spatial frequencies in the transverse direction. $C(m, p)$ is called the transfer function for the given system and describe the strength of a periodic component in the image. For a cylindrically symmetric two-dimensional system, the transfer function behaves as the convolution of the pupil function (P_1 and P_2) as follows (same with equation 2.27b):

$$C(l) = [\tilde{U}_1(M_1 l)P_1(\lambda f l)] \otimes \otimes [\tilde{f}_1(M_1 l)P_2(\lambda f l)] \quad (5.8)$$

Super-resolving effects would be obtained if a physical phase mask is applied to the pupil function [112,113]. Therefore, the super resolving main lobes would be obtained in the thesis due to the proposed modified aperture synthesis method by applying the digital phase masks. However, this digital phase modulation is applied after the light from all the sub-apertures is integrated into the pinhole, while physical phase masks are used before the light is integrated into the pinhole. This difference results in weaker superresolving effects of our method than by physical phase masks. Nevertheless, digitally applying the phase mask has the advantage of less signal loss compared with the physical phase mask.

Like other pupil filtering methods, side lobe artifacts are an inherent drawback of our method. The side lobe artifacts could be suppressed by averaging images obtained by distinct digital pupil filters. Note that the images mentioned above are originated from data obtained during one cross-sectional scan, so it does not affect the imaging speed. Another limitation of the proposed method is that at least one guide star point is required to obtain the optimized phase masks, which may limit its application in those scenarios where a guide star cannot be confidently identified.

In conclusion, the modified aperture synthesis method can effectively improve the transverse resolution of OCT. The side lobe artifacts issue is mitigated by averaging images of distinct pupil filters. This method is suitable for high-resolution retinal imaging, where physical phase masks might be too power-inefficient, and photoreceptors can serve as guide stars. Although the improvement in the transverse resolution is incremental compared with case without any digital refocusing. But if this resolution improvement is translated to the improvement in the depth of focus, we have

50% larger depth of focus, which is pretty significant especially for high resolution OCT where depth of focus is the bottleneck.

Chapter 6

Conclusion and recommendations

6.1 Conclusion

Spectral-domain optical coherence tomography (SD-OCT) imaging system with high resolutions, a large ranging depth, and low-cost are critical for the diagnosis of human diseases. The thesis aims to develop and validate optical imaging methods to address the penetration limitation and reduce the cost of high-resolution SD-OCT systems. To summarize:

Development and validation of a low-cost SD-OCT system using an all-reflective-optics based spectrometer. Comparing with the typical applied refractive optics based spectrometer, this design improved the optical transmission by 26% and reduce the cost by 16%, while maintains a comparable ranging depth and high axial resolution. The meaning of this study is to provide a simple design that enables researchers to customize their own spectrometer by themselves. The low-cost approaches with high performances make the research viable for clinical translation and commercialization.

Development and validation of a dual-spectrometer SD-OCT system and imaging processing methods to solve the limited ranging depth and DOF. The developed dual-spectrometer system provides a maximum ranging depth of about 3.5 mm and a 6-dB roll-off range of about 1.44 mm, which are 94% and 78% larger than the single spectrometer system, respectively. In addition, the focal range (Rayleigh range) was extended by 8 times by employing the forward-model based digital refocusing method. This technology was validated the feasibility in intact pig eyes ex vivo. Imaging the cellular structures of the posterior cornea, particularly the endothelial cells, is critical for the evaluation of the corneal grafts during corneal transplantation and diagnosis of corneal diseases.

Development and validation of a multi aperture synthesis (MAS) combined with digital phase pupil filters. This technique significantly improved the transverse resolution through the whole transverse field-of-view with 24% reduction in the main lobe width and 1dB and the sidelobe suppression. The proposed method may helpful for retina disease diagnosis like photoreceptor degenerations which are the most common form of blindness in western world due to the death of retinal photoreceptors.

6.2 Recommendations for future research

Spectrometer design with low-cost and high performance

A high-resolution SD-OCT system with low-cost, high-performance, and high-speed is meaningful for a variety of applications. More future work should be focused on the development of an advanced SD-OCT system featured with high-resolution, high-efficiency, high-speed, and low-cost.

The two spectrometer designs discussed in this thesis can be combined to an optimal dual-spectrometer design. In this new design, a reflective parabolic collimator and low-cost refractive camera lens may be used (Figure 6.1). In addition, the input beam transverse shape can be shaped from circular to elliptical. This elliptical beam is advantageous in that firstly it requires smaller grating size, and secondly, it mitigates the spherical aberrations along the minor axis direction. The camera lens may be composed of two off-the-shelf lenses: a plano-convex lens and an achromatic lens, which provide the optimal transmission efficiency while maintaining diffraction-limited spot size in the x-direction (direction of the linear sensor). A commercially available detector (Teledyne e2v) with a high acquisition time of 250 kHz will be used in this spectrometer design.

Figure 6.1 Spectrometer design: Ray tracing of the designed spectrometer obtained using Zemax software. For the dual-spectrometer design: part number 1 for the

wavelength range of 0.745 μm to 0.87 μm and part number 2 for the wavelength range of 0.835 μm to 0.96 μm .

The elliptical input beam is achieved by polishing the input fiber tip by 37.5° , so that the fiber numerical aperture is 0.13 in the y-direction (minor axis) and 0.28 in the x-direction (major axis). The effective NA of the collimation is 0.08 (FWHM). The required grating area is about $3.9 \text{ mm} \times 13 \text{ mm}$, which is 50% of that for a circular input beam. The grating is 1500 l/mm. The camera lens is composed of an off the shelf plano-convex singlets and an achromatic lens (part number 1 for the wavelength range of 0.745 μm to 0.87 μm ; part number 2 for the wavelength range of 0.835 μm to 0.960 μm) (Figure 6. 1). The simulation results demonstrate that the average spot diameter that encircles 50% energy, for the two spectrometers, are about 6.63 μm and 7.52 μm (Table 6. 1). This design reduces the cost of the grating by half and can provide high-performances (thanks to the small spot size) for an SD-OCT system. In the future, the detailed performance of this design based dual-spectrometers will be tested in a high-resolution SD-OCT system.

Wavelength (μm)	Diameter (μm) Y-Enclosed energy in fraction of 0.5	Dual-Spectrometers
0.745	5.78	Spectrometer 1
0.81	5.78	
0.87	8.32	
0.835	6.34	Spectrometer 2
0.897	7.76	
0.96	8.46	

Table 6. 1 The spot diameter when Y-Enclosed energy is in the fraction of 0.5 of the total energy.

Development of μOCT system for retina imaging

A μOCT system that gives access to cellular details is meaningful for the investigation of major eye diseases. More future work should be focused on the

development of a μ OCT system with an improved transverse resolution for retinal imaging, such as for photoreceptors imaging.

Firstly, we will apply a reported non-iterative digital aberration correction approach which corrects the wavefront distortions in the pupil plane through phase conjugation to obtain aberration-free cellular-level resolution for photoreceptors imaging [116, 117]. Besides, the developed digital phase pupil filters discussed in this thesis will be used and validated for further improving the transverse resolution.

Author's Publications

Journal Articles

- **Lulu Wang**, Qiaozhou Xiong, Xin Ge, En Bo, Jun Xie, Xinyu Liu, Xiaojun Yu et al. "Cellular resolution corneal imaging with extended imaging range." *Optics Express*, 27, no. 2 (2019): 1298-1309.
- **Lulu Wang**, Xiaojun Yu, Xin Ge, Xuan Wu, Xianghong Wang, En Bo, Nanshuo Wang, et al. "Design and optimization of a spectrometer for high-resolution SD-OCT." *Laser Physics Letter*, 2019, 4(16), 045603.
- **Lulu Wang**, Xin Ge, Xinyu Liu, and Linbo Liu, "Improving transverse resolution of multiple aperture synthesis optical coherence tomography using digital phase pupil filter", in preparation.
- Bo, En, **Lulu Wang**, Jun Xie, Xin Ge, and Linbo Liu. "Pixel-Reassigned Spectral-Domain Optical Coherence Tomography." *IEEE Photonics Journal* 10, no. 2 (2018): 1-8.
- Yu, Xiaojun, Hongying Tang, Chi Hu, Qianshan Ding, **Lulu Wang**, Xianghong Wang, Zeming Fan, and Linbo Liu. "Multiscale skin imaging in vivo using optical coherence tomography." *Laser Physics Letters* 15, no. 7 (2018): 075601.
- Yu, Xiaojun, Qiaozhou Xiong, Yuemei Luo, Nanshuo Wang, **Lulu Wang**, Hong Liang Tey, and Linbo Liu. "Contrast enhanced subsurface fingerprint detection using high-speed optical coherence tomography." *IEEE Photonics Technology Letters* 29, no. 1 (2017): 70-73.
- Mengjia Zheng, Hao Chang, Sharon W.T. Chew, **Lulu Wang** et al, "Osmosis-powered hydrogel microneedles for microliters of skin interstitial fluid extraction within minutes", *Advanced Materials*, submitted.

Conference Proceedings

- **Lulu Wang**, et al. "CoSrneal imaging with extended imaging range using dual spectrometer high-resolution SD-OCT." *CLEO*, Poster presentation, 2019.
- **Lulu Wang**, et al. "Design and Optimization of Spectrometer with High Efficiency for SD-OCT." *CLEO-PR, OECC and PGC*, Oral presentation, 2017.

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