Developing polarization sensitive micro optical coherence tomography – towards high contrast, resolution and sensitivity optical imaging

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**DEVELOPING POLARIZATION SENSITIVE
MICRO OPTICAL COHERENCE
TOMOGRAPHY – TOWARDS HIGH
CONTRAST, RESOLUTION AND
SENSITIVITY OPTICAL IMAGING**

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Acknowledgements

I still remembered every important moment: the midnight I got my first result; the afternoon I submitted my first manuscript; the first presentation I made in the conference to a crowded room of people; the first idea turned out to work; the first acceptance letter I got from the editor. Who did I firstly want to let know in these moments? The answer is my supervisor, Prof. Liu Linbo. I feel extremely lucky to have him as my supervisor. People say research will treat arrogance. But mine is not. Prof. Liu is there to help in any situation, cheers up me upon every failed attempt, and prevents me from the down side of research. During my first year, he trained me hand in hand from the basic optical alignment to academic writing. In the second year, he encouraged me to develop my own idea, try to independently conduct experiments. Every time I bought some components but later turned out just another bad idea, he said that was a good try and laughed. In the third year, he helped me connect to Dr. Brett Bouma in Harvard Medical School. With his support, I went to Boston for a visit study in my third year. After I was back, he allowed me to work in a small team, and provided me every opportunity to maximize my potential. As a student of him, I can feel myself growing up. Finally, now I'm ready to graduate. I'll use the skills, the supports and the passions I got from him to keep working on and make him proud of me.

In Boston, I met Dr. Martin Villiger, who turned out to be one of the most influential persons to me. In Boston, I'm lucky to join a project to make PS-OCT phantoms. Martin was the leader of that project. Like a character in the movie, he is super smart and sharp, invincibly strong in his expertise. Later I became interested in the polarization sensitive imaging technologies. Though Martin was busy and strict in time management, he was very generous in spending time helping others who wants to learn. I learned my PS-OCT knowledge from Martin. Later we began to discuss ideas, most of which were immature. But every discussion helped me and gave me a better understanding

of things. He set a role model for me both in academic achievements and personalities.

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During the PhD study, my wife, Dr. Zhang Chen, supports on every aspect and gives me the courage to keep doing the right things. My parents in China are ready to receive my calls whenever I need to talk.

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Summary

Optical coherence tomography (OCT) has become established as a high-resolution three-dimensional imaging modality for the diagnosis of various human diseases. This thesis focuses on the technical development of current OCT systems, and proposes several improvements to OCT in terms of contrast, resolution and sensitivity.

Polarization sensitive-OCT (PS-OCT) needs two different polarization states illuminating on the same sample spot to solve the local birefringence properties, specifically, local retardation and local relative optic axis. Current PS-OCT uses a polarization modulator to change the polarization state of adjacent A-scan lines, or encodes various polarization detections on different imaging depth. However, these methods are not applicable for high resolution OCT. The bandwidth of a modulator limits the system resolution lower than about 5 μm . The depth range of an OCT is determined by the wavelength resolution of the spectral detection. For high resolution OCT with an ultra-broadband source, there are little margins left for extra depth multiplexing. To overcome these problems, this thesis describes a new reasoning methodology, derived from the polarization reciprocity theorem, termed as “holding point reasoning”. This reasoning theorem states that, for a sample with random birefringence layer structures (no diattenuation), the backscattered polarization state evolving trace along the depth is consisted discontinuous arcs lying on the Poincare sphere. In a unitary backscattering system, the circles that the arcs are on all pass through the “holding point”, which is the Q-U plane reflection of the input polarization state. In the second chapter of this thesis, I described both the experiments and theoretical confirmation of this reasoning method. This reasoning technique enables the single input PS-OCT to resolve local birefringence properties, open a way towards the construction of high resolution polarization sensitive optical coherence tomography. In this chapter, the prototype of such system based on holding point reasoning was demonstrated.

Collaborating with one of the leading research groups, I developed an approach to fabricate a tissue-like OCT birefringence phantom, a key protocol for characterizing and calibrating the absolute detection value, sensitivity and range of PS-OCT. Taking use of the stress-induced birefringence, the leveled birefringence regions were created by stretching the polycarbonate sheet to different lengths. Several pieces of polycarbonate sheet with different birefringence and different patterns were fabricated. To package the target, the birefringent samples were embedded into matrix made of epoxy. Scatters were added into the polycarbonate and epoxy to mimic the scattering property of the biological tissue. The fabrication, test and demarcation of the target were described in the third chapter.

In fiber-based OCT, the interference fringes suffer from the fading effect due to misalignment of the light polarization states between the reference and sample arms, resulting in sensitivity degradation and image intensity variation. We theoretically and experimentally analyzed the relation between the misalignment and the fading coefficient. Assuming that the variation of the light polarization in single mode fiber was a random process, we statistically quantified the fading effect. Furthermore, in OCT configuration based on Michelson interferometer, we reported an interesting observation, that the polarization states of light travelling a round trip in SMF are not evenly distributed on the Poincare sphere. Based on this observation, we demonstrated the existence of an optimal output polarization state of the reference arm to mitigate the fading effect. We demonstrated that in an optimal setup, the statistical average SNR could be 3.5 dB higher than a setup without proper polarization management. The fourth chapter of the thesis discusses the management of polarization fading problem in OCT.

Besides from polarization detection, to maintain and improve the resolution of OCT, I developed a spectral estimation (SE-OCT) algorithm to super-resolve the layer structures in transparent samples. This algorithm extrapolates the interferometric fringes outside the source bandwidth, thereby improving the resolving ability of OCT. Based on the assumption that the sample was

consisted of sparse layers, the algorithm can super resolve two close surfaces within 200 nm, far under the physical resolution. SE-OCT shows good performance in transparent tissues such as corneal. The discussion of SE-OCT is included in the fifth chapter.

In this thesis, a method to improve the sensitivity of spectral domain OCT is also included. Termed as spectral encoded extended source OCT (SEES-OCT), this new configuration is an attempt to improve signal strength for ophthalmic imaging. By introducing a dispersive element in the infinity space of the sample arm, the scanning laser spot was dispersed with a visual angle of 7.9 mrad. The maximum permissible exposure (MPE) of such an extended source is 3.1 times larger than that of a “standard” point source OCT, which corresponds to sensitivity improvement of 5 dB. The advantage of SEES-OCT in providing superior penetration depth over a point source system was demonstrated.

In conclusion, this thesis provides a step forward to develop a viable OCT with high resolution, sensitivity and contrast with the discussion and solutions to several technical problems. The feasibility and effectiveness of the proposed solutions were demonstrated with phantoms and ex vivo animal imaging in the context of relevant biological diseases.

List of Abbreviations

ABS	Acrylonitrile Butadiene Styrene
AOM	Acousto-Optic Modulator
APES	Amplitude and Phase Estimation of a Sinusoid
ARMA	Autoregressive Moving Average
BS	Beam Splitter
CCD	Charge-Coupled Device
DFT	Discrete Fourier Transform
DOP	Degree of Polarization
FD-OCT	Fourier Domain Optical Coherence Tomography
FDML	Fourier Domain Mode Locking
FWHM	Full Width at Half Maximum
IAA	Iterative Adaptive Approach
IVOCT	Intravascular Optical Coherence Tomography
MPE	Maximum Permissible Exposure
MUSIC	Multiple Signal Classification
OCE	Optical Coherence Elastography
OCT	Optical Coherence Tomography
OFDI	Optical Frequency Domain Imaging
ONH	Optical Nerve Head
PC	Polycarbonate
PDD	Polarization Diversity Detection
PDF	Probability Density Function
PMD	Polarization Mode Dispersion
PS-OCT	Polarization Sensitive Optical Coherence Tomography
PS- μ OCT	Polarization Sensitive Micro Optical Coherence Tomography
PSF	Point-Spread Function
QWP	Quarter Wave Plate
SD-OCT	Spectral Domain Optical Coherence Tomography
SE-OCT	Spectral Estimation Optical Coherence Tomography
SEES-OCT	Spectrally Encoded Extended Source OCT
SLD	Superluminescent Diode
SMF	Single Mode Fiber

SNR	Signal-to-Noise Ratio
SS-OCT	Swept Source Optical Coherence Tomography
UHR-OCT	Ultra-High-Resolution Optical Coherence Tomography
μ OCT	Micro Optical Coherence Tomography

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Chapter 1 Introduction

1.1 Optical coherence tomography

It has been 25 years since the introduction of optical coherence tomography (OCT) [1]. As a representative of bio-medical research, later turning to a successful commercial clinical tool, OCT technology attracts a large attention from both commercial and research communities in these two decades [2]. The development of this imaging technique was dramatically rapid, intensive, multi-directional and fruitful in these dozens of years.

One of a significant event in the development history of OCT technology is the invention of Fourier domain OCT (FD-OCT) [3], which records the interference fringe data in the spectral domain and digitally retrieves the sample depth profile using inverse Fourier transform. Until 2003, the community realized the sensitivity advantage of Fourier domain OCT over time domain OCT, thanks to the discussion of [4,5], and later the experimental validated by [6]. Briefly, the sensitivity advantage comes from the large number of multiple detections. The inverse Fourier transform coherently adds up the signal meanwhile incoherently averaging the noise. It is proved an FD-OCT usually provides more than 20 dB higher sensitivity than a time-domain OCT (TD-OCT) under the same source condition. The improvement is critical for OCT image quality. Another advantage brought by FD-OCT is the imaging speed [7,8]. Unlike time domain OCT, the recording happens in wavelength domain rather than spatial domain, thus no motion device or scanner is needed. The wavelength sweeping laser or high speed spectrometer can achieve an Aline scanning speed of more than 100 kHz [9,10], opening the possibilities of reliable clinical in vivo imaging in the human body.

FD-OCT has become new generation of the technology and outperforms TD-OCT in most aspects. Therefore, in this thesis, all the experiments, simulations and discussions are based on the context of the FD-OCT. Particularly, there are two main implementations of FD-OCT. One is based on high speed spectrometers, the so called spectral domain OCT (SD-OCT). This kind of OCT uses a spectrometer to record the spectral interference fringes. The other is based on high speed wavelength scanning laser, termed as swept source OCT (SS-OCT). This kind of OCT relies on the wavelength sweep of the laser to record the fringes thereby using a single set of detectors. Generally, SS-OCT's detectors have higher dynamic range and better sensitivity than normal line scan camera. With the development of Fourier domain mode-locking (FDML) laser [7], the wavelength scanning speed can be at MHz level maintaining an excellent line-width. SS-OCT usually has higher dynamic range, better sensitivity, larger roll-off imaging depth and faster speed [11]. However, recently advances in high speed camera and low noise spectrometer is minimizing the performance difference between SS-OCT and SD-OCT. What's more, wavelength sweeping laser has limited overall sweeping bandwidth. On the contrary, broadband sources are more available, including superluminescent diode array [12] and supercontinuum source [13,14]. Thus, currently SD-OCT has a higher resolution than SS-OCT. In addition, many functional OCT, such as OCT angiography [15], optical coherence elastography (OCE) [16] and polarization sensitive OCT (PS-OCT) [17] take use of the phase information of the interference fringes. These technologies favor the SD-OCT since the better phase stability.

1.2 State of the art

1.2.1 Micro-OCT

The high-resolution variant of OCT is Micro-OCT (μ OCT), which can achieve a resolution of close to 1 micrometer. First introduced by [18], the concept of μ OCT

is based on SD-OCT, pushing the resolution with a super continuum source and a high NA objective lens. With isotropic resolution in three scanning directions, μ OCT promises to see cellular and sub-cellular structure in many human organs, thus providing valuable information on disease diagnosis including cancer. μ OCT has been demonstrated to be critically useful in cardiology [18], ophthalmology [19], pulmonology [20].

However, due to the contrast mechanism of OCT, the image is formed by the information-carrying speckles, which undermine the micro structure visibility, limiting the true resolving power of μ OCT. In addition, the intensity image of OCT sometimes lacks the contrast from structures of similar scattering properties. Therefore, one of the ongoing research fields about μ OCT focuses on the reasons behind the contrast and speckle reduction [21]. Another topic of μ OCT is to extend the depth of focus. Due to the use of high NA objectives, the depth of focus is highly limited. The depth of focus is a big obstacle preventing μ OCT from clinical applications. Techniques are being developed to extend the depth of focus of μ OCT.

1.2.2 Polarization sensitive OCT

Polarization sensitive optical coherence tomography (PS-OCT) was proposed in 1992 [22], adding additional polarization modification on the input light and uses polarization sensitive detecting scheme to record the polarization state of the output light. Therefore, PS-OCT can provide the contrast of tissue birefringence properties, in addition to the conventional intensity contrast. Specifically, PS-OCT can detect tissue birefringence properties including retardation and diattenuation between the two orthogonal light propagating modes, the orientation of the fast optics axis and the degree of polarization.

A common kind of PS-OCT, known as “single input state PS-OCT”, uses circular polarization light to probe the tissue [23]. As the existence of birefringence, the

polarization state of the light will change from circular to elliptical while propagating in the tissue. The depth-resolved polarization states of backscattered light can be extracted from the fringes recorded by detectors. Detectors in PS-OCT usually comprises of a polarization beam splitter which splits the horizontal and vertical components of the light beam and separately records the interference fringes in each channel.

As the probing light are focused into the tissue structure and scattered back upon collection, the direct interpretation of the depth resolved output polarization states of the light leads to the result of accumulated birefringence properties of the tissue, including the cumulative retardation, cumulative optical axis. However, in most occasions, the tissue being examined has varies structures and rich inhomogeneous layers, making it difficult to read the maps of cumulative properties. On the other hand, the map of local birefringence properties of the tissue inherently relates to structure of the certain location and provides direct information of the region of interest. Therefore, extracting the depth resolved local birefringence properties of the tissue provides a more intuitive and accurate representation of the tissue structure and function [24].

1.3 Goals and structure of this thesis

My efforts in my PhD research is to technically improve the OCT image quality and application scope. My interest first focuses on the sensitivity, then resolution, and finally the functional birefringence contrast. This thesis is organized as a combination of my journal paper results from the latest to the oldest.

In the second chapter, I reported an interesting constraint of polarization reciprocity. In a reciprocal unitary backscattering light circuit, where there is no polarization dependent loss, the polarization state evolvment of backscattered light must pass or must tend to pass a certain polarization state, corresponding to a point on the Poincare sphere, termed as polarization holding point. This effect

reveals a relevant constraint to the polarization evolution trace of the backscattered light in a birefringent light circuit, providing a satisfying explanation to several previously observations. Furthermore, in polarimetry imaging, this constraint bridges directly the birefringence property of the remote target to the measured Stokes vectors, rather than accessing to the Jones or Mueller matrix, thereby enabling straightforward measurement and analysis of the birefringence of the target. I theoretically and experimentally confirmed this effect. As a demonstration, I performed local birefringence imaging of the birefringent phantom and ex-vivo swine retina using a single input state ultra-high resolution PS-OCT. Such local birefringence imaging with single input polarization state was believed a challenge. Furthermore, for conventional two input polarization states PS-OCT, we demonstrated an improvement of the detection accuracy of relative optic axis by applying the polarization holding constraint.

In the third chapter, I present a method to fabricate stable, tissue-like birefringence phantoms that contain multiple well-defined regions of distinct birefringence. Polarization sensitive (PS) measurements with optical coherence tomography (OCT) provide valuable insight into the microscopic structure and organization of many biological tissues. Over time, an array of different PS-OCT implementations and reconstruction strategies has been developed. Wave plates are frequently used for the characterization of PS systems, but their specular reflections are very different from the backscattering encountered in biological tissues. Alternatively, muscle and tendon tissue is used for qualitative demonstration of imaging performance. However, the underlying birefringence of these samples is not known and the sample is perishable, precluding cross-validation of PS systems. I think these robust phantoms I made will prove very valuable for the testing, validation, comparison, and optimization of PS-OCT imaging systems and reconstruction strategies.

In the fourth chapter, I firstly theoretically and experimentally analyzed and quantified the fading effect. The polarization induced fringe fading problem in white light interferometer had been noticed about 30 years ago. In terms of OCT biomedical imaging, this fading effect brings serious image SNR degradation, which is also noticed and reported by several groups. However, though an old problem, the fading effect seems never been fully studied and attended by the whole OCT community, especial for the community using Fourier domain OCT with broadband sources, the polarization state of the light is always not properly managed. For Michelson type OCT, which is favored by the high resolution OCT community, I demonstrated an optimal polarization management, bringing a statistical 3.5 dB SNR improvement to a common system. I believe this model will help the OCT community gain a full insight to polarization fading effect, as well as suggesting an easy way to optimize the system SNR if the configuration is applicable to their systems.

In the fifth chapter I revisited the autoregressive spectral estimation method and applied it in FD-OCT to instantly achieve an axial super-resolution of 2~4 fold, as well as eliminating the sidelobe artifacts. Standard OCT still cannot always fulfil its promise as ‘optical biopsy’ with a 5-15 μm axial resolution. Current clinical reliable FD-OCT provides a ~ 4 μm axial resolution in ophthalmic imaging and ~ 10 μm for intravascular imaging (in air). I demonstrated that without any extension in source bandwidth, the corneal endothelium cells can be resolved and the thickness of the endothelium cells can be measured to be 3.1 μm using this method. For intravascular imaging, the plague of cholesterol crystal can be distinguished from other structure as the layer pattern appeared after resolution enhancement. The results were registered with a specially designed 1.3 μm OCT device.

In the sixth chapter, I developed an extended source optical coherence tomography (SEES-OCT) technique in an attempt to improve signal strength for ophthalmic imaging. A line illumination with a visual angle of 7.9 mrad is produced by

introducing a dispersive element in the infinity space of the sample arm. The maximum permissible exposure (MPE) of such an extended source is 3.1 times larger than that of a “standard” point source OCT, which corresponds to sensitivity improvement of 5 dB. The advantage of SEES-OCT in providing superior penetration depth over a point source system is demonstrated using swine eye tissues ex vivo.

In the last chapter, I concluded my efforts in improving the current OCT and proposed some future paths opened by the theories and technologies I proposed in this thesis.

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Chapter 2 Holding point effect

2.1 Motivation and background

The reciprocity is a fascinating property of wave propagation, stating the natural symmetry of waves transporting forward and backward in a medium, constantly attracting researchers' attention in interpreting [25], applying [26] and even breaking [27] it. In polarimetry, the most well-known understanding of reciprocity may be the transpose symmetry of the Jones matrix when light is travelling bi-directionally in the medium with symmetric dielectric tensor [28]. Driven by the development of optical devices, polarimetry sensing and imaging has been widely applied in the fields of astronomy [29], remote sensing [30], biological imaging [31] and medical diagnosis [32]. Reciprocity is inevitably one of the most fundamental principles in detecting inaccessible remote targets via backscattered signals.

Due to the complexity of the light-medium interacting in polarization, considerable efforts have been made on reliably extracting local physical properties of the target from measured data. Basic scattering theorems, such as vector radiative transfer theory [33], have been developed to interpret the light-target interacting, and practical techniques, such as various target decomposition methods [34] have been developed to reliably extract relevant parameters. Recently, borrowing the methods and techniques from the radar polarimetry, biological polarimetry sensing and imaging have been extensively investigated [35,36]. In terms of polarimetry, biological tissues are complicated media, where ballistic and multiple scattering [37], linear birefringence [35], optical activity [38], circular and linear dichroism [39] and depolarization [40] may simultaneously exist, making it a puzzle extracting and analyzing the relevant specific properties.

Polarization sensitive optical coherence tomography (PS-OCT) is an emerging polarimetry imaging technique based on backscattering light, with light source and detector located at the same side of the sample. PS-OCT has been widely applied in the field of in-vivo diagnostic imaging, including ophthalmology [41], cardiology [42], pneumology [43] and dermatology [44]. Due to the common path of illumination and detection beams, polarization dependent scattering is minimized, furthermore cancelling the optical activity and circular dichroism. The coherence gate rejects most of the multiple-scattering light, which is regarded as no more than noise [45]. Therefore, literally linear birefringence and dichroism would pronounce in PS-OCT. Interestingly, according to recent reviews [17,46], in PS-OCT, the polarization dependent absorption, or dichroism, is proved to be very small comparing to the retarding effect of linear birefringence.

2.2 The reciprocal birefringent medium

Figure 2.3 shows a birefringent medium modelled by a retarder stack, where A is the surface, B and C are two scattering interfaces in the sample. According to [47], without diattenuation, the Jones matrix of layer AB can be expressed as a multiplication of a retarder and a rotator.

$$\mathbf{J}_{AB} = \mathbf{J}_{\text{retarder}(AB)} \mathbf{J}_{\text{rotator}(AB)} \quad \dots(2.1)$$

According to the reciprocity [25], the backscattered Jones Matrix

$$\mathbf{J}_{ABA} = \mathbf{J}_{AB}^T \mathbf{J}_{AB} = \mathbf{J}_{\text{retarder}(AB)}^T \mathbf{J}_{\text{rotator}(AB)}^T \mathbf{J}_{\text{rotator}(AB)} \mathbf{J}_{\text{retarder}(AB)} \quad \dots(2.2)$$

The Jones matrix of a rotator is orthogonal, so $\mathbf{J}_{\text{rotator}(AB)}^T = \mathbf{J}_{\text{rotator}(AB)}^{-1}$

$$\mathbf{J}_{ABA} = \mathbf{J}_{\text{retarder}(AB)}^T \mathbf{J}_{\text{retarder}(AB)} \quad \dots(2.3)$$

In OCT measurement, the birefringence of the sample is constructed from the round-trip detection. Unavoidably, the circular birefringence is missing. This is

also known that PS-OCT cannot detect circular birefringence in backscattering since circular birefringence is cancelled between forward and backward trips.

To further illustrate the polarization state evolvement in a birefringent medium. I constructed a sample with three layers with different optic axis orientations, shown in **Figure 2.1**. By Jones matrix calculation, one can get the true polarization evolving trace. The trace is plotted on the Poincare sphere (**Figure 2.1**). The round trace of light backscattered from sample layer A follows the trace OI-IA-AI-IO. To reconstruct the single trip Jones matrix of OA, one can find the trace O-A'-A_{double}. The latitude of reconstructed A' is correct, but the longitude of A' is ambiguous because of the cancelling of the rotation matrix.

Figure 2.1 | Polarization state involvement in a birefringent medium. The medium consists of three birefringent layers with different optic axis orientations. The true evolvement traces are simulated by Jones calculation, the reconstructed cumulative traces are simulated without rotation matrix.

2.3 Holding point theory

Here we reported an interesting constraint of polarization reciprocity. In a reciprocal unitary backscattering light circuit, where there is no polarization dependent loss, the polarization state evolvement of backscattered light must pass or tend to pass a certain polarization state, corresponding to a point on the Poincare sphere, termed as polarization holding point. This effect reveals a relevant constraint to the polarization evolvement trace of the backscattered light in a birefringent light circuit, providing a satisfying explanation to several previously observations [48–51]. Furthermore, in polarimetry imaging, this constraint directly bridges the birefringence property of the remote target to the measured Stokes vectors, rather than accessing to the Jones or Mueller matrix, thereby enabling straightforward measurement and analysis of the birefringence of the target. In this chapter, we theoretically and experimentally confirmed this effect. As a demonstration, we performed local birefringence imaging of the birefringent phantom [52] and ex-vivo swine retina using a single input state ultra-high resolution PS-OCT. High-resolution local birefringence imaging with single input polarization state was believed a challenge before [53,54]. Furthermore, for conventional two input polarization states PS-OCT, we demonstrated an improvement of the detection accuracy of relative optic axis by applying the polarization holding constraint.

In several literatures [48–51], observation was reported that the polarization states of the light back reflected from single mode fiber (SMF) are not randomly uniformly distributed on the Poincare sphere. M. Oskar *et al.* summarized this observation in his paper [50] that the polarization state showed a preference for one side of the Poincare sphere, and one particular point was crossed many times. More recently, our group has taken advantage of this effect to optimize the fading degradation of OCT signal [51]. Further study of this observation led us to discover this interesting so-called polarization holding effect.

To confirm the existence of the polarization holding effect, a simple optical circuit is built, containing about 10-meter SMF and a reflector, shown in **Figure 2.2a**. A fully polarized light beam is collimated into the fiber. A polarization-diverse detector is used to detect the polarization state of the reflected light. We randomly twisted the paddles of polarization controller and recorded the polarization state evolvement. Shown in **Figure 2.2b**, the polarization state evolving trace on the Poincare sphere crossed a point (polarization holding point in **Figure 2.2b**) several times. This experiment is a simple demonstration of the existence of polarization holding effect.

Assuming there is no depolarization, the evolvement of polarization state in a birefringent medium can be represented by the movement of Stokes vector on the Poincare sphere. A retarder corresponds to a circular rotation of Stoke vector about the relative optic axis. Many optical media can be modelled as a stack of retarders with different optic axis orientations, such as the single mode fiber [55] or biological tissue [56]. In a backscattering system, the light double passes the medium, forwardly and then backwardly, thereby cancelling the circular birefringence. If the sample was modelled as a stack of linear retarders with respectively random optic axis, in PS-OCT, the evolving trace of the polarization state of the double-pass light along depth would be a connection of small circular arcs. Each arc corresponds to each linear retarder layers in the sample.

The polarization holding effect reveals that in a reciprocal unitary backscattering circuit without depolarization, the rotational circles of the backscattered polarization state, corresponding to retarder layers, will all pass one point on the Poincare sphere, termed as the polarization holding point. If the input polarization state is arbitrarily $\mathbf{s}_{in} = [s_1, s_2, s_3]^T$, the corresponding polarization holding point is $\mathbf{s}_{PHP} = [s_1, s_2, -s_3]^T$.

Figure 2.3 shows an optical sample modelled by a retarder stack, where A is the surface, B and C are two scattering interfaces in the sample. The medium between

BC is assumed to be a pure retarder with optic axis oriented at θ and retardance δ . The input polarization state at the sample surface is $\mathbf{s}_{\text{in}}$, after passed through medium AB, becomes $\mathbf{s}_{\text{BCin}}$. We know $\mathbf{s}_{\text{BCin}} = \mathbf{M}_{\text{AB}}\mathbf{s}_{\text{in}}$, where $\mathbf{M}_{\text{AB}}$ is the 3 x 3 Mueller matrix of the medium AB. To prove that the rotation circle of BC passed through the holding point (noted as $\mathbf{s}_{\text{PHP}} = \mathbf{D}\mathbf{s}_{\text{in}}$, where $\mathbf{D} = \text{diag}[1 \ 1 \ -1]$), is to say that we want to find a retardance α , where when $\delta = \alpha$, $\mathbf{s}_{\text{out}} = \mathbf{D}\mathbf{s}_{\text{in}}$. To avoid the complexity of flipping the coordinate system in backscattering, we mirrored the stack by the interface C, assuming the existence of B' and A' where the light polarization state at B' and A' is identical to the polarization state of $\mathbf{s}_{\text{BCout}}$ and $\mathbf{s}_{\text{out}}$. Analogy to the well-known transpose symmetry of Jones matrix saying $\mathbf{J}_{\text{CB}'} = \mathbf{J}_{\text{BC}}^T$, the backward Muller matrix of layer BC, $\mathbf{M}_{\text{CB}'}$ is equals to $\mathbf{D}\mathbf{M}_{\text{BC}}^T\mathbf{D}$, where T noted the transpose operation. The Mueller matrix of BC can be represented as

$$\mathbf{M}_{\text{BC}} = \mathbf{M}_{\text{retarder}}(\theta, \delta) = \begin{bmatrix} \cos^2(2\theta) + \cos(\delta)\sin^2(2\theta) & \cos(2\theta)\sin(2\theta) - \cos(2\theta)\cos(\delta)\sin(2\theta) & \sin(2\theta)\sin(\delta) \\ \cos(2\theta)\sin(2\theta) - \cos(2\theta)\cos(\delta)\sin(2\theta) & \cos(\delta)\cos^2(2\theta) + \sin^2(2\theta) & -\cos(2\theta)\sin(\delta) \\ -\sin(2\theta)\sin(\delta) & \cos(2\theta)\sin(\delta) & \cos(\delta) \end{bmatrix} \quad \dots(2.4)$$

The round trip Muller matrix of BC layer

$$\mathbf{M}_{\text{BCB}'} = \mathbf{M}_{\text{CB}'}\mathbf{M}_{\text{BC}} = \mathbf{D}\mathbf{M}_{\text{BC}}^T\mathbf{D}\mathbf{M}_{\text{BC}} = \mathbf{M}_{\text{retarder}}(\theta, 2\delta). \quad \dots(2.5)$$

Assuming $\mathbf{S}_{\text{inBC}} = [s_1, s_2, s_3]$, we find that when

$$\delta = \alpha = \frac{1}{2} \arctan \frac{2s_3(s_1 \sin 2\theta - s_2 \cos 2\theta)}{s_3^2 - (s_1 \sin 2\theta - s_2 \cos 2\theta)^2}, \quad \dots(2.6)$$

substituting α into

$$\mathbf{s}_{\text{BCout}} = \mathbf{M}_{\text{retarder}}(\theta, 2\delta) \mathbf{s}_{\text{BCin}}, \quad \dots(2.7)$$

noting

$$\sin 2\alpha = \frac{2s_3(s_2 \cos 2\theta - s_1 \sin 2\theta)}{(s_1 \sin 2\theta - s_2 \cos 2\theta)^2 + s_3^2} \quad \dots(2.8)$$

and

$$\cos 2\alpha = \frac{(s_1 \sin 2\theta - s_2 \cos 2\theta)^2 - s_3^2}{(s_1 \sin 2\theta - s_2 \cos 2\theta)^2 + s_3^2}, \quad \dots(2.9)$$

we get $\mathbf{s}_{BCout} = \mathbf{D}\mathbf{s}_{BCin}$. Further, the output polarization state at the sample surface $\mathbf{s}_{out} = \mathbf{M}_{B'A'} \mathbf{s}_{BCout}$. When $\delta = \alpha$, knowing $\mathbf{s}_{BCout} = \mathbf{D}\mathbf{s}_{BCin}$, then $\mathbf{s}_{out} = \mathbf{M}_{B'A'} \mathbf{D}\mathbf{s}_{BCin} = \mathbf{D}\mathbf{M}_{AB}^T \mathbf{D}\mathbf{M}_{AB} \mathbf{s}_{in}$. Note that $\mathbf{M}_{AB}$ is orthogonal, $\mathbf{s}_{out} = \mathbf{D}\mathbf{M}_{AB}^T \mathbf{M}_{AB} \mathbf{s}_{in} = \mathbf{D}\mathbf{s}_{in}$. To conclude, a δ can always be found, making that $\mathbf{s}_{out} = \mathbf{D}\mathbf{s}_{in}$ for every layer of retarder $\mathbf{M}_{BC}$, meaning that the polarization state evolvement trace of each layer will eventually reach the point $\mathbf{D}\mathbf{s}_{in}$ if enough birefringence were presented.

Revisiting the literatures [48–51], no matter the Rayleigh backscattering along the fiber or changing the birefringence of the fiber using a polarization controller, the fiber can always be modelled as a retarder stack, exactly fulfilling the unitary backscattering conditions. That's why the similar observations to Figure 2.2 were made in the literatures.

Figure 2.2 | Demonstration of polarization holding effect. **a**, Schematic of optical system used in the demonstration experiment. P: polarizer, PC: polarization controller, SMF: single mode fiber, L: lenses, M: mirror. **b**, Polarization state evolving trace on the Poincare sphere. The polarization holding point was crossed several times.

To validate this effect, we made a phantom containing three layers of linear retarders with different optic axis orientations, shown in **Figure 2.4a**. The phantom was probed with light of different polarization states (circular in **Figure 2.4b**, elliptic in **Figure 2.4c** and linear in **Figure 2.4d**).

Backscattered light was recorded by a set of polarization-diverse detector. Applying PS-OCT techniques, coherence gate was used to differentiate the light backscattered from different depths. Shown in **Figure 2.4b-d**, the polarization state evolution traces along the depth were plotted on the Poincare spheres, color-coded according to the retarder layers in **Figure 2.4a**. We fitted a circle (purple circles in **Figure 2.4b-d**) to the measured polarization state evolution for every retarder layer. As our prediction, the circles all crossed at the polarization holding point s_{PHP} (blue arrows in **Figure 2.4b-d**), which is exactly the QU plane mirror of the input polarization state s_{in} , confirming $s_{\text{PHP}} = Ds_{\text{in}}$.

Figure 2.3 | Retarder stack model of optical sample. A is the surface of the sample. B - C is the layer under investigation. B' and A' are imaginary mirrors of B and A about boundary C in order to avoid coordinate system transformation in backscattering.

Figure 2.4 | Holding point effect validation using three layer retarder phantom. **a**, Schematic sketch of three layer phantom sample. Each layer has a different optic axis orientation. **b-d**, Polarization state evolving trace for each layer on the Poincaré sphere with different input polarization state (**b**, circularly polarized input light; **c**, elliptically polarized input light; **d**, linearly polarized input light). Purple lines are the fitting circles of the polarization state evolution of each retarder layer. Holding point and input polarization state are indicated by red and blue arrows, respectively. It can be noticed that the holding point and input point are symmetric with respect to the QU plane. The colors of the measured points correspond to the colors of layers in **a**.

2.4 Holding point in PS-OCT

2.4.1 Single input state PS-OCT

Figure 2.5 | Local birefringence reasoning using single input state PS-OCT. For a small sample slice $z - \Delta z$ to $z + \Delta z$, the polarization state evolved from point A to B. P was the polarization holding point. Then the rotation axis can be determined by $\vec{r} = \overrightarrow{PA} \times \overrightarrow{PB}$. The inset figure indicates the definition of s_z , $s_{z-\Delta z}$ and $s_{z+\Delta z}$ (Polarization states of light backscattered from depth z , $z - \Delta z$ and $z + \Delta z$, respectively).

Conventional single input state PS-OCT only reconstructs cumulative retardation and assumes a constant orientation of the optic axis along depth [57]. Recently, increasing effort has been directed to reconstructing local retardance [53,56], which offers a more intuitive view of tissues having a layered architecture. However, the reconstruction of local birefringence is usually based on Jones [56] or Mueller [10,24] formulism, which requires at least two input states.

A straightforward application of the holding point effect is the direct solving of the local birefringence using a single input state, without accessing to Mueller matrix of the sample.

The depth polarization evolvement of the light backscattered from a sample can be reconstructed from the PS-OCT depth A-scan. To resolve local birefringence, a small depth interval Δz (**Figure 2.5**) is defined, usually several times larger than the intensity resolution. The sample slice in the interval Δz is assumed a pure retarder. For this retarder, the input and the output states are known as $\mathbf{s}_{z-\Delta z}$ and $\mathbf{s}_{z+\Delta z}$. The reasoning is clearer in geometric representation, shown in **Figure 2.5**. On the Poincare sphere, A and B corresponds to the polarization states $\mathbf{s}_{z-\Delta z}$ and $\mathbf{s}_{z+\Delta z}$ and P corresponds to holding point. The rotational axis can be calculated from $\mathbf{r} = \overrightarrow{PA} \times \overrightarrow{PB}$. Once $\mathbf{r}$ is known, the double-pass retardation can be solved as the angle δ between vectors $O'A$ and $O'B$ [10], where O' is the center of the polarization evolving circle.

Figure 2.6 | Phantom imaging using single input state PS-OCT. **a-b**, Schematic sketch of two layer phantom sample. Red arrow indicates the light scanning path. **c-d**, cumulative retardation images reconstructed by the conventional algorithms for single input state PS-OCT. **e-f**, local birefringence images reconstructed using the polarization holding effect theorem. Scalebars in **c** of **c-f**, vertical: 200 μm , horizontal: 400 μm .

To test the imaging performance, we made a phantom with two birefringent layers, respectively consisting of a long birefringent band and four elements with distinct birefringence levels (**Figure 2.6a,b**). The detailed procedure to make such a phantom has been reported. To compare the local birefringence measurements, the phantom was scanned from both sides (red arrows in **Figure 2.6a,b**). **Figure 2.6c,d** are the cumulative retardation images reconstructed by the conventional algorithms for single input state PS-OCT [57]. **Figure 2.6e,f** are local birefringence images reconstructed from the proposed method. We can see that local birefringence images (**Figure 2.6e,f**) provide a more insightful structure information of the phantom. In addition, comparing **Figure 2.6e** and **Figure 2.6f**, we confirm that the local birefringence value is correctly resolved in multi-layer samples. However, it should be mentioned for single input PS-OCT, blind areas exist in the image, termed as holding point artifacts. Specifically, when the output polarization state is overlapped with or close to the holding point, the local birefringence cannot be reliably reasoned.

The retinal nerve fiber layer (RNFL) is a fibrous structure lying on the surface of the retina. The thickness change of RNFL is a diagnostic indicator of glaucoma [58], additionally may also relate to other nerve diseases [59]. Clinically, the thickness of RNFL can be detected by scanning laser polarimetry [60] through cumulative retardation and OCT morphological imaging [61]. The PS-OCT

provides a technical advance to simultaneously evaluate both parameters measured by the two apparatus [62]. Moreover, recent investigations are trying to figure out whether the birefringence degradation happens before the thickness degradation, aiming for early diagnosis of glaucoma. However, it has been confirmed that the cumulative retardation is highly correlated with thickness [63]. Efforts have been directed to the measurement of the local birefringence image using two input states PS-OCT [54].

Figure 2.7 | Cross-sectional and en face images of swine retina. **a-c**, Cross-sectional intensity images. **d-f**, Cross-sectional local birefringence images. **g-i**, Cross-sectional relative optic axis projections. **j**, En face intensity projection of the RNFL layer, blue lines from left to right indicate locations of cross-sectional images **a**, **b**, **c**, respectively. **k**, Measured RNFL thickness heatmap. Dark tracks indicate position of the blood vessels. **l**, Cumulative retardation of the RNFL. **m**, Average local birefringence within the RNFL. **n**, En face projection of relative optic axis within the RNFL. Scalebars in **a** of **a-i**, vertical: 200 μm , horizontal: 400 μm . Scalebars in **j** of **j-n**, X: 400 μm , Y: 400 μm .

Knowing the polarization holding point, the measurement of local birefringence of RNFL can be achieved using the single input ultra-high-resolution OCT. We performed ex-vivo imaging on swine retina. **Figure 2.7a-i** are three sets of cross-sectional images of the swine retina around the nerve head (locations indicated by blue lines in **Figure 2.7j**), including the conventional intensity images (**Figure 2.7a-c**), local birefringence images (**Figure 2.7d-f**) and relative optic axis projection (**Figure 2.7g-i**). The RNFL can be clearly seen in the intensity images (**Figure 2.7a-c**). The local birefringence images further confirm the RNFL on the surface of the retina, with obviously higher birefringence than the tissue below. The relative optic axis images provide a relative contrast of the fiber orientation, which is more obvious in its en face projection (**Figure 2.7n**). **Figure 2.7j** is the en face intensity projection of the RNFL layer. We manually segmented the RNFL layer and measured the RNFL thickness, which is shown in **Figure 2.7k**. **Figure 2.7l** and **Figure 2.7m** show the cumulative retardation of the entire RNFL and the average local birefringence within the RNFL, respectively. Consistent with previously report [63], the cumulative retardation and morphological thickness of RNFL are highly correlated. However, we find that the local birefringence projection of the RNFL is decoupled with the thickness map, maybe providing more information to evaluate the nerve fiber structure.

2.4.2 Two input state PS-OCT

Many PS-OCT systems probe the sample with two input polarization states, either sequentially on successive A-lines using a polarization modulator [64], simultaneously by multiplexing in frequency [65,66], or by rapidly varying the polarization state within individual A-lines [67].

Figure 2.8 | Improvement of two input states PS-OCT using the holding point effect. **a**, Method reasoning diagram. **b**, Schematic sketch of two layer phantom sample. Orange arrow indicates the light scanning path. **c**, Optic axis projection of cross-sectional images from conventional reasoning. **d**, Optic axis projection of cross-sectional images from holding point reasoning. **e-g**, pixel value distribution of optic axis orientation in each birefringent region indicating in **c-d**. The birefringence of each region is indicated in the title of **e-g** as $\Delta n = n_e - n_o$. Scalebars in **c** of **c-d**, vertical: 200 μm , horizontal: 400 μm .

As we have demonstrated, if the holding point of the system is known, information from single input measurement is enough for reasoning optic axis orientation and local birefringence. However, one advantage of two input states PS-OCT can be free of holding point artifacts. For two input states PS-OCT, it is straightforward to get an idea to use the holding point to increase the accuracy of birefringence measurement. Shown in **Figure 2.8**, in a conventional two input states PS-OCT [10], the relative optic axis is solved by $\mathbf{r} = \mathbf{a}_3 \times \mathbf{a}_6$. When we know the holding point of each input state, we can solve the relative optic axis by a least square manner. According to reference [10], it's better to weight the vector by its length. The relative optic axis is solved by $\min(\mathbf{r}) \|\mathbf{A} \cdot \mathbf{r}\|_2^2$, where $\mathbf{A} = [\mathbf{a}_1, \dots, \mathbf{a}_6]^T$; $\mathbf{a}_1$ - $\mathbf{a}_6$ are 6 vectors labeled in **Figure 2.8a**.

Figure 2.8b shows the phantom image reported previously with known nominal birefringence values. We used the PS-OCT system reported before [24] with the benchtop scanning head to image the phantom. As a proof of concept, we used a ~ 30 nm bandwidth source by applying a tight Gaussian window to the fringe to avoid the complexity in polarization mode dispersion correction [68,69]. The holding point is measured by a calibration process like the experiment shown in **Figure 2.2**. **Figure 2.8c,d** show the optic axis projection of cross-sectional images from conventional reasoning (**Figure 2.8c**) and holding point reasoning (**Figure 2.8d**). The pixel value distribution of optic axis orientation in each birefringent region is shown in **Figure 2.8e-g**. We can see when the birefringence is small, the optic axis resolved by holding point reasoning (**Figure 2.8e**, yellow histogram) is more accurate than the conventional method [10] (**Figure 2.8e**, blue histogram). In addition, when the birefringence is high, these two methods provide similar results (**Figures 2.8c&d**, arrows **b, c**), though the holding point reasoning shows slightly more accurate, which can be seen from the distribution histograms (**Figure 2.8g**).

2.5 Discussion

Though our demonstrations focus on the application in biological birefringence imaging, we believe the application of the polarization holding effect can be wide and various. The observations of this effect originated from studies on optical fiber. Since the polarization evolution along the wavelength is an analogy to spatial polarization evolution in some cases, such as the polarization mode dispersion (PMD) measurements [70]. We noticed that the holding point also exists in the wavelength domain, which may help the measurement and analysis of PMD in fiber optical communication.

In birefringence imaging, the holding point effect opens new possibilities for single input state imaging. However, to our understanding, the holding point artifact is a main drawback, hindering the practical applications for fiber based systems, which is one of our next objectives to achieve. Moreover, for two input states PS-OCT, the holding point shows obvious improvement in optics axis reasoning when the local birefringence is low. In the retardation image, the improvement is limited because the retardation is anyway small with no regarding to accuracy of the optic axis measurement. Nevertheless, we believe there still exist potential benefits of knowing the system polarization holding point.

In this chapter, we reported a polarization holding effect in reciprocal unitary backscattering. Specifically, in an optical circuit without polarization dependent loss, the polarization state evolution of the light backscattered from remote targets is constrained by a holding point, which is determined by the polarization state of the input light. We theoretically proved and experimentally validated this effect. Furthermore, we demonstrated three applications related to medical birefringence imaging. This holding effect is an interesting behavior of light polarization evolution and can potentially contribute to the analysis, optimization and development of polarimetry systems.

Chapter 3 Tissue-like birefringence phantoms

3.1 Motivation

Resolution test targets and tissue-mimicking phantoms are essential to test, optimize, calibrate, and evaluate optical imaging systems [71]. The USAF 1951 resolution test target is frequently used to test an imaging system's lateral resolution. In optical coherence tomography (OCT), the axial resolution often exceeds the resolution in the lateral direction, and the axial point spread function (PSF) can be readily measured from a reflecting surface. To directly assess the three dimensional PSF, targets containing point-like scatters, such as laser marks or beads embedded in a transparent matrix can be used [72,73]. Beyond characterization of the PSF, to evaluate the structural and functional imaging performance, phantoms that mimic the optical, structural, and possibly mechanical properties of real biological tissue are highly valuable [71]. Curatolo *et al.* demonstrated an OCT phantom using lithographic casting to produce three-dimensional structures embedded in a silicone matrix [74]. Strupler *et al.* used a diamond-turned aluminum multi-segment mirror covered by a tissue-mimicking material along with a calibration algorithm to determine volumetric field-of-view, axial resolution, and image curvature [75]. Bisailon *et al.* discussed different strategies to create phantoms of coronary arteries [76]. To test imaging performance and software segmentation accuracy, Baxi *et al.* developed a phantom precisely replicating retinal morphology [77].

An important extension of conventional OCT is polarization sensitive OCT (PS-OCT) [22,78] which employs polarization-diverse detection to measure the polarization state of light backscattered by the sample. To make the measurements tolerant to layered samples with varying polarization properties, many PS-OCT

systems probe the sample with two input polarization states, either sequentially on successive A-lines using a polarization modulator [79], simultaneously by multiplexing in frequency [66,80], or by rapidly varying the polarization state within individual A-lines [81]. PS-OCT complements the conventional backscatter intensity images with additional contrast, such as retardation [10], degree of polarization (DOP) [40,82], and optic axis orientation [83]. Birefringence corresponds to the difference of the refractive indices ($\Delta n = n_e - n_o$) experienced by light prepared along the extraordinary (n_e) and the ordinary (n_o) polarization states, respectively. In a uniaxial material without diattenuation, these two principle states are orthogonal to each other. Retardation, δ , denotes the phase delay accrued over a distance L between light polarized along each of the two principle states, and is related to the birefringence by $\delta = 2\pi\Delta nL/\lambda$, with λ the central wavelength of the light. In biological tissues, birefringence occurs mostly in regularly arranged fibrillary tissues, such as collagen, muscle, or myelin. Accordingly, PS-OCT, recently reviewed by de Boer *et al* [17]. and Baumann [46], has attracted attention for imaging a range of samples, including the cornea [84], retina [41], skin burns [85] and scars [44], airway smooth muscle [86], coronary atherosclerosis [42], and breast tumor [87,88].

Over time, an array of different PS-OCT implementations and reconstruction methods has been developed. For their validation, wave plates [89] and biological tissues, such as muscle [57] or tendon [53], are frequently used as test samples. Although a wave plate is stable and well-controlled, its specular reflections are a poor approximation of the backscattering properties of biological tissues and do not necessarily reveal the shortcomings of a PS-OCT imaging system [90]. Muscle and tendon generate the typical speckle signal of biological tissue and exhibit birefringence. However, their nominal amount and distribution of birefringence are unknown, and they are perishable, precluding repeated and controlled assessment and cross-validation of PS-OCT imaging systems.

We previously have used rubber phantoms for PS-OCT [24,82]. By stretching the rubber sample to different lengths, we could control the amount of stress-induced birefringence and the optic axis orientation. However, controlling the applied stress and holding the sample in the stretched position proved to be challenging and complicated the assembly of phantoms containing regions with distinct birefringence levels. Furthermore, for un-vulcanized stretched rubber samples, we observed significant birefringence decay within just a few hours due to stress relaxation, impeding attempts to produce stable phantoms with well-defined amounts of birefringence.

In this chapter, we describe a method to fabricate robust and durable phantoms containing spatially defined elements of predetermined birefringence and optic axis orientation. We developed an approach to induce a controlled amount of stable birefringence in polycarbonate films, overcoming the drawbacks encountered with the rubber. We present phantoms comprising multiple elements of distinct birefringence in two layers with different optic axis orientation, tailored for either galvanometer-based or catheter-based PS-OCT imaging. We performed depth-resolved birefringence imaging of the phantoms using our implementation of PS-OCT [24] and analyzed the birefringence of the different elements.

3.2 Fabrication process

3.2.1 Imaging system

Throughout this work, we used a polarization-modulated, fiber-based optical frequency domain imaging (OFDI) setup, similar to the one described in [24]. In short, the system employed a wavelength-swept laser source centered at 1300 nm, with a tuning range of 115 nm, leading to an axial resolution of $< 10 \mu\text{m}$ in tissue, and a repetition rate of 103.6 kHz. An acousto-optic modulator (AOM) was inserted in the reference arm for resolving depth degeneracy. The polarization of the light in the sample arm was modulated with an electro-optic modulator between

linear and circular polarization states in adjacent A-lines, before directing the light through a circulator to either a benchtop unit, composed of two galvanometric mirrors and a scan lens (LSM03, Thorlabs), or a motor drive unit (Terumo) interfaced with commercial intravascular catheters (FastView, Terumo).

Recorded data were processed with spectral binning [24] to obtain maps of depth-resolved birefringence and DOP. We used one-fifth of the original spectral bandwidth (i.e., nine overlapping spectral bins), a lateral Gaussian filter having a full width at half maximum equal to 12 adjacent A-lines, and an axial offset of 48 μm to derive birefringence. A reduction of the DOP indicates a randomization of the detected polarization states within a small neighborhood around each pixel. This depolarization can serve as an independent contrast mechanism [40], but strong depolarization also precludes the reconstruction of meaningful birefringence. We used DOP to validate the reliability of the reconstructed birefringence. Birefringence and DOP are displayed using a near isoluminant color map, overlaid with the intensity tomogram [24].

For birefringence imaging with PS-OCT, an ideal phantom should 1) exhibit sufficient back-scattering to generate an appreciable OCT signal; 2) contain several laterally and axially well-defined regions with distinct, but homogenous birefringence; 3) have a non-birefringent scattering host matrix. The scattering properties of both the birefringent elements and the background matrix should result in a fully-developed speckle pattern in the reconstructed tomogram to best mimic biological tissues. The spatial confinement of the birefringent elements enables segmentation and evaluation of the accuracy of birefringence measurement within these areas.

For single input state PS-OCT, which reconstructs cumulative retardation and assumes a constant orientation of the optic axis along depth, a single birefringent layer would be sufficient. Recently, increased effort has been directed to reconstructing local retardation (i.e. birefringence) [33], offering a more intuitive

view of tissues having a layered architecture. Furthermore, the optic axis orientation can also be reconstructed in a depth-resolved manner [83]. To accommodate these capabilities, we aimed for two axially superposed birefringent layers with distinct optic axis orientations, as shown schematically in **Figure 3.1**. One layer is a long birefringent band, and the other consists of four birefringent elements with distinct birefringence levels. The angle of the optic axes between the two birefringent layers is maximized at 45 degrees. The birefringent elements are embedded into a non-birefringent matrix and offer a convenient geometry for benchtop imaging. To also enable catheter-based PS-OCT imaging, we integrated a similar arrangement of birefringent elements into the background matrix coated along a glass capillary tube.

Figure 3.1 | Birefringent elements arrangement in the phantom. Layer 1 consists of a long birefringent band; Layer 2 consists of four birefringent elements with distinct birefringence levels.

3.2.2 Annealing, heating and stretching

Stress induces birefringence in many polymers by increasing the alignment of the polymer chains, an effect known as photo-elasticity. The birefringence Δn across a band of photo-elastic material is linearly correlated to the stress σ [91]:

$$\Delta n = C\sigma, \quad (3.1)$$

where C is known as the stress optical coefficient. Further, the stress is linearly related to the strain for an elastic material. We have previously used the elasticity of rubber latex to conveniently generate controlled amounts of birefringence. However, for un-vulcanized rubber samples, we observed a slow but significant decay of the induced birefringence continuing for hours and days. We attribute this to the two competitive effects that are taking place in a stretched rubbery material, as illustrated in **Figure 3.2(a)**. The first effect is elastic deformation, which increases the alignment of the polymer chains, thereby creating birefringence; the second is viscous flow, whereby molecule chains slide against each other and decrease the birefringence. This second effect leads to the so-called “stress relaxation” in viscoelastic materials.

The rubbery state of polymers is an intermediate phase between the glassy solid state and the liquid phase, as summarized in **Figure 3.2(b)**. The temperature separating the glassy state from the rubbery state is the glass-transition temperature. Un-vulcanized rubber (polyisoprene, glass-transition temperature of -70 °C [92]) resides in the rubbery phase at room temperature and exhibits the typical viscoelastic properties of this state.

Figure 3.2 | (a) Behaviour of polymer chains of rubbery state polymers under tension. (b) Physical state transitions in polymers as a function of temperature. Figures reproduced from [52]

To overcome the shortcomings of the rubber’s viscoelasticity, we opted for a polymer with a higher glass-transition temperature, i.e. above room temperature. We chose polycarbonate (PC), which has a glass-transition temperature of 145°C. Both scattering and transparent PC films are commercially available with various thickness options. Here, we used films with a thickness of 250 μm (CT301326, Goodfellow Inc). Scattering PC films generate a suitable OCT signal with fully-developed speckle and are easy to cut to desired dimensions using scissors or a paper trimmer. Heating PC above 145°C and converting it to the viscoelastic state enables controlled stretching without necessitating significant force. Cooling the stretched material and returning it to the glassy state ‘freezes’ the molecular arrangement and maintains the induced birefringence, but releases the contracting force induced by the elastic deformation.

The manufacturing process of PC films appears to induce residual stress that results in a birefringence pattern visible in cross-sectional images (**Figure 3.3(a)**). To create samples with homogeneous and controllable birefringence and optic axis orientation, the residual birefringence should be removed before stretching. Taking advantage of the stress relaxation phenomenon, we cut the PC film into 10-mm wide strips, sandwiched them between two metal plates, and put them into an oven at 150°C for 24 hours of annealing. This process completely removes the residual

birefringence stress and provides samples with homogenously low birefringence at baseline (**Figure 3(b)**).

Figure 3.3 | PS-OCT cross-sections showing (a) birefringence patterns present in commercial PC films due to residual stress and (b) homogenously low birefringence after annealing strips of PC. Horizontal and vertical scale bars: 2 mm and 250 μm , respectively. Figures reproduced from [52].

The schematic of the setup used to stretch PC strips, along with the detailed procedure, is shown in **Figure 3.4(a)**. We used weights secured directly to the PC strip to apply a constant traction force. Rails were used to avoid swinging of the weights. To apply stress to the PC strip only after the transition to the rubbery state, we placed the entire stretching setup in the oven at 150°C , while supporting the weights with a spacer. After heating for 30 minutes, the spacer was removed to apply the stress, which elongated the strip. The amount of strain was controlled by the height of the spacer. Once the weights reached their lower position, we opened the door of the oven and cooled down the setup and the PC sample immediately.

The stress, σ , is defined by the force of gravity pulling down the weights, G , and the cross-section of the strip, A , and is given by $\sigma = G/A$. To obtain birefringence comparable in magnitude to that of most biological tissues (i.e. 0 to $\sim 2 \times 10^{-3}$), a strain of $\sim 5\%$ proved to be sufficient for PC. Therefore, the change of cross-section during the stretching can be neglected, and the retardation δ can be controlled by adjusting the width w of the strip as:

$$\delta \propto 1/w. \quad (3.2)$$

In practice, it is challenging to accurately adjust the elongation. However, the width of each strip can be varied to generate different levels of birefringence while applying the same amount of elongation (**Figure 3.4(b)**). The strips can then be cut

into smaller elements suitable for integration into the final phantoms. Specifically, in our experiment, we prepared a series of PC strips with widths ranging from 2 to 7 mm. The strips were then cut into smaller rectangular areas (2×4 mm for slab and 1×2 mm for cylindrical phantoms), and assembled into phantoms.

Figure 3.4 | (a) Schematic of the stretching apparatus and steps of pre-heating, stretching, and cooling. (b) Photograph of three PC strips of varying widths after stretching along with corresponding cross-sectional PS-OCT images showing the obtained birefringence levels. Figures reproduced from [52].

3.2.3 Matrix

Suitable candidate matrix materials for optical phantoms have been reviewed by Pogue *et al.* [93]. For our application, we chose epoxy (20-3440-032, Buehler Inc.), which is durable, transparent, and non-birefringent. More importantly, the epoxy resin is initially liquid and solidifies within 9 hours of curing after mixing with the epoxy hardener (20-3442-016, Buehler Inc.), allowing a convenient time window to cast the phantoms. Specifically, to make the matrix, we mixed 40 ml epoxy resin with 0.5 g TiO_2 powder (634662, Aldrich Inc.). To uniformly mix the particles into

the epoxy, we ultrasonicated the mixture for 2 hours in a 60°C water bath. The hardener was then added to the resin at a volume ratio of 1:2, and poured into the mold. Molds were prepared from rubber latex (S25719, Fisher Science Education) to define the outer shape of the phantom. Rubber latex is frequently used to make molds, and is easy to separate from the cast after curing. The birefringent PC elements were placed and arranged within the mold as designed in **Figure 3.1** before pouring the epoxy. Slab phantoms were straightforward to cast, using an open rectangular mold. For cylindrical phantoms, we used glass capillaries (22-260-943, Fisher Scientific Inc.) as the innermost layer. The birefringent elements were glued to outer surface of the capillary using epoxy. The capillary with the attached birefringent elements was then embedded in a cylindrical rubber mold and immersed in epoxy.

3.3 Phantom imaging

To verify the relation between stress and induced birefringence, we prepared a wedge-shaped PC strip, as shown in **Figure 3.5(a)**. Its linearly varying width is expected to result in an inversely related retardation. The resulting birefringence in the en-face projection indeed exhibits the expected behavior (**Figure 3.5(b)**). However, reconstruction of the local retardation has its own limitations and potential artifacts. To more directly assess the retardation of the light passing through the stretched wedge sample, we sandwiched the sample between two glass slides and determined the polarization state of the light reflected from the first surface of the top slide and the second surface of the bottom slide (**Figure 3.5(c)**). To reduce the strong specular reflections and avoid detector saturation, the entire sample assembly was immersed in water for imaging. The two modulated input polarization states of our PS-OCT instrument allowed computation of the cumulative retardation between these two interfaces. Limiting the analysis to the central area along the strip (dot-dashed blue line in **Figure 3.5(b)**), averaging between 20 adjacent A-lines along the width, and unwrapping the retardation from

the widest side of the strip resulted in a linear relation between $1/w$ and the retardation ($R^2 = 0.997$), as shown in **Figure 3.5(d)**. This confirms the expectation and demonstrates that the birefringence can be quantitatively controlled by adjusting the width of the strip.

Figure 3.5 | (a) Photograph of the wedge sample after stretching and (b) matching en-face projection of birefringence obtained from volumetric PS-OCT dataset. (c) A representative cross-sectional image illustrating the reference surfaces (arrows) used to measure the cumulative retardation. Horizontal and vertical scale bars: 1 mm and 250 μm , respectively. (d) Measured cumulative retardation against $1/w$, where w is the width of the strip, along with linear regression. Figures reproduced from [52].

3.3.1 PS-OCT imaging

Figure 3.6 presents intensity, birefringence and DOP images obtained from a slab (a-c) and cylindrical (d-e) phantom, respectively. For the slab phantom, **Figure 3.6(a)** displays cross-sections through all the birefringent regions. **Figures 3.6(b) & (c)** are two en-face images, whose depth locations are indicated in **Figure 3.6(a)**. **Figure 3.6(d)** shows cross-sections of the cylindrical phantom imaged with a rotational scan of the side-looking fiber probe in the intravascular catheter. **Figure 3.6(e)** presents a corresponding longitudinal section generated from the volumetric data set recorded while rotating and pulling back the fiber probe.

Backscattering from the birefringent segments is slightly stronger than from the matrix, and already delineates the individual elements. The birefringence maps present more clearly the individual elements and their varying birefringence levels. The DOP indicates uniform polarization states, except at the vertical edges of the embedded segments. Although the refractive indices of the PC strips and the matrix material appear very close, it is likely their residual difference that perturbs the polarization states in these areas. This effect is more pronounced in catheter-based imaging, where the probing beam propagates through this interface, not merely along it as in case of benchtop imaging. Nevertheless, within the birefringent elements, the DOP remains high, validating the reconstructed measures of birefringence.

Figure 3.6 | Intensity, birefringence and DOP images of the slab (a-c) and cylindrical (d-e) phantoms. (a) Representative cross-sectional images of the birefringence phantom for galvanometer-scanning system. (b) & (c) En-face images at different depths as indicated by the dashed red lines in (a). Horizontal and vertical scale bars for (a-c): 2 mm and 250 μm , respectively. (d) Representative images obtained from one rotational scan with the catheter. Scale bar: 1 mm. (e) Longitudinal sections obtained from a pull-back data set, with its corresponding location indicated by the dashed red line in (d). Radial and horizontal scale bars: 250 μm and 1 mm, respectively. Figures reproduced from [52].

3.3.2 Image analysis

The clear spatial definition of the homogeneously birefringent elements enables convenient quantitative analysis of these regions. Using the slab phantom, we manually segmented three-dimensional regions of interest by identifying the corners of each birefringent element. **Figures 3.7(a) & (b)** display volume renderings of the elements and their segmentations. For each segment, the histogram of the measured birefringence was computed. Results for all regions are displayed in **Figure 3.7(c)**. The mean of measured birefringence values in each segmentation is regarded as the nominal Δn of each element. The standard deviation (std) offers insight into the measurement uncertainty.

Figure 3.7 | (a) & (b) Volume rendering of the slab phantom along with the segmentation of the individual birefringent elements. (c) Histogram of the birefringence measurements for each segmented region. std: Standard deviation.

3.4 Discussion

Reconstruction of cumulative retardation or birefringence in PS-OCT is subject to many system and processing-dependent artifacts. The birefringence phantoms that we fabricated generate a tissue-like backscattering signal and contain spatially confined areas with a homogeneous, predictable amount of birefringence, in a range comparable to the birefringence reported in biological tissues such as muscle and collagen [94,95]. These phantoms can help to benchmark different PS-OCT architectures and reconstruction strategies. Segmentation of the individual elements facilitates quantitative analysis and enables comparison of imaging systems. The stability of the phantoms even allows comparing systems that are in separate locations, and could enable calibration of birefringence measurements.

Although we put great effort into generating homogeneously birefringent elements, it is likely that a residual variation within the strips remains, which may be more appreciable with higher-resolution PS-OCT. The unique capability of PS-OCT to measure polarization signatures from scattering media makes it challenging to independently validate the generated levels of birefringence. The scattering properties of the utilized PC films exclude polarized light microscopy. Transparent PC films feature a different stress optical coefficient. And cutting thin slices of the material may induce additional stress that alters the observed birefringence. Whereas our current analysis focused on birefringence, these phantoms also enable assessing the reconstruction of the optic axis orientation. Similar phantoms could also be used for other implementations of tissue polarimetry, where stretched elastic materials have been used to induce birefringence [96].

The PC material used in this study generates tissue-like scattering in the 1300-nm wavelength region. Preliminary imaging with a system operating at 800 nm suggested that the resulting backscattering may be too strong, compromising the typical OCT imaging depth. Acrylonitrile butadiene styrene (ABS) films (5751T,

McMaster-Carr) offer an interesting alternative in this case. This ABS film is slightly more transparent, resulting in better imaging performance at 800 nm, and can be stretched above its glass-transition temperature (105 C°) in the same way that PC can be processed. In addition, we also noticed that in order to induce the same amount of birefringence, the required elongation for ABS is slightly larger than that of PC.

Chapter 4 Polarization management in OCT

4.1 Motivation and background

Flexible fiber-optic based imaging probes, including handheld scanning head, medical-compatible endoscopes and intravascular catheters have significantly broadened the clinical application scope of optical coherence tomography (OCT), enabling high-resolution imaging of several regions in human body. A typical fiber-optic probe contains a single mode fiber (SMF) cable of several meters' length, to deliver the probing light to the region of interest and return the light backscattered from the tissue structures. SMF provides OCT probes with a high degree of flexibility. Furthermore, circumferential or linear scanning can be achieved with an actuator at the proximal end, such as optical rotary junction and pull-back translation stage, by mechanically conveying the scanning motion from the proximal end to the imaging spot through the probe structure [97].

Light propagating in SMF is supported by two orthogonal polarization modes [70]. Due to the asymmetries of fiber geometry and stress, such as elliptical cross-sections, bending and twisting, the light of two polarization modes travels at different phase velocities, resulting in the presence of fiber birefringence. Thus, in OCT probes, the polarization states of light at the output of SMF are unpredictable and very sensitive to probe motion [98]. Scanning and flexing of the OCT probes results in variations in the polarization states of light from the sample arm [98]. When the polarization states of light from the sample are misaligned with those of the reference arm, the interference fringes would fade out, leading to intensity variations and SNR degradation in OCT images [99].

Early OCT devices used Faraday rotators to compensate the birefringence of the round-trip SMF [100], which is impossible to be integrated into a miniature probe.

Polarization diversity detection (PDD), usually using two sets of detectors, respectively aligned with two orthogonal polarization directions, can thereby eliminate the fringe fading effect [99]. However, the PDD scheme of current polarization sensitive OCT (PS-OCT) increases the system complexity and cost. Particularly for spectral-domain OCT systems, PDD needs two pixel-aligned, synchronized spectrometers, tremendously increasing the cost of the entire setup. Besides the PDD approach, common path probe is free of the fading problem [101], which relies on the advance of micro-optics to integrate the reference reflector into the probe distal end.

In this chapter, from the statistical view, we characterized the intensity fluctuation and average signal-to-noise ratio (SNR) loss induced by the fringe fading of the sample arm polarization randomization. By taking advantage of an interesting observation, that the output polarization states of round-trip sample arm SMF are not uniformly distributed on the Poincare sphere [102], an optimum polarization state of reference arm can be found for the Michelson-type OCT configuration. From our analysis, we also suggested that the light source with low degree of polarization such as super-continuum source, or light source with long fiber, should be carefully managed to achieve the optimal SNR, which is consistent with previous observations [103]. We demonstrated our optimal polarization management, using two additional polarizers, could statistically provide a 1.5 times increase in fringe amplitude, corresponding to 3.5 dB increase in system sensitivity.

Figure 4.1 | **a.** Schematic of a fiber-coupler based OCT setup; **b.** Stokes representation of misalignment of reference and sample polarization states on the Poincare sphere; **c-e.** Three fading cases with different A , where A is the angle between the polarization states of reference s_{ref} and sample s_{mirror} (a mirror) on the Poincare sphere. F_x and F_y were simulated fringes obtained from two spectrometers (SP X and SP Y), $F_x + F_y$ was a simulation of fringe if using a single spectrometer (Single SP). SP: spectrometer, PBS: polarizing beam splitter. Figures reproduced from [104].

4.2 Polarization modelling and validation

Figure 4.1a shows the scheme of a fiber-coupler based OCT setup. Assuming a fully polarized light source and neglecting the polarization mode dispersion (PMD), we have $\mathbf{j}_{ref} = \mathbf{J}_{out} \mathbf{J}_{ref}^T \mathbf{J}_{ref} \mathbf{j}_{in}$, and $\mathbf{j}_{sam} = \mathbf{J}_{out} \mathbf{J}_{sam}^T \mathbf{J}_{tis} \mathbf{J}_{sam} \mathbf{j}_{in}$, where the superscript T stands for transpose operation. $\mathbf{j}_s$, $\mathbf{j}_{ref}$ and $\mathbf{j}_{sam}$ are the Jones vectors of the input light, the parts of the output light from the reference arm and sample arm, respectively. $\mathbf{J}_{in}$, $\mathbf{J}_{ref}$, $\mathbf{J}_{sam}$ and $\mathbf{J}_{out}$ are the single-trip Jones matrices of the four fiber tails of the coupler, labeled in **Figure 4.1a**. $\mathbf{J}_{tis}$ is the round-trip Jones matrix of the tissue. According to [105], $\mathbf{J}_{tis} = \int \chi(z) \begin{bmatrix} e^{i2k n_o z} & 0 \\ 0 & e^{i2k n_e z} \end{bmatrix} dz$, where $\chi(z)$ is the scattering profile along the depth z . In the context of a non-PS-OCT, the tissue is assumed to be non-birefringent. Taking $n_o = n_e = n$, $\mathbf{J}_{tis}$ is degenerated to an identity matrix with a factor $F = \int \chi(z) e^{i2k n z} dz$. The detected complex fringe $f(k)$ after background subtraction can be written as

$$\begin{aligned} f(k) &= \mathbf{j}_{ref}^\dagger \mathbf{j}_{sam} = (\mathbf{J}_{out} \mathbf{J}_{ref}^T \mathbf{J}_{ref} \mathbf{j}_{in})^\dagger (\mathbf{J}_{out} \mathbf{J}_{sam}^T (F(k) \mathbf{I}) \mathbf{J}_{sam} \mathbf{j}_{in}) \\ &= \mathbf{j}_{ref}^\dagger \mathbf{j}_{mirror} F(k) \end{aligned} \quad (4.1)$$

where $\mathbf{j}_{mirror}$ is the Jones vector of the light polarization state when the sample is a single reflector. The superscript $\dagger$ is the conjugate transpose. The measured depth profile can be obtained by the inverse Fourier transform of $F(k)$. $\mathbf{j}_{ref}^\dagger \mathbf{j}_{mirror}$ is a complex number, thus eq. (1) can be written in the form of

$$f(k) = P \exp(i\psi) F(k) \quad (4.2)$$

From eq. (2), the polarization state misalignment between the reference and sample arms adds a fading coefficient P and a phase ψ to the complex interference fringe. Transforming the Jones vectors $\mathbf{j}_{ref}$ and $\mathbf{j}_{sam}$ to their Stokes representations $\mathbf{s}_{ref}$, and $\mathbf{s}_{mirror}$, we found

$$P = \cos(A/2) \quad (4.3)$$

where A is the angle between $\mathbf{s}_{ref}$ and $\mathbf{s}_{mirror}$ on the Poincare sphere, indicated in **Figure 4.1b**. It is more straightforward to interpret the fading effect by the misalignment angle A in the Stokes representation, shown in **Figures 4.1b-e** with several specific cases, no fading (**Figure 4.1c**), half fading (**Figure 4.1d**) and full fading (**Figure 4.1e**).

The reference arm is usually stationary, making $\mathbf{s}_{ref}$ a fixed point on the Poincare sphere. While in a flexible probe system, $\mathbf{s}_{mirror}$ is constantly changing during the scanning. For different A-lines, the fading coefficient P is random. From the statistical view, the statistical distribution of P determines the average SNR loss induced by the fading effect.

A white light Michelson-type interferometer with PDD shown in **Figure 4.2a** was built to study SMF induced polarization randomization. Light from a supercontinuum source was modified to be circular polarized after passing through a polarizer (P1) and a quarter wave plate (QWP), and then was divided into two beams, focused into the reference and sample arms respectively. Each arm contained an identical 1.5 m-long SMF cable and a back-reflector, to balance the material dispersion between the two arms. By inserting a 45° polarizer (P2) before the reference arm fiber, the birefringence effect of the reference arm was eliminated. To simulate a flexing probe, a 3-paddle polarization controller (PC, Thorlabs, FPC030) was used to randomly twist and bend the sample arm SMF. The interference fringes at two orthogonal polarization directions, $F_x(k)$ and $F_y(k)$ were recorded by two carefully aligned customized spectrometers, responding to a wavelength band of 680 - 900 nm. Borrowed from the PMD measurement methodology of PS-OCT [68], the Jones vector $\mathbf{j}_{mirror}(k)$ along the wavelength of the round-trip sample light can be measured as $[\mathcal{H}[F_x(k)] \quad \mathcal{H}[F_y(k)]]^T$, where $\mathcal{H}[\cdot]$ is the Hilbert transformation. To mimic the output of a non-PS-OCT, we directly added the fringes from two spectrometers, $F_{nops}(k) = F_x(k) + F_y(k)$. The fading coefficient P was measured as the ratio between the peak amplitude of $\mathcal{F}[F_{nops}(k)]$

and the summation of peak amplitudes of $\mathcal{F}[F_x(k)]$, $\mathcal{F}[F_y(k)]$, where $\mathcal{F}[\cdot]$ is the Fourier transformation.

Figure 4.2 | **a.** Setup for measurement of the round-trip SMF polarization randomization; **b.** Measured fading coefficient P against $\cos(A/2)$; **c.** Measured DOP distribution; **d.** Measured DGD distribution and theoretical Rayleigh PDF. QWP: quarter wave plate, P1-2: polarizers, BS: beam splitter, PBS, polarizing beam splitter, L1-6: lenses, M1-3: mirrors, SP: spectrometer, PC: polarization controller, SMF: single mode fiber, DOP: degree of polarization, DGD: differential group delay. Figures reproduced from [104]

We recorded 200 fringes with randomly different positions of the polarization controller paddles. **Figure 4.2b** is the measured fading coefficient P against the value of $\cos(A/2)$, where A is the misalignment angle between vectors $\mathbf{s}_{\text{ref}}$ and $\mathbf{s}_{\text{mirror}}$ on the Poincare sphere. Though the overall measured points were regressing well to the theoretical linear relation (red line in **Figure 4.2b**), it was noticed that disagreement existed in some individual points. One reason of this deviation may be the PMD effect of the fiber, which means the $\mathbf{s}_{\text{mirror}}$ were varying at different wavelengths. Thus the fading coefficient P was varying along the k -space. We used the degree of polarization (DOP) to characterize the PMD effect over the entire spectrum. In the Stokes representation, the DOP is defined as $DOP = \sum_k \sqrt{s_1^2(k) + s_2^2(k) + s_3^2(k)} / \sum_k s_0(k)$. From the DOP-color-encoded measurement points (**Figure 4.2b**), lower DOP resulted in larger deviation. In **Figure 4.2c** we counted the distribution of DOP of the 200 measurements. The measured DOP was high (close to 1) in most cases. Therefore, it is acceptable to ignore the PMD effect in the theoretical analysis and numerical simulation. To further quantify the PMD effect and the birefringence of the round-trip sample arm fiber, we modelled this fiber as a pure retarder [106] and reconstructed the Jones matrix $\mathbf{J}_{\text{sam}}$ of a particular wavelength (860 nm in this calculation). By $\text{DGD} = 2\sqrt{\det(d(\mathbf{J}_{\text{sam}})/d\lambda)}$ (Where $\det$ is the determinant of a matrix), we measured the differential group delay (DGD) and plotted its distribution in **Figure 4.2d**. The mean DGD was measured to be 9.9 fs and the distribution was a good fit (p value = 0.46 with χ^2 test) to a Rayleigh distribution, which is the theoretical probability density function (PDF) of DGD [106]. The PMD measurement is necessary to confirm the birefringence of 1.5 m fiber was large enough to fully randomize the output light polarization state. In addition, as previously reported [103], PMD effect results a variation of the fading coefficient P in k -space, inducing amplitude and phase modulations on the fringe, which degrades the axial point spread function.

4.3 SNR optimization

As reported in several literatures [48,102,106], an interesting observation was that the output polarization states of the round-trip light from a SMF cable were not evenly distributed on the Poincare sphere, which can be observed in **Figure 4.3a** where the k-space-average polarization states of the 200 measured fringes were plotted on the Poincare sphere. We can see polarization states tended to concentrate to the point $[-0.54 \ -0.23 \ -0.8]^T$. To further study this observation, we numerically simulated the process of monochromatic light travelling a round trip in a piece of SMF fiber. The fiber was modelled using a randomly generated Jones matrix following [55]. Based on 5000 runs of simulation, two statements were concluded. (1) For round-trip SMF with an arbitrary input polarization state, $[s_1 \ s_2 \ s_3]^T$ in Stokes representation, the highest probability output polarization state will be $[s_1 \ s_2 \ -s_3]^T$, on the contrary, the lowest probability point will be $[-s_1 \ -s_2 \ s_3]^T$. (2) The probability of the output polarization state would be mono-decreasing with its angle to $[s_1 \ s_2 \ -s_3]^T$ on the Poincare sphere.

Based on the above observation and eq. (3), there exists an optimal output reference polarization state maximizing the average SNR of the fringes. We noted the optimal reference polarization state as $\mathbf{s}_{ORPS}$ (Stokes vector of optimal reference polarization state). Based on our analysis, $\mathbf{s}_{ORPS}$ should be equal to $[s_1 \ s_2 \ -s_3]^T$, given $[s_1 \ s_2 \ s_3]^T$ is the input state. We took three cases to illuminate and evaluate this optimization. For each case, the angle between $\mathbf{s}_{ref}$ and $\mathbf{s}_{ORPS}$ on Poincare sphere, noted as A_{ref} , was 0° , 90° and 180° , respectively. We estimated the PDF of the fading coefficient P in each case using the kernel density estimation method. Based on the 5000 runs of numerical simulation, without regard to the PMD effect, using the ‘box’ kernel, the empirical PDFs of P in each case were plotted in **Figures 4.3b-d** with green lines, with the mean and variance labeled by their sides. In the optimal case (**Figure 4.3b**), the average SNR is 3.97 dB higher than the worst case (**Figure 4.3d**).

Figure 4.3 | a. Normalized polarization states distributed on the Poincare sphere of a piece of round-trip SMF. The input polarization state was s_{input} . Most output polarization states were distributed close to s_{ORPS} ; **b-d.** The empirical PDFs based on numerical simulation and corresponding measured histograms of the fading coefficient P , respectively when $A_{ref} = 0^\circ$, 90° and 180° . A_{ref} is the angle between the reference output s_{ref} and s_{ORPS} . Figures reproduced from [104]

To verify the results of the numerical simulation, we used the previously measured 200 Stokes vectors to test the empirical PDFs. In our setup, the sample arm input polarization state was expected to be right circular polarized, which fell at $+V$ point on the Poincare sphere. Therefore, the optimum reference output polarization state should be left circular polarized, at $-V$ point on Poincare sphere. Due to imperfection of the optical components and optics alignment, there obviously existed an error. The s_{ORPS} was measured to be $[-0.54 \ -0.23 \ -0.8]^T$. To match the three simulation cases, we calculated the angles between the measured Stokes vectors and three polarization states of $[-0.54 \ -0.23 \ -0.8]^T$ ($A_{ref} = 0^\circ$), $[0.58 \ 0.58 \ -0.56]^T$ ($A_{ref} = 90^\circ$), and $[0.54 \ 0.23 \ 0.8]^T$ ($A_{ref} = 180^\circ$). We plotted the histograms together with respect to empirical PDFs, shown in **Figures 4.3b-d**.

However, not all light sources are fully polarized. An extreme example is super-continuum sources, which inherently generates light by a random process, thereby producing unpolarized light. Other cases such as superluminescent diodes (SLD) produce substantially polarized light. Meanwhile, many light source manipulations would depolarize the light, such as the light combination of an SLD array, or simply passing the light through a long piece of SMF.

Fully unpolarized light source can be modelled as a combination of two cross-polarized beams without statistical correlation between each other [107]. We assume $\mathbf{j}_{ref}$ and $\mathbf{j}_{mirror}$ are the corresponding arbitrary Jones vectors if the source is switched to fully polarized. Given that the cross-polarization state of a Jones vector $\mathbf{j}$ is $(\text{anti-diag}\{-1,1\}) \cdot \mathbf{j}^*$, where $\text{anti-diag}\{\cdot\}$ is an anti-diagonal matrix. Superscript $*$ is the conjugate operator. Therefore, for OCT using unpolarized light, eq. (1) can be re-written as

$$\begin{aligned} f_{upo}(k) &= \frac{\mathbf{j}_{ref}^\dagger \mathbf{j}_{mirror}}{2} + \frac{\left(\begin{pmatrix} 0 & -1 \\ 1 & 0 \end{pmatrix} \mathbf{j}_{ref}^* \right)^\dagger \left(\begin{pmatrix} 0 & -1 \\ 1 & 0 \end{pmatrix} \mathbf{j}_{mirror}^* \right)}{2} \\ &= \frac{\mathbf{j}_{ref}^\dagger \mathbf{j}_{mirror}}{2} + \frac{\mathbf{j}_{mirror}^\dagger \mathbf{j}_{ref}}{2} \\ &= \Re[\mathbf{j}_{ref}^\dagger \mathbf{j}_{mirror}] F(k) \end{aligned} \quad (4.4)$$

where $\Re[\cdot]$ is the real part. $f_{upo}(k)$ is the detected complex fringes after background subtraction. We cannot find a concise representation for the fading coefficient P_{upo} in Stokes. From $P_{upo} = \Re[\mathbf{j}_{ref}^\dagger \mathbf{j}_{mirror}]$, we can see that the probability distribution of P_{upo} is also affected by the $\mathbf{j}_{ref}$, meaning that we can change the distribution of P_{upo} by changing the reference SMF. However, there is no any obvious configuration to maximize P_{upo} . Specifically, no matter how we configure $\mathbf{j}_{ref}$, the distribution of P_{upo} is always nearly central-symmetric with a mean around 0.5.

We built up a beam-splitter-based single-spectrometer OCT system to demonstrate the proposed optimal polarization management. Shown in **Figure 4.4a**, the light source was a super-continuum source centered at 800 nm. A free-space beam-splitter was used for convenient management of the polarization states. Two polarizers were inserted into the input port (P1) and the reference port (P2). Based on our analysis, if the input polarization state $\mathbf{s}_{in}$ is linear polarized, the optimal reference output polarization state $\mathbf{s}_{ORPS}$ should be equal to $\mathbf{s}_{in}$. Thus, the optimization can be achieved by aligning the optic axes of the two polarizers, P1

and P2, forcing $\mathbf{s}_{ref} = \mathbf{s}_{in} = \mathbf{s}_{ORPS}$. We recorded 1024 fringes while randomly changing the polarization controller (PC) in the sample arm. To make a comparison, we also recorded fringes under other two configurations. One was a sub-optimal configuration where the angle of the two polarizers were around 45° , corresponding to $A_{ref} = 90^\circ$, the other was using an unpolarized source achieved by removing the two polarizers (P1 & P2). In all these recordings, the light intensity illuminated on the detectors from the sample and reference arms were adjusted to be identical. The measured normalized fading coefficients are shown in histograms in **Figure 4.4b**. Conforming with our prediction, aligning $\mathbf{s}_{ref}$ to $\mathbf{s}_{ORPS}$ resulted in the highest average SNR, and the optimal average amplitude was 1.5 times higher than an unpolarized source OCT without any polarization management, corresponding to 3.5 dB sensitivity improvement.

Figure 4.4 | **a.** A beam-splitter-based single-spectrometer OCT setup; **b.** Measured fading coefficient histograms with optimal & sub-optimal reference output polarization states and unpolarized light source. PC: polarization controller. L1-4: lenses, P1-2: polarizers. Figures reproduced from [104]

4.4 Conclusion

In this chapter, we quantified the polarization randomization induced SNR loss in fiber-based OCT and proposed a polarization management method to mitigate the SNR loss. Based on our analysis, we suggested the development of broadband fiber couplers integrating two inline polarizers, one at input tail and one at output tail, for the construction of a robust, high SNR, low cost and flexible OCT system.

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Chapter 5 Spectral estimation for resolution enhancement

5.1 Motivation and background

Understanding and diagnosing human diseases often requires three-dimensional visualization of tissue microstructures *in vivo*. Modern biomedical imaging techniques, such as sonography, computed tomography and nuclear magnetic resonance imaging have revolutionized healthcare and life science research. However, the spatial resolution of these methods is not sufficient to visualize cellular-level details associated with the pathophysiological processes that underlie human diseases.

Over 25 years optical coherence tomography (OCT) has become established as a high-resolution three-dimensional imaging modality for the diagnosis of various human diseases [108–110]. The introduction of Fourier-domain OCT (FD-OCT), including spectral domain OCT and swept source OCT, has resulted in the rapid development of ophthalmic and intravascular imaging [111–113]. Unlike the conventional light microscopy methods, OCT provides non-invasive and non-contact imaging of the elastic light scattering properties of a sample in three dimensions [114]. In the depth direction, interferometric measurements of optical delay provide depth-resolved scattering profiles (A-line) of the sample. In the lateral direction (*en face* plane), confocal gating by beam focusing and pinhole coupling provide transverse discrimination of signals. Without using exogenous contrast agents, OCT could potentially be used as the non-invasive ‘optical biopsy’ [114–116], complementing conventional, invasive biopsy and histopathology. However, this potential is still not fully achieved. One of the reasons is that the depth resolution of OCT is insufficient to identify micrometer-

sized cellular-level structures. The typical depth resolutions of current ophthalmic and intravascular OCT are 4–7 μm (in air) [115,117] and 10–15 μm (in air) [118,119], respectively.

The depth resolution of the current OCT technology is governed by the coherence length of the light source, which is further determined by the source center wavelength and the spectral bandwidth. Sustained efforts have been made to improve the depth resolution to 1–2 μm in air by using light sources with a shorter center wavelength and a broader bandwidth, enabled by the advances in laser technology, such as UHR-OCT and Micro-OCT [20,112,120–123]. This improved resolving power has enabled the visualization of cellular and extracellular structures in multiple organs, including the eye [120,124], respiratory airways [20,123], and arteries [20,125].

However, because the depth resolution of UHR-OCT and Micro-OCT is still limited by the coherence length of the light source, compromises have to be made in the imaging range [20,125], sensitivity [121,126], system complexity [125], and cost [125] to maintain the improvement in resolution. Unfortunately, these compromises are difficult to accommodate in clinical settings. Therefore, a method that overcomes the coherence-length limitations without compromising other imaging parameters significantly benefits the clinical applications.

In FD-OCT, the depth profile of the sample is encoded in the periodicities in the wavenumber space of a broadband illumination. These spectral interference signals (interferograms) are conventionally transformed into depth profiles of a sample using inverse discrete Fourier transform (DFT), which provides a depth resolution limited by the coherence length of the light source [127,128]. Although the DFT-based spectral analysis method provides reasonable results, it has two inherent limitations [129]: the low frequency resolution, which corresponds to the low depth resolution of FD-OCT; and the ‘frequency leakage’, which corresponds to the sidelobe artifacts in the OCT depth point-spread function (PSF). The sidelobe

artifacts are the spurious ‘satellite’ peaks appearing in the depth PSF undermining the adjacent features in an OCT image [130]. Modern spectral estimation techniques have been developed as alternatives of the DFT-based method to alleviate its limitations. Generally, these methods can be categorized into two classes: the parametric methods, such as autoregressive, autoregressive moving average (ARMA), and the multiple signal classification (MUSIC) [131] etc., and non-parametric methods, such as Copan [132], the amplitude and phase estimation of a sinusoid (APES) [133], and the recently developed iterative adaptive approach (IAA) [134] etc. A previous study that was conducted by Takahashi et al., used the maximum entropy method to enhance the depth resolution of OCT. However, the potential of this class of powerful techniques has not been fully explored in OCT imaging [135].

We present a novel modality that improves the depth resolution by up to 4.7 times and suppresses axial (depth) sidelobes without altering the hardware system or compromising sensitivity and imaging range of OCT. This new modality, named spectral estimation OCT (SE-OCT), employs an established parametric spectral estimation method, the autoregressive method [136], to replace the conventional inverse DFT for the depth profile retrieval. The autoregressive spectral estimation was originally developed in the field of geophysics [137] and has been widely used in the signal processing of radar [138], sonar, Doppler ultrasound [139], spectroscopy [140], and biomedicine [141]. The term ‘spectral estimation’ often refers to the estimation of the frequency components of a temporal or spatial signal. However, in our application, the spectral estimation is used to estimate the frequency components of the interferograms in the optical spectral domain, hereby producing the depth profiles of the sample. We further show that the superresolution performance of SE-OCT is depended on the signal-to-noise ratio (SNR) of the feature of interest. This superresolution technique is readily incorporated into FD-OCT devices and may make it possible for a cellular-level inspection of several microstructures in the eye and blood vessel.

5.2 Spectral estimation and validation

Figure 5.1 | Principles of FD-OCT and SE-OCT. (a) A Michelson interferometer-based OCT hardware system and the spectral interference. (b) FD-OCT uses inverse DFT to retrieve depth profiles from the interferograms. (c) SE-OCT uses the autoregressive spectral estimation technique to retrieve the depth profiles, including modeling the reshaped interferograms to an autoregressive model and calculating the depth profiles using the model parameters. Figures reproduced from [73].

A typical spectrometer based OCT imaging system is depicted in **Figure 5.1a**. In this schematic, the light source provides broadband illumination to both the sample arm and the reference arm. Spectral interference occurs between photons that are reflected from the reference mirror and those that are backscattered from the refractive-index inhomogeneities of the structures within the sample. The spectral interference signals (interferograms) are detected using a spectrometer, followed by uniformly resampling in the wavenumber space (k-space) (**Figure 5.1b**). The only difference between SE-OCT and FD-OCT is in the spectral interference signal analysis. In brief, the FD-OCT approach uses inverse DFT to retrieve depth information from the k-space interferograms. In SE-OCT, the k-space interferograms are reshaped to a uniform distribution, and then modeled to an autoregressive process, followed by calculation of the depth profiles using the autoregressive model parameters (**Figure 5.1c**).

After pre-conditioning steps, the uniform reshaped k-space interferogram was fitted to an autoregressive model, which can be expressed recursively as

$$x_n = -\sum_{m=1}^p a_m x_{n-m} + n_n \quad \dots(5.1)$$

where x_n is the data sequence of the interferogram, n_n is the white noise sequence with a variance of σ^2 , driving the p order autoregressive process. a_m and σ^2 are the parameters to be estimated.

Estimating the parameters of an autoregressive model is one of the key steps in the autoregressive spectral estimation. For this purpose, several approaches have been developed [142], such as the Burg's approach, the Yule-Walker approach, the covariance approach, and the modified covariance approach. The study conducted by Takahashi et al. [135] used the maximum entropy method, a mathematical equivalent to the autoregressive spectral estimation with the model parameters estimated by Burg's method. However, Burg's method suffers from the peak-splitting problem [136], which may cause misinterpreting of the underlying layer

structure. In SE-OCT, we used the modified covariance method [142] to estimate the model parameters which does not have the peak-splitting issue. Another key step is reshaping the interferograms into a uniform distribution. Since the interferograms are modulated by the spectral shape of the light source, the uniform reshaping of the interferograms before modeling can satisfy the stationary requirements of the autoregressive process, thus dramatically improving the estimation fidelity of SE-OCT.

After a model is estimated, the frequency contents of the interferogram, or the depth profiles of backscattering light intensity $I_{AR}(z)$ can be calculated from the model parameters

$$I_{AR}(z) = \frac{\sigma^2}{|1 + \sum_{m=1}^p a_m \exp(-j2\pi z m \Delta k)|} \quad (5.2)$$

where z is the depth, Δk is the sampling interval of the interferogram in the k -space. In the practical calculation of SE-OCT images, $I_{AR}(z)$ was continuous along the z axis and had a range of $-\pi/\Delta k \leq z \leq \pi/\Delta k$, according to the Nyquist–Shannon sampling theorem. To produce the digital images, we sampled the function $I_{AR}(z)$ to a discrete series within its range at a length 8-times longer than that of the input interferogram. Finally, an averaging down sampling was performed to resize the image to a suitable aspect ratio.

To characterize the resolution superiority and sidelobe suppression of SE-OCT, we conducted two phantom studies, the air wedge and calibration particles. To demonstrate the clinical potential of SE-OCT, we conducted four imaging experiments: the rat corneal endothelium, the swine aortic endothelium, the cholesterol crystals in the human atherosclerotic plaques, and the polymer coating of a drug-eluting stent *ex vivo*. The OCT imaging system used in this study assumed the schematic shown in **Figure 5.1a** with the following additional features. (1) Two diode array sources and a broadband spectrometer were employed to achieve a coherence-length limited depth resolution of 1.3 μm in air.

It's worth mentioning that the system is equivalent to a Micro-OCT setup when operates in FD-OCT mode. (2) By numerically or physically reducing the detected spectral bandwidth, we could also reproduce the depth resolution of the clinical FD-OCT devices, specifically, 4-5 μm for rat corneal tissues. (3) Another spectrometer with relatively narrow detection bandwidth was dedicated to reproduce the depth resolution of the intravascular OCT (10 μm in air) for imaging the vascular tissues. We demonstrate that SE-OCT was capable of visualizing the above-mentioned cellular and extra-cellular details, which was not possible using the corresponding FD-OCT with the same spectral bandwidth. In our study, the 1.3- μm FD-OCT images served as the ground truth, together with the histology image, validating and confirming the structures in bandwidth-reduced SE-OCT and FD-OCT images.

5.2.1 Air wedge

Figure 5.2 | Air wedge between two glass surfaces, imaged with FD-OCT vs. SE-OCT. (a&b) Cross-sectional images produced by FD-OCT (a) and SE-OCT (b). **(c)** Normalized Depth line profiles at the scan length of 7.5 mm in the FD-OCT image (red) and SE-OCT image (blue). **(d)** Measured wedge thickness using FD-OCT (red) and SE-OCT (blue) as a function of the calculated true thickness. **(e)** Plots of depth resolutions of FD-OCT (red) and SE-OCT (blue) as a function of the signal-to-noise ratio predicted by numerical simulation, with verifying experimental results (Squares: FD-OCT; Circles: SE-OCT). Figures reproduced from [73].

An air wedge was formed by stacking two optical flats, with a small angle between them. This simple phantom is ideal for characterizing the depth resolution, because the spacing between the two air-glass interfaces of the wedge increases linearly from 0 along the transverse beam scanning direction (scan length). We numerically reduced the detected spectral bandwidth by applying a ‘tight’ Gaussian window to provide a coherence-length limited depth resolution of $4.8\text{ }\mu\text{m}$ in air. With the same bandwidth-reduced interferograms, SE-OCT provided significantly greater depth-resolving power than FD-OCT (**Figures 5.2a,b&c**). The fringe patterns (blue arrows in **Figures 5.2a&b**) in the transverse direction were formed by the coherence superposition of reflected wavefronts of the upper and lower surfaces of the air wedge, which are known as speckles in the depth direction.

In autoregressive spectral estimation, the magnitude of the estimated spectrum is not linearly related with the original spectrum. Therefore, the full-width at half-maximum of the depth point-spread function was not a valid indicator of the depth resolution of SE-OCT. We defined the resolution of OCT as the minimum distance where two interfaces were resolvable. Using this definition, the depth resolution of both FD-OCT and SE-OCT could be characterized and compared by plotting the measured wedge thickness as a function of the true thickness (**Figure 5.2d**). The first total constructive interference between the two reflected wavefronts, which demarcates resolvable thickness and unresolvable thickness, occurred at a true thickness of $4.77\text{ }\mu\text{m}$ in the FD-OCT image (**Figure 5.2d**). By contrast, SE-OCT clearly resolved the two interfaces up to a true thickness of $1.03\text{ }\mu\text{m}$ – a resolution 4.7-times better than FD-OCT. Note that this result was under the condition of a signal-to-noise ratio of 45 dB of each surface.

It was reported that the signal-to-noise ratio affects the results of autoregressive spectral analysis [143]. Therefore, it is important to investigate the influences of the noise on the super-resolving power of SE-OCT. We firstly conducted a numerical analysis in which normally distributed random noise was added to a

simulated interferogram. The SE-OCT depth resolution was higher than the coherence-length limited depth resolution over the entire tested range of signal-to-noise ratio, and the superresolution factor increased as this ratio increased (**Figure 5.2e**). We then conducted imaging experiments using signal-to-noise ratios of 35 dB to 45 dB, and the results matched our theoretical prediction very well (**Figure 5.2e**, squares and circles).

5.2.2 Polystyrene beads

Figure 5.3 | Comparison between SE-OCT and FD-OCT analyses of polystyrene calibration particles, from the same interferogram data. (a, b & c) Cross-sectional images of calibration particles in water using FD-OCT (a), Gaussian reshaped FD-OCT (b) and SE-OCT (c). Scale bar: 20 μm . Insets: magnified views of two particles. (d) Original (left), Gaussian reshaped (middle) and uniform reshaped (right) interferograms from one location after background subtraction. (e) Depth profiles in the images of FD-OCT (red), Gaussian reshaped FD-OCT (black) and SE-OCT (blue) at the location indicated by the red (a), gray (b), and blue (c) vertical lines in a, b, and c, respectively. The coherence-length limited resolution (CLLR) of FD-OCT was 4.1 μm before spectral reshaping, which was measured at the water/air interface. The nominal optical height (3.2 μm) of the polystyrene particles fell below the CLLR and cannot be resolved by FD-OCT. By contrast, SE-OCT could resolve the two surfaces of the beads and correctly measure their size. Yellow arrows: two particles were overshadowed by the sidelobe artifacts of the water surface in FD-OCT and can be seen in Gaussian reshaped FD-OCT and SE-OCT. Gaussian reshaped FD-OCT suppressed the sidelobe artifacts at the cost of slight degradation of depth resolution. Orange circles: spurious peaks. Figures reproduced from [73].

To verify that the superresolution of SE-OCT is also valid for spherical phantoms that resemble biological cells, we imaged polystyrene calibration particles (80177, Fluka, diameter 2 μm) in water (**Figures 5.3a,b&c**). To physically reduce the light source spectral bandwidth, we used a superluminescent diode array with the spectral band from 755 nm to 860 nm, corresponding to a measured coherence-length limited depth resolution of 4.1 μm . The nominal optical height of the polystyrene particles was 3.2 μm with a refractive index of 1.58 at 810 nm. FD-OCT was unable to resolve the top and bottom surfaces of the particles (**Figure 5.3a**). By contrast, with the same spectral bandwidth (**Figure 5.3d**), SE-OCT could clearly resolve the top and bottom surfaces of each particle (**Figure 5.3c** and inset) and correctly measure the height of the particles (**Figure 5.3e**), demonstrating the superior depth resolution.

Since the spectral shape of the light source was non-Gaussian (**Figure 5.3d**), sidelobe artifacts appeared in FD-OCT images (**Figures 5.3a,e**), which seriously degraded the image quality. In FD-OCT practices, a Gaussian window is usually applied to the interferograms to suppress the sidelobe artifacts at the cost of depth resolution degradation (**Figure 5.3b**). In the SE-OCT images, we found the sidelobes were also completely suppressed, allowing clearer visualization of relatively weakly scattering particles (**Figure 5.3c**, yellow arrows) which would otherwise be overshadowed by the sidelobes of neighboring strongly scattering objects (**Figure 5.3a**, yellow arrows), without affecting the resolution. We also found that in SE-OCT, superresolution and sidelobe suppression were achieved without affecting the noise floor or signal strength (**Figure 5.3e**), although spurious peaks might appear as additional noise (**Figure 5.3c**, orange circles and **Figure 5.3e**).

5.3 Biological tissue imaging

5.3.1 Corneal imaging

Figure 5.4 | Fresh corneal tissue from a normal rat *ex vivo*: SE-OCT vs. FD-OCT. (a-c) Cross-sectional images of a corneal central area produced by the 1.3- μm FD-OCT (a), 4.1- μm FD-OCT (b), and SE-OCT (c). Inset in (a): A three-dimensional rendering of the corneal tissue with blue and pink planes indicating the cross-sectional plane and the en face plane, respectively. (a'-c') Magnified views of posterior corneal tissue indicated by the orange boxes in (a, b & c). (a''-c'') Corresponding *en face* images of the corneal endothelium produced by flattening the endothelium boundary. Hexagonally shaped cells can be discerned, confirming endothelial cells. (d) Representative histology of the rat cornea. EP: epithelium, S: stroma, DM: Descemet's membrane, ED: endothelium, CLLR: coherence-length limited resolution. Scale bars: (a-c) Horizontal: 60 μm , vertical: 10 μm . (a'-c') Horizontal: 40 μm , vertical: 5 μm . (a''-c'') 60 μm . (d) 5 μm . Figures reproduced from [73].

The corneal endothelium plays a vital role in maintaining corneal transparency. Therefore, it is important to visualize and evaluate the morphology of endothelial cells when diagnosing corneal decomposition and during pre-operative and post-operative assessment of corneal and intraocular surgeries. Unfortunately, current ophthalmic OCT, with a typical coherence-length limited resolution of 4-7 μm is unable to clearly visualize this monocellular layer.

Firstly, we used the OCT system to acquire three-dimensional images of fresh corneal tissue from a normal rat *ex vivo*. In the images of FD-OCT with a coherence-length limited depth resolution of 1.3 μm (for short, note as 1.3- μm FD-OCT in this chapter), the corneal epithelium, stroma, Descemet's membrane, and endothelium can be clearly seen in the representative cross-sectional images (**Figures 5.4a&a'**). The corneal endothelium and Descemet's membrane can be observed as two weakly scattering layers defined by three strongly scattering interfaces: the interface between the stroma and Descemet's membrane, the basolateral surface of the endothelium, and the apical surface of the endothelium. These structures were confirmed by clear images of the hexagonally shaped endothelial cells in the corresponding reconstructed *en face* images (**Figure 5.4a''**), and a representative histology image (**Figure 5.4d**).

To reproduce the depth resolution of a typical ophthalmic OCT, we digitally truncated the spectral bandwidth by applying a relatively 'tight' Gaussian window to the interferograms detected by the spectrometer, thereby producing images with a coherence-length limited resolution of 4.1 μm (**Figures 5.4b&b'**). In these 4.1- μm FD-OCT images, the characteristic anatomical features including Descemet's membrane and the surfaces of endothelium could not be clearly seen. In addition, the contrast of the corresponding *en face* image (**Figure 5.4b'**) was degraded with respect to the 1.3- μm depth resolution image even though the transverse resolution was not altered.

By contrast, with the same spectral bandwidth, SE-OCT was able to break the coherence-length resolution limitation, eliminate the sidelobe artifacts, and improve the image quality to be comparable to 1.3- μm FD-OCT images (**Figures 5.4c,c'**). The contrast of the *en face* image (**Figure 5.4c''**) was also enhanced due to higher depth resolution. In SE-OCT images (**Figure 5.4c'**), the thickness of Descemet's membrane and the endothelial layer was measured to be 3.1 μm and 2.3 μm , respectively (refractive index = 1.37).

5.3.2 Artery imaging

Though intravascular OCT (IVOCT) is the highest-resolution coronary imaging modality [144], it is still limited by a depth resolution of 10-15 μm [145,146]. This limitation in depth resolution hinders our ability to visualize cellular level details associated with coronary atherosclerosis. To explore the potential of SE-OCT for visualizing cellular and extra-cellular details, we imaged fresh aortic tissues from a normal swine, human atherosclerotic aortic tissues, and a drug-eluting stent.

Firstly, we acquired three-dimensional images of the sample tissues using the OCT system with a coherence-length limited depth resolution of 10 μm . The recorded spectral interference signals (interferograms) were processed using inverse DFT and the spectral estimation techniques to produce FD-OCT and SE-OCT images, respectively. Secondly, FD-OCT images with a coherence-length limited depth resolution of 1.3 μm were also acquired to serve as the high resolution reference.

Figure 5.5 | Swine aortic tissue, human atherosclerotic aortic tissue and drug-eluting stent: SE-OCT vs. FD-OCT. (a-c) Cross-sectional images of a fresh swine aortic tissue imaged by the 1.3- μm FD-OCT (a), 10- μm FD-OCT (b), and SE-OCT (c). (a'-c') Magnified views of aortic endothelium within the orange boxes in (a, b & c). (d) *En face* plane of the surface of the swine aorta by flattened 1.3- μm FD-OCT images. The pavement-like arrangement and the consistent cell orientation confirmed that the bright 'caps' in (a' & c') were endothelial cells. The yellow dotted line indicates the location where the cross-sectional images (a, b & c) were taken. (e-g) Cross-sectional images of a human atherosclerotic plaque, produced by the 1.3- μm FD-OCT (e), 10 μm FD-OCT (f), and SE-OCT (g). (e'-g') Magnified views of cholesterol crystals within the orange boxes in (e, f & g). (h, i) The measured angles confirming two cholesterol crystal plates indicated by the arrows in (e' & g'). (k) Three-dimension rendered view of the drug-eluting stent struts from the 1.3 μm FD-OCT image. The blue plane indicates the cross-sectional images in (j). (j) Cross-sectional images of a drug-eluting stent strut produced by the 1.3- μm FD-OCT, 10- μm FD-OCT, and SE-OCT. ED: endothelium, IEL: internal elastic lamina, P: polymer; SS: stent strut; MI: mirror images, CLLR: coherence-length limited resolution. Scale bars: (a-d) 40 μm , (a'-d', h & i) 20 μm , (e-g, m) 50 μm , (e'-g') 30 μm .

Arterial endothelial cells keep low-density lipoprotein and leukocytes from entering into the intima. The damage of endothelial cells is considered as the first step of atherosclerosis development. In addition, the presence of endothelial cells is an indicator of arterial healing after stent implantation.

In the 1.3- μm FD-OCT images of fresh swine aorta, the endothelial cells could be visualized as bright ‘caps’, lining at the surface of the vessel wall (**Figures 5.5a&a’**, yellow arrows) above the internal elastic lamina. This was confirmed by their pavement-like arrangement and the consistent cell orientation in the flattened *en face* image of the vascular wall surface (**Figure 5.5d**). The *en face* image was produced by a flattening algorithm in which the luminal surface was aligned to a flat plane. The internal elastic lamina appeared as a high scattering layer which agrees with the findings in Micro-OCT images and is also confirmed by the fact that the elastic fibers were parallel to the direction of luminal flow.

Unfortunately, in 10- μm FD-OCT images (**Figures 5.5b&b’**), the visibility of the endothelial cells was undermined by the mixture of the strongly scattering cell surface with the relatively weakly scattering connective tissues beneath the cells. By contrast, in SE-OCT images (**Figures 5.5c&c’**), we could visualize the monolayer cells as bright ‘caps’ similar to those found in the 1.3- μm FD-OCT images (**Figures 5.5a&a’**). This finding was confirmed by the cellular-level one-to-one match among the SE-OCT image, the 1.3- μm FD-OCT images, and the surface flattened *en face* image (**Figures 5.5c’&d’**, yellow arrows).

The cholesterol crystals in the vascular wall may be confused on occasion with macrophage accumulations. The key to minimizing this risk of confusion would be to resolve the linear appearance of the cholesterol crystals [146]. However, the thickness of cholesterol crystals is typically much smaller than 10 μm , beyond the current resolution limit of IVOCT.

Serving as the ground truth, the 1.3- μm FD-OCT images showed that cholesterol crystals could be characterized by the linear structures formed by intense reflections from their top and bottom surfaces in the cross-sectional images (**Figures 5.5e&e'**) and the parallelogram shape with an acute angle of 79.81 ± 2.98 degrees in the *en face* plane (**Figures 5.5h&i**) [147].

In the 10- μm FD-OCT cross-sectional images (**Figures 5.5f&f'**), the characteristic linear morphological information was lost. However, the SE-OCT image was able to reveal the linear appearance of the cholesterol crystals (**Figures 5.5g&g'**), although some fine structures were less clear than the 1.3- μm FD-OCT images.

Drug-eluting stents are coated with a polymer that controls their drug-eluting profile to inhibit neointimal hyperplasia. Clinically, the polymer coating is occasionally broken at the time of stent implantation, especially for calcified lesions. Visualizing this polymer coating may help us further understand the *in vivo* behaviors of drug-eluting stent, and contribute to better design of drug-eluting stent. Yet current IVOCT cannot clearly distinguish the polymer layer from stent struts, because the polymer thickness is equivalent to or smaller than the depth resolution of current IVOCT.

We acquired three-dimensional images (**Figure 5.5k**) of an undeployed, non-biodegradable drug-eluting stent. The stent was manually expanded, opened and flattened to create the clearance for the abluminal beam scanning. Under the 1.3- μm FD-OCT, the measured optical thickness of the polymer was 8.2-10.8 μm (**Figure 5.5j**, upper panel).

The 10- μm FD-OCT could not clearly resolve the polymer coating (**Figure 5.5j**, middle panel). However, in the corresponding SE-OCT image (**Figure 5.5j**, bottom panel), we can clearly see the polymer layer defined by the air-coating interface and the abluminal surface of the strut, with an equivalent contrast to that of the 1.3- μm FD-OCT.

5.4 Discussion

In this study, we improved the performance of OCT through modeling the reshaped spectral interferograms to autoregressive processes, and the results indicate the clinical potential of SE-OCT to provide cellular-level information. For ophthalmic imaging, without any change in the current OCT hardware, SE-OCT allows a quantitative evaluation of corneal endothelial damage, by clearly visualizing the endothelial layer and measuring its thickness. This technical advance can help surgeons plan the targeted replacement of corneal tissue and confirm the benefit of selective transplantation of the endothelial layer, i.e., endothelial keratoplasty [148]. For intravascular imaging, SE-OCT may be used to evaluate the endothelial coverage after stent implantation. SE-OCT can also enhance the visibility of the cholesterol crystal, which is one of the specific components of high-risk atherosclerotic plaques and most wanted by cardiologists. Furthermore, the ability to identify the drug-eluting stent polymer would facilitate accurate quantification of stent thickness and evaluation of stent pathology after coronary implantation.

In FD-OCT, using the inverse DFT for depth profile retrieval assumes the spectral data out of light source spectral band to be zeros. By contrast, In SE-OCT, the autoregressive modeling procedures estimate the interferogram data out of the light source spectral band, thereby virtually broadening the spectral bandwidth in favor of the depth resolution. However, the spectral interferogram is actually formed by composition of periodicities in wavenumber space, thus is not a true autoregressive process. Therefore, we use a high order autoregressive model to approximate the OCT interferogram. This approximation may lead to two drawbacks. One is that the estimated signal magnitude, or light intensity in the SE-OCT images, may not be proportional to the true intensity backscattered from the samples. Take bead 1 in **Figure 5.3e** as an example, the signal intensity of its two surfaces has a significant difference which should be small. This issue may not affect the sample

morphology presented in SE-OCT images, but quantitative analysis based on the image intensity should be carefully taken. The other drawback is that spurious peaks appear in SE-OCT images (**Figures 5.3c**, orange circles & **5.3e**) as additional noise, especially when a high order was used. However, we found this one-pixel width noise may not significantly affect the image quality and can be easily suppressed by frame averaging, which is widely used in OCT images to suppress the speckle noise. In addition, the order selected for autoregressive modeling affects the result of SE-OCT: a lower order results in fewer details, while a higher order leads to increased spurious peaks. In SE-OCT application, we found it was a good balance to set the order to be the 1/3 of the length of the interferogram samples.

The performance of SE-OCT depends on the signal-to-noise ratio of the microstructure of interest (**Figure 5.2e**). Specifically, SE-OCT shows better performance for the highly scattering microstructures, such as those demonstrated in **Figures 5.4 & 5.5**, than the weakly scattering microstructures. This SNR dependence may limit the application of SE-OCT to relatively highly scattering microstructures. Nevertheless, over the SNR range > 15 dB, SE-OCT demonstrates superior depth resolution than the corresponding FD-OCT.

In our study, the lateral resolution of the OCT system was set to be $2.5\text{ }\mu\text{m}$, higher than that of the clinical systems. This was necessary for the one-to-one confirmation of the cellular and extracellular structures from the *en face* view, especially the corneal and aortic endothelial cells, and the cholesterol crystals. Since these cellular and extra-cellular details have relatively large lateral sizes (corneal endothelium: $20\text{-}30\text{ }\mu\text{m}$, aortic endothelium: $15\text{-}20\text{ }\mu\text{m}$, cholesterol crystals: $>50\text{ }\mu\text{m}$ and stent polymer: $>100\text{ }\mu\text{m}$), we were wondering if our superresolution method may enable visualization of these cellular-level structures in a FD-OCT system with the spatial resolutions equivalent to those of a clinical OCT system ($15\text{-}30\text{ }\mu\text{m}$). To give a simple evaluation of this hypothesis, we

convoluted the swine aortic images with a 2-dimensional Gaussian function with the full-width at half-maximum of $20\text{ }\mu\text{m}$ in the lateral direction to approximate the images acquired from a system with a $20\text{ }\mu\text{m}$ lateral resolution. In the convoluted images, the characteristic features of aortic endothelial cells can still be identified in the cross-sectional SE-OCT image, even though those in the *en face* image was completely blurred. However, the above-mentioned incoherent convolution cannot replace the coherent convolution which is the actual physical process of lateral OCT image formation. Therefore, near-term future developments for SE-OCT will be testing the efficacy of cellular-level imaging in clinical OCT systems *in vivo*. Moreover, improvement of processing speed using parallel computing and achieving nanometer depth resolution using supercontinuum generation could help the method become even more attractive for wide use.

Chapter 5. Spectral estimation for resolution enhancement

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Chapter 6 Spectrally encoded extended source OCT

6.1 Motivation and background

Optical coherence tomography (OCT) has been established as an imaging tool for diagnosis of various eye diseases. Current Fourier domain OCT, including spectral domain OCT and swept source OCT, is capable of performing three-dimensional (3D) imaging of human eyes *in vivo* and visualizing many tissue structures in the posterior segment [113,149]. However, there are two unmet clinical needs: visualizing deep tissue structures [150–152] and faster scanning to reduce motion artifacts in 3D images [153,154], both of which require better sensitivity. The sensitivity of an existing ophthalmic OCT system is mainly limited by the maximum permissible radiant exposure imposed by the laser safety standards [155]. As an extended source is less likely to cause thermal damage to the eye tissues than a point source with the same input power at the cornea, higher MPE is allowed for an extended source than that of a point source [155]. Therefore, an extended source OCT technology will effectively enhance the sensitivity, which can be used to increase either penetration depth or imaging speed or both simultaneously.

In this Letter, we report a spectrally encoded extended source OCT (SEES-OCT) technique that allows significantly higher MPE than that provided by commercial ophthalmic OCT systems. To the best of our knowledge this has never been reported before. Imaging was performed using optic nerve head (ONH) tissues from enucleated swine eyes to demonstrate a sensitivity advantage of 7-dB over the corresponding point source system without significant degradation in transverse resolution. The hardware system of SEES-OCT device is almost the same as the

current point source system except for one additional element in the infinity space. Therefore, any existing ophthalmic OCT system can be easily modified so that it can be switched between the point source mode and the extended source mode. Based on its merits in sensitivity and flexibility, this technique may help visualize deep ocular tissue structures which are at risk of damage in glaucoma such as the lamina cribrosa, the posterior sclera, and the retro-laminar tissues [150,152,156].

6.2 Extended source and validation

Figure 6.1 | Schematic of SEES-OCT system. AO: analog output. BS: non-polarizing beam splitter. IMAQ: image acquisition board. L1-5: achromatic lens. DAQ: data acquisition card. NDF: neutral density filter. PC: personal computer. CL: Camera Link cable. RM: reference mirror. Figures reproduced from [157].

The SEES-OCT setup assumes a free-space Michelson interferometer design (**Figure 6.1**). The output of a superluminescent diode (SLD) array (Exalos Ultra-Broadband EBS4C32) is collimated by lens L1 (AC050-010-B, $f=10$ mm, Thorlabs Inc.) with optical power of 5.72 mW over a 3-dB spectral range of 950-1130 nm. A 50:50 non-polarizing beam splitter (BS) splits the source beam into a reference beam and a sample beam. An equilateral prism (#43-494, N-SF18, Edmund Optics) transforms the collimated sample beam into a fan beam by separating radiation of different wavelength over a propagation angular range of 7.9 mrad (Full-angle at half maximum). By use of an objective lens of 50-mm focal length (AC254-050-B, Thorlabs Inc.), the full-length at half maximum of the illumination line on the sample is ~ 380 μm . Light backreflected from the reference arm and backscattered from the sample arm is recombined through the beam splitter before directed to a spatial filter consisting of a focusing lens L4 (AC050-010-B, $f=10$ mm, Thorlabs Inc.), a 10 μm pinhole, and another collimation lens L5 (AC127-030-B, $f=30$ mm, Thorlabs Inc.). The spectrometer is composed of an 1450 lines/mm volume phase holographic transmission grating (1030 nm, Wasatch Photonics Inc.), a camera lens (Nikon AF Nikkor 50 mm $f/1.8\text{D}$), and a linear camera (Sensors Unlimited GL2048L). We used 2048 camera pixels to detect a total spectral range from 925-1150 nm and each camera pixel corresponds to a spectral band of ~ 0.11 nm. We digitized the detected signal at 12-bit resolution and transferred it to the personal computer through the camera link cables and an image acquisition board (KBN-PCE-CL4-F, Bitflow). The camera and the galvo scanners are synchronized by a triggering signal generated by the computer. It's worth mentioning that by replacing the prism with a mirror a SEES-OCT device can be changed to a 'standard' point source OCT device (**Figure 6.1**, dashed box).

Figure 6.2 | (a) Schematic of wavelength sweeping. L3: objective lens. Black arrow: direction of the fast scan; (b) 512 consecutive spectra acquired when scanning across a pattern of the resolution target. Green arrow: edge line; (b') Spectra in (b) after remapping. Yellow arrow: scan margin. (c) Time delay of different spectral bands. Figures reproduced from [157].

The illumination line is aligned in the same direction as the fast scanning direction of the galvo scanners, so that at a given sample point O the wavelength sweeps over time as the fast scanning mirror scans the sample beam across it (**Figure 6.2(a)**). As a result, at the point O the arrival time (A-line sequence number) of different spectral bands is dependent on the refractive index of the prism and the galvo scanning speed. **Figure 6.2(b)** shows a B-scan frame consisting of 512 consecutive spectral lines acquired when the fast scanning mirror scans the sample beam across a chromium-coated pattern of a United States Air Force (USAF) 1951 resolution target with the reference path blocked. Then the B-scan frame was remapped by arrival time so that every vertical spectral line contained the spectrum from only one sample point (**Figure 6.2(b')**). Specifically, an edge curve of the coated pattern (green arrow in **Figure 6.2(b)**) was used to extract the delay time n (in A-line cycle) of different spectral bands arriving at the same point with regard to the first spectral band. The edge curve was first extracted using image processing method. Note that the edge curve was actually the spectrum from one

single point on the sample. After the arrival time remapping, the points on the curve should be in the same A-line, meaning a straight vertical line in the B-scan frame. Therefore, the A-line number difference between m^{th} camera pixel and the 1st pixel on this curve was the time delay n of the corresponding spectral band detected by the camera pixel m . This discrete time delay data was further processed using 6th order polynomial fitting to obtain a continuous curve (**Figure 6.2(c)**), which was used as a look-up table to remap spectral data. The B-scan range was 4.37 mm so that the spectral remapping process results in a scan margin of 50 A-lines (yellow arrow, **Figure 6.2(b')**) where axial resolution is degraded.

Figure 6.3 | Spatial resolution: SEES-OCT vs. point source OCT. (a) An en face view of a 3D image of USAF 1951 resolution target acquired using SEES-OCT; (b) Axial point-spread function of SEES-OCT (blue solid curve) compared with that of the point source OCT (green dashed curve). Figures reproduced from [157].

6.3 Imaging of swine optical nerve head

Figure 6.4 | Cross-sectional (longitudinal) images of a swine ONH tissue acquired using (a) the point source OCT (0.4 mW sample power), (b) SEES-OCT (2.1 mW sample power), and (c) the point source OCT (2.1 mW sample power), respectively. (a'-c') corresponding en face images of the lamina cribrosa (LC) region. PL: pre-lamina tissue; S: sclera; RL: retro-lamina; V: central retinal vessels. Figures reproduced from [157].

To test whether the transverse resolution of SEES-OCT may be degraded with respect to the corresponding point source OCT system, we scanned the resolution target using the SEES-OCT system. We predicted the transverse focal spot of the point source system to be $21 \mu\text{m}$ ($1/e^2$ power level) based on the mode field diameter of the input single mode fiber ($4.2 \mu\text{m}$ @ 980 nm , Corning HI 980) and the magnification of the sample arm optics (0.2X). In **Figure 6.3(a)**, the vertical direction is the direction of the illumination line. Therefore, the vertical line patterns reflect the resolving power of SEES-OCT while the horizontal line patterns reflect that of the corresponding point source OCT. We observed slight degradation in transverse resolving power in the vertical direction with regard to the diffraction-limited resolution in the horizontal direction. The chromium

patterns of element 6 in group 4 (with a line spacing of 17.5 μm) can be clearly resolved. To characterize the axial resolution of the system, we investigated the axial point spread functions (PSF) by measuring the A-line profiles of a partially reflecting mirror sample using both SEES-OCT and the point source OCT. The axial resolution of SEES-OCT was tested to be 4.6 μm in air (**Figure 6.3(b)**), which is 10% lower than that of the point source system. This may be because in SEES-OCT the effective spectral bandwidth of the sample field reflected from a specular object is smaller than that of the point source case. Since different spectral bands are reflected at different locations of the galvo mirror, at a given sample point the incident angles of different spectral bands are slightly different. Therefore, when the spectral bands are reflected by the specular sample, the detection efficiency drops slightly for the spectral bands at both ends of the spectral range where the reflection angles are suboptimal for coupling into the pinhole. However, such a problem will be minimized for backscattered signal.

The MPE at the cornea for an extended source is obtained by multiplying the MPE of a small source by a parameter C_E , which is a function of the visual angle α (**Figure 6.1**) [155]: $C_E = \alpha / \alpha_{min} = 0.667\alpha = 5.3$. Assuming the maximum input power at the cornea limited by the MPE of the point source OCT is 0.4 mW, the corresponding maximum permissible optical power for the SEES-OCT is 2.1 mW. To characterize the sensitivity of the SEES-OCT system, we measured the signal to noise ratio (SNR) using a partially reflecting mirror sample (-46.4 dB reflectivity) when the path length difference between the mirror sample and reference arm was maintained at 0.3 mm. The sensitivity was measured to be 102.2 dB. In order to understand the measured sensitivity, we analyzed the SNR using the following formulas [158]:

$$S[\text{dB}] = 10 \times \log \left(\frac{N_{\text{ref}} \times \sum N_S}{N_{\text{sh}}^2 + N_{\text{el}}^2 + N_{\text{RIN}}^2} \right) \quad (6.1)$$

$$N_{\text{RIN}} = (f/\Delta\nu)^{1/2} N_{\text{ref}} \quad (6.2)$$

$$N_{\text{sh}} = (N_{\text{ref}})^{1/2} \quad (6.3)$$

where N_{ref} is the number of electrons per pixel generated by the reference arm light, N_{el} is the electrical noise of the photodetector, N_{sh} is the number of shot noise electrons, N_{RIN} is the relative intensity noise, f is the reciprocal of twice the exposure time of the CCD, $\Delta\nu$ denotes the FWHM spectral bandwidth of the reference light received by a single pixel, $\sum N_{\text{S}}$ denotes the sum of electrons over the entire array generated by the sample arm light returning from a 100% reflector. The full well depth of the camera pixel is $2.1\text{M } e^-$. With a spectrometer efficiency of 0.64 and an average fill factor of 0.7, $N_{\text{ref}} = 937,440 e^-$ and $N_{\text{sh}} = 968 e^-$. The line rate and the exposure time were 20,000 Hz and $45 \mu\text{s}$ respectively. Therefore, the N_{RIN} was calculated to be $665 e^-$. The total electrical noise was found to be $872 e^-$. $\sum N_{\text{S}}$ was measured to be $5.95 \times 10^{10} e^-$. Using Eq. (6.1), the sensitivity was estimated to be 104 dB which was in agreement with our experimental results.

In order to demonstrate the potential of SEES-OCT for visualizing deep tissue structures of the posterior eye segment, we imaged a swine ONH *ex vivo* using both the SEES-OCT system and the corresponding point source system. Freshly enucleated swine eyes were dissected to expose the ONHs to the focused beam. 3D images were acquired using the point source system at sample power of 0.4 mW (**Figures 6.4(a)&(a')**), the SEES-OCT system at sample power of 2.1 mW (**Figures 6.4(b)&(b')**), and the point source system at sample power of 2.1 mW (**Figures 6.4(c)&(c')**), respectively. The image size was 4.37 mm by 4.37 mm by 2.45 mm (x by y by z) with a frame rate of 40 Hz and 512 A-lines per frame. We found that SEES-OCT can image both the lamina cribrosa and the retro-laminar tissues (**Figure 6.4(b)**) and that penetration was significantly greater than that of the point source OCT system due to a higher MPE (5.3 times). The image quality achieved by the SEES-OCT setup is comparable with that of the point source setup operation at the same sample power (**Figures 6.4(b-c)**).

SEES-OCT has the potential to reduce transverse motion artifacts in a 3D image caused by involuntary fixational eye movements (e.g. micro saccades and drift),

but it is more sensitive to the axial motion artifacts than its point source counterpart due to effectively prolonged exposure time for a given sample point. Due to this issue, SEES-OCT is better suited as an add-on to point source devices. A simple switch mechanism (**Figure 6.1**, dashed box) will be enough to integrate SEES-OCT in an existing device. By doing this, higher penetration and potentially higher speed can be achieved in the extended source mode without affecting the functions of the point source devices.

6.4 Conclusion

In conclusion, we have developed a spectrally encoded extended source OCT technology and demonstrated its potential to image deeper than the existing OCT technology. SEES-OCT can be easily integrated into the existing point source OCT devices as an add-on option with minor hardware changes. Therefore, this technology may help visualize tissue structures that may not be readily seen using a point source system.

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Chapter 7 Summary and future works

7.1 Summary

My efforts in my PhD study is to improve the current OCT technology, in terms of sensitivity, resolution and polarization contrast. Along with the technical works, I was also making considerable efforts working on translational tissue imaging, including corneal, aorta, esophagus, tumor. I didn't include them in my thesis because I'd like to make my thesis consistent in content and focusing on technical improvements of OCT.

The concept of polarization holding point proposed in this thesis may be impactful to the current OCT. First, the concept, in principle, enables improved optical performance for simpler and less expensive OCT architecture; second, it may improve the performance of conventional polarization-sensitive OCT systems, especially those utilizing endoscope and catheter probes; the last, it helps to alleviate the polarization fading problem in single camera fiber-coupler system.

The technical innovation of extended source and spectral estimation OCT can be attractively implemented in existing ophthalmic systems through a minor modification of the scan head along with the inclusion of refined software reconstruction algorithms to increase the imaging performance.

The birefringence phantoms may be widely adopted in polarimetric imaging labs as a stable and transferable sample.

7.2 Limitations and future works

In chapter 2, I reported a reciprocal property of the polarized light travelling in backscattering unitary circuit. This property is a fundamental effect that originates

from the reciprocity of light. In birefringence imaging, the holding point effect opens new possibilities for single input state PS-OCT detection. However, to our understanding, the holding point artifact is a main drawback, hindering the practical applications for catheter-based systems. We believe this problem can be overcome by careful design of probe, distributing the input polarization states to different wavelengths. For two input state PS-OCT, the holding point shows obvious improvement in optics axis reasoning when the local birefringence is low. In the retardation map, the improvement is limited because the retardation is anyway small, with no regarding to accuracy of the optic axis measurement. But we believe there still exist potential benefits of utilizing the system polarization holding point to improve the detection accuracy.

In chapter 3, I reported a method to fabricate birefringence phantoms. This chapter focuses on the protocol of reproducing such a phantom, while leaves weak analysis of the physical modeling of the birefringence generation. There is still some space to further investigate the birefringence stability, based on more rigor physical dynamic modeling of the molecule chains behavior.

In chapter 4, I discussed the polarization fading problem in a single channel OCT system. The polarization management has an application scope that the system must be fiber-coupler based on Michelson type interferometer. If the system is based on Mach-Zahnder interferometer, the method proposed is not applicable.

In chapter 5, SE-OCT is proposed. To demonstrated the capability of this method, I chose layer-structural samples based on the autoaggressive model assumptions. However, in real biological tissues, there is no such prerequisites except for some special tissue architectures. SE-OCT's performance depends on the tissue structure, limiting the application scope of this method in clinic.

In chapter 6, SEES-OCT actually has significant clinical values. This work has been patented and may be applied to clinical systems soon. However, the only

regret of this method is the encoding approach in wavelength domain, which only works for spectral domain OCT (SD-OCT) but not works for swept source OCT (SS-OCT).

This thesis is a technical thesis for OCT improvement. However, in OCT field, clinical needs and translational improvements are even more impactful than technical improvements. In the next step, I will further investigate what can be brought to clinic by having a polarization sensitive OCT with ultra-high resolution.

In conclusion, this thesis provides a step forward to develop a viable OCT with high resolution, sensitivity and contrast with the attempts and solutions to several technical problems. The feasibility and effectiveness of the proposed solutions were demonstrated with phantoms and ex vivo animal imaging in the context of relevant biological diseases.

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