Title
In vitro rabbit trachea imaging using long-range optical coherence tomography

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In vitro rabbit trachea imaging using long-range optical coherence tomography

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Biomedical Engineering

by

Mengke Zhang

Thesis Committee:
Professor Zhongping Chen, Chair
Professor William C. Tang
Associate Professor Michelle Digman

2016
DEDICATION

To

my parents and friends

in recognition of their love, support, trust and help.
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ABSTRACT OF THE THESIS

*In vitro* rabbit trachea imaging using long-range optical coherence tomography

By

Mengke Zhang

Master of Science in Biomedical Engineering

University of California, Irvine, 2016

Professor Zhongping Chen, Chair

Diagnostic imaging of the trachea can help in identifying a variety of intrinsic and extrinsic abnormalities of the trachea. Imaging diagnosis of trachea has been accomplished using magnetic resonance imaging (MRI), X-ray cephalometry and computed tomography (CT). However CT and X-ray cephalometry require the use of ionizing radiation and MRI typically requires sedation of the patient to prevent motion artifacts. Long-range optical coherence tomography (OCT) has the potential to provide high-speed three-dimensional tomographic images with high resolution and without the use of ionizing radiation. Analogous to ultrasound, OCT measures backscattered light intensity using coherence interferometry to construct topographical images of complex tissue. Since OCT uses infrared light rather than acoustic waves, its spatial resolution (~10 µm) is exceptionally high. In this study, I present work on the development of a long-range OCT endoscopic probe with 1.47 mm OD and 11.5 mm working distance used in conjunction with a Swept Source/ Fourier domain OCT system to acquire structural and anatomical datasets of the rabbit trachea *in vitro*. 

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CHAPTER 1: Introduction

1.1 Principles of optical coherence tomography

Optical coherence tomography (OCT) is an emerging non-invasive three-dimensional imaging technique. High-resolution cross-sectional images through inhomogeneous samples, such as biological tissues can be produced by OCT[2].

The imaging process in OCT is very similar to the ultrasound imaging, in which the time travelled by a sound wave from its emission until its detection after being reflected by a sample is measured. Reconstructions of the structure of a certain sample are achieved by computing the time it takes for a light beam to travel the optical path due to the reflection of an object and the magnitude of the backreflected light[1][4]. Because it is not possible to precisely measure the time values, interferometry and low-coherence light become significant techniques for OCT.

In ophthalmology, OCT is widely used for non-invasive structural and quantitative imaging of the anterior and retina segment. OCT allows identification of pathologies for disease diagnosis and monitoring response to therapy[3]. Because of the characteristics of OCT, it is an extremely attractive non-invasive imaging procedure that can be used in many areas.
1.1.1 Physical fundamentals of optical coherence tomography

As an interferometric technique, OCT is based on interference between a split and later recombined broadband optical field. A classic OCT schematic is shown in Figure 1.1.

Oct works by first taking a light beam, emitted by the light source, and splitting it on a beam splitter. One of the split beam is sent into a reference path and the other is sent into a sample path. Beam in the reference path is reflected by a reference mirror and the beam in the sample path is reflected from the different layers within a sample[2]. The reflected beams are coupled and later detected by a photo detector, in which an interference pattern is obtained. Intensity peaks can be achieved from sharp refractive index variations between layers of the sample. As a result, it is possible to infer the structure of the studied sample.

Figure 1.1 A typical OCT system which is based on a Michelson interferometer.
It is important to know that the interference between the reflected light only happens when the difference between optical path lengths of the reference and sample arms is no more than a value equal to the coherence length of the light. As a result, the axial resolution of an OCT system is determined by the temporal coherence of the light source[2].

In this outline, the reference mirror position is changed within a determined range of values corresponding to an imaging depth range of interest so as to get a complete A-scan profile. The A-scan is a figure of the reflectivity of the sample in an axial depth direction[4]. Cross-sectional images can be achieved by laterally scanning the incident optical beam and performing sequential A-scans, as shown in Figure 1.2.

This generates two-dimensional data sets that represent the optical backscattering in a cross-sectional plane through the sample. Images can be shown in gray scale in order to picture the internal tissue structure of the pathology. Three-dimensional, volumetric data sets are produced by acquiring sequential cross-sectional images, scanning the incident optical beam in a raster or other two-dimensional pattern. Three-dimensional OCT (3D-OCT) data contain comprehensive volumetric structural information which can be displayed similarly to MR or CT images[5].
Figure 1.2 Optical coherence tomography (OCT) produces cross-sectional and three-dimensional images by measuring the magnitude and echo time delay of light. Measurements of backreflection versus depth are known as A-scans. Cross-sectional images are generated by scanning the OCT beam in a lateral direction to acquire a series of axial scans. This produces a B-scan that can be displayed as a gray scale image. Three-dimensional volumetric data sets (3D-OCT) can be achieved by raster scanning to generate a series of two-dimensional data sets (B-scans)[17].
1.1.2 Mathematical formulation

In this section, I will show the mathematical formulation of OCT systems following the treatment presented by Wang[2].

Considering the OCT system represented previously, the optical field at the input of the interferometer is characterized by:

$$E_{in}(\omega, t) = s(\omega)e^{-i\omega t}$$

(1)

where $\omega$ is the frequency, $s(\omega)$ is the source field amplitude spectrum and $t$ is the time variation. The phase term is ignored, since the initial phase of the incident wave is arbitrary.

At a beam splitter, the incident wave $E_{in}(\omega, t)$ is split in a reference beam $E_r(\omega, t, \Delta z)$, and at a sample beam $E_s(\omega, t)$. A reference mirror reflects the reference beam, and the sample beam is reflected by the structures within the sample:

$$E_r(\omega, t, \Delta z) = (T_r T_s)^\frac{1}{2}E_{in}(\omega, t)e^{-i\phi(\Delta z)}$$

(2)

$$E_s(\omega, t) = (T_r T_s)^\frac{1}{2}E_{in}(\omega, t)H(\omega)$$

(3)

Here $T_r$ and $T_s$ represent the reference and sample arms intensity transmission coefficients; and $H(\omega)$ represents the frequency domain response function of the sample. The scanning of the mirror position, by a geometric distance $\Delta z = \Delta tc/n_{air}$, results in a phase accumulation $\phi(\Delta z)$ that is determined as:
\[ \phi(\Delta z) = \frac{2\omega n_{\text{air}} \Delta z}{c} \] (4)

The frequency domain response function of the sample, \( H(\omega) \), that describes its internal structure is characterized by \( r(\omega, z) \), the backscattering coefficient from the sample structural features, and by \( n(\omega, z) \), the frequency dependent, depth varying group refractive index:

\[ H(\omega) = \int_{-\infty}^{+\infty} r(\omega, z) e^{i2n(\omega,z)\omega z/c} \, dz \] (5)

At the output of the interferometer, the optical field is a consequence of the interference between the sample and reference beams:

\[ E_{\text{out}}(\omega, t, \Delta z) = E_r(\omega, t) + E_z(\omega, t, \Delta z) \] (6)

The photo detector records an optical intensity proportional to a time average of the output electrical field multiplied by its complex conjugate:

\[ I(\omega, \Delta z) = \langle E_{\text{out}} E_{\text{out}}^* \rangle = \langle E_z E_z^* \rangle + \langle E_r E_r^* \rangle + 2\Re\{\langle E_z E_r^* \rangle\} \] (7)
where the first two terms correspond to the self-interference term and the last to the real part of the cross-interference.

Considering the equations describing the reference and sample optical fields and substituting $s(\omega)$ by the source power spectrum $S(\omega) = |s(\omega)|^2$, recorded optical intensity is given by:

\[
I(\omega, \Delta z) = T_r T_s S(\omega) |H(\omega)|^2 + T_r T_s S(\omega) + 2T_r T_s \Re\{S(\omega) H(\omega) e^{-i\phi(\Delta z)}\} \tag{8}
\]
1.1.3 Optical coherence tomography modalities

The information about the optical structure of a sample can be retrieved from measurements in both time and frequency domains. These techniques are considered Time-Domain OCT (TD-OCT) and Fourier-Domain OCT (FD-OCT). Depending on the area of application, other modalities, such as Polarization Sensitive OCT (PS-OCT), Quantum OCT (Q-OCT) or Doppler OCT (D-OCT), can be used.

1.1.3.1 Time domain OCT

In a Time-Domain OCT (TD-OCT) system, the reference mirror is moved in order to match the optical path from the reflections within the different layers of sample[2]. Considering an ideal situation in which the OCT system is operated on air and there are no losses on the beam splitter (with a 50:50 ratio) and assuming writing the optical intensity is recorded by the photo detector as a function of the mirror displacement, the obtained interference pattern in each axial scan can be described as:

\[ I(\Delta z) = I_0 + R\{\Gamma(\Delta z)\} \]

where \( I_0 \) is the self-interference term, and \( \Gamma(\Delta z) \) is the cross-interference term:

\[ I_0 = \frac{1}{4} \int_{-\infty}^{\infty} s(\omega)(|H(\omega)|^2 + 1)d\omega \]

\[ R\{\Gamma(\Delta z)\} \]
\[ \Gamma(\Delta z) = \frac{1}{2} \int_{-\infty}^{+\infty} H(\omega)S(\omega)\cos\{\phi(\Delta z)\} d\omega \]  

(11)

If we consider a sample composed of \( N \) individual layers, the sample’s response function can be obtained from equation (5), using the layer interface’s reflectivities \( (r_j) \), according to:

\[ H = \sum_{j=1}^{N} r_j \exp \left\{ i 2 \frac{\omega}{c} \sum_{m=1}^{j} n_m z_m \right\} \]  

(12)

where \( z_m \) is the thickness of each layer and \( n_m \) is the refractive index. The reflectivites \( r_j \) are determined by the Fresnel’s equations:

\[ r_j = \frac{n_{j+1} - n_j}{n_{j+1} + n_j} \]  

(13)
1.1.3.2 Fourier domain OCT

In a Fourier-Domain OCT (FD-OCT) the measurements are taken in the frequency space and the reference arm of the system is fixed.

In order to obtain the intensity spectrum of the reflected light, the reference mirror is at a fixed position without being moved, which is different from TD-OCT and the light is detected by a spectrometer. The intensity spectrum \( I(\omega) \) is Fourier transformed \( (FT) \) to obtain a time domain interference pattern \( I(t) \):

\[
I(\omega) = \frac{1}{4} S(\omega)\{H(\omega) + 1\}^2
\]

\[
I(t) = FT\{I(\omega)\}
\]

Figure 1.3 A FD-OCT system. The output light field is split by a diffraction grating, and component frequencies are detected by a linear detector array.
Figure 1.3 exemplifies an FD-OCT system using a linear detector array. The output intensity spectrum is a set of \( N \) data points, in which each point matches a recorded intensity at each detector of the array. As the time domain interference pattern is obtained from the output intensity spectrum, using the Fourier Transform, it will have only \( N/2 \) data points corresponding to a time interval \( \Delta \tau \) given by the detected spectral width (\( \Delta \Omega \)):

\[
\Delta \tau = \frac{2\pi}{\Delta \Omega} \tag{16}
\]

\[
\Delta \Omega = 2\pi c \frac{\Delta \lambda}{\lambda^2} \tag{17}
\]

The conversion from time to spatial domain is achieved by multiplying both sides of equation (17) by \( c/n_{ave} \), where \( n_{ave} \) is the average sample refractive index. Therefore, the maximum depth \( z_{max} \) is determined by multiplying equation (17) by the number of time domain points \( N/2 \) and dividing by 2 to take into account the double pass of the light through the sample:

\[
Z_{max} = \frac{1}{4n_{ave} \Delta \lambda} N \tag{18}
\]

It follows that the maximum probing depth depends linearly on the number of detector elements \( N \).

It is also possible to implement an FD-OCT system using a single detector. This is done by sequentially recording the optical intensity in a photo detector while synchronously sweeping the wavenumber of a narrowband swept laser source[7]. This modality, Swept-Source OCT (SS-OCT), is discussed in detail later.
1.1.4 Applications of optical coherence tomography

As a high-resolution and fast acquisition technique, OCT can be used in a lot of applications. OCT was initially applied for ophthalmologic imaging, and today it is also used in biomedicine. Compared with other imaging techniques like CT and MRI, the independence of the depth resolution with the sample beam aperture, the high axial and lateral resolution, the high probing depth and the possibility to create function dependent image contrast make OCT a much attractive imaging technique[6].

OCT was first applied for imaging in the eye and the first in vivo tomograms of the human optic disc and macula were demonstrated in 1993[7]. OCT is an important tool for the early diagnosis and analysis of ocular diseases. It also facilitates the monitoring of therapies and surgical procedures. For example, it can be used to guide laser treatment and non-invasively monitor patient performance before, during and after surgery[2].

It is also achievable to use OCT as an alternative to the customary histology and excisional biopsy methods. OCT is valuable in imaging tissue pathologies in situations where conventional excisional biopsies would be dangerous, guiding conventional biopsy to reduce false negative rates from sampling errors and guiding the detection of early neoplastic changes[7]. Studies have shown that cancer tissues absorb and scatter near-infrared light more than the surrounding healthy tissues. So it is possible to use OCT in order to diagnose tumors. What’s more, OCT has been applied to miniature and flexible imaging probes, enabling the acquisition of images of internal organ systems. In vivo imaging of animal and human gastrointestinal and genital tracts has already been performed.

Concerning tissue engineering and biomaterials, OCT is adequate for the setup of automated, non-invasive, and precise measuring systems used for quality control of engineered tissue for
surgical implantation. Additionally, OCT can be applied in nanotechnology, and developments have been made in analyzing the complex flow dynamics within microfluidic structures, using Doppler OCT.

OCT has also been proposed as a suitable technology for high-density data storage on multilayer optical discs, the nondestructive study of polymer matrix composites, and evaluation of paints, coatings, plastics and ceramics[2][7].
1.2 Swept Source/Fourier Domain OCT

Swept source/Fourier domain (SS-OCT) uses an interferometer with a frequency swept, narrow-bandwidth light source. The sweep in frequency with time essentially labels different time delays in the light beam, which can then be detected by interference. The output from a narrow-bandwidth, frequency swept light source is divided into a sample path and a reference path. The light in the sample arm goes to the sample to be imaged and is back-reflected from internal structures at different depths. The light in the reference arm is reflected from a reference mirror at a fixed delay without changing. The sample and reference arm have a time offset determined by the difference between two paths, which is related to the depth of the structure in the sample. As the frequency of the light is swept as a function of time, the light echoes in the sample arm will have a frequency offset from the reference beam. When the sample and reference arms interfere, a modulation or beat in intensity is generated at a frequency, which is given by this frequency offset. As a result, different echo delays will produce different frequency modulations. The A-scan can be measured by digitizing the photo detector signal over a single frequency sweep of the light source, correcting any nonlinearity in the frequency sweep as a function of time and then Fourier transforming this beat frequency signal. This results in an A-scan measurement of the magnitude and echo delay of light from the sample.

Frequency swept light sources typically produce frequency sweeps that are non-linear in time. As a result, it is important to rescale the digitized interference signal so that it is sampled with equal frequency or k intervals rather than equal time intervals. Once a calibration of frequency versus time is available, this resampling can be performed by numerically processing the interference signal. However, modern SS-OCT avoids this computational cost by clocking the A/D at equal frequency or k intervals. This optical clocking is typically performed using a Mach-Zehnder
interferometer to detect the frequency sweep and clock the A/D at a variable rate corresponding to the frequency sweep rate. The technique of optical clocking has the advantages of increasing data processing speed by removing the computationally expensive step of resampling the interference signal and also reducing the amount of data that is acquired, but it requires special A/D instrumentation that can clock accurately with a variable clock rate. Furthermore, the optical and electronic propagation delays in swept source/Fourier domain instruments must be carefully managed because a timing mismatch between the clock and interference signal results in sampling at incorrect times which distorts the interference signal. The requirement of high-speed A/D as well as precise synchronization makes swept source/Fourier domain detection more challenging than spectral/Fourier domain detection.

Like spectral/Fourier domain detection, swept source/Fourier domain detection measures all of the optical echoes at the same time, rather than sequentially as in time domain detection. This enables a dramatic improvement in detection sensitivity. SS-OCT have the advantage in that they can be used in the 1,310 nm and 1,000 nm wavelength ranges where silicon-based cameras lack sensitivity and more expensive InGaAs cameras are demanded. The axial resolution and axial scan rate in swept source OCT are determined by the sweep repetition rate and the sweep range of the laser. If the laser can achieve high sweep repetition rates, imaging can be produced much faster than SD-OCT, which is limited by the camera read rates[5].
1.3 Image Resolutions and Depth of Field

Image resolution is one of the most important factors affecting OCT image quality. Developing a method to achieve high resolution was a major work of early research. Comparing to standard microscopy, OCT can achieve good axial resolution independent of the beam focusing and beam spot size. The axial resolution in OCT is determined by the measurement resolution for echo time delays of light. In low-coherence interferometry, the axial resolution is determined by the width of the field autocorrelation function, which is inversely proportional to the bandwidth of the light source. Considering a Gaussian-shaped spectrum, the axial resolution is

\[
\Delta z = \frac{2\ln 2}{\pi} \cdot \frac{\lambda^2}{\Delta \lambda}
\]  (19)

where \(\Delta \lambda\) is the full-width-at-half-maximum of the power spectrum, \(\Delta z\) is the full-width-at-half-maximum of the autocorrelation function, and \(\lambda\) is the center wavelength of the light source[1]. Figure 1.4 shows a plot of axial resolution versus light source bandwidths for center wavelengths of 850 nm, 1,060 nm, and 1,310 nm. Since axial resolution is inversely proportional to the bandwidth of the light source, broad bandwidth light sources are used to get a much higher axial resolution[5].
The lateral resolution in OCT imaging is determined by the diffraction limited spot size of the focused beam. The diffraction limited minimum spot size is proportional to the wavelength and inversely proportional to the numerical aperture or the focusing angle of the beam. The lateral resolution is given by the following equation:

$$\Delta x = \frac{4\lambda}{\pi} \cdot \frac{f}{d}$$

(19)
where d is the size of the incident beam on the objective lens, λ is the wavelength, and f is the focal length. High lateral resolution can be achieved by using a large numerical aperture that can focus the beam to a very small spot size. At the same time, because of diffraction, the lateral resolution also governs confocal parameter b, which is two times the Rayleigh range:

$$ b = 2z_R = \frac{\pi \Delta x^2}{\lambda} $$

(20)

As a result, there is a trade-off between depth of field and lateral resolution, which means increasing the lateral resolution decreases the depth of field. Usually, OCT imaging is equipped with low numerical aperture focusing in order to have a large depth of field. The confocal parameter is larger than the coherence length (b > \Delta z) and the axial resolution is determined by the measurement resolution for echo time delays of light. Comparing to microscopy, OCT can achieve high axial resolution independent of the numerical aperture of the focusing. This feature is particularly powerful for applications such as ophthalmic imaging in which numerical apertures are limited. Low numerical aperture focusing also limits the lateral resolution because the focused spot sizes are large[5].
1.4 Sensitivity of OCT system

The sensitivity $S$ of an OCT system is also an important feature that can be described as the ratio of maximum signal over noise floor. As the signal powers are proportional to the matching reflectivity, the sensitivity is determined by the following equation:

$$ S = \frac{1}{R_{\text{min}}} \bigg| \text{SNR} = 1 $$

(21)

Considering a Michelson interferometer with an ideal and symmetric beam splitter, and if considering system is operated in a short noise dominant regime, the sensitivity is determined by the following equation:

$$ S = \frac{\alpha}{4} \cdot \frac{P_{\text{source}}}{q_e} \cdot \frac{1}{B} $$

(22)

It is significant to note that the sensitivity of a FD-OCT system depends on sample depth. The roll-off of sensitivity can be described as the decreasing visibility of higher fringe frequencies corresponding to great sample depths[5].
CHAPTER 2: Materials and Methods

2.1 Apparatus

The long-range OCT system design is depicted in Figure 2.1. All the optical components are assembled on a portable Newport optical breadboard. Output light from a 1300 nm MEMS VCSEL swept laser source (25 mW average output power, 100 kHz A-scan rate, over 100 mm coherence length, Thorlabs, Newton, New Jersey) is split by a 99:1 coupler (Thorlabs, Newton, New Jersey) into the sample and reference arms. In the reference arm, light travels through an optical delay line (OZ Optics, Carp, Canada) and is reflected by a fiber mirror (Thorlabs, Newton, New Jersey). In the sample arm, light travel through a rotary joint down to the end of the probe and is reflected by samples. Reflected lights is coupled by a 50:50 coupler (Thorlabs, Newton, New Jersey) and converted to electrical signal by a 1.6 GHz detector (Thorlabs, Newton, New Jersey). Then the signal is digitalized by a 12 bit, 1.8 GS/s digitizer (Alazar Technologies Inc., Pointe-Claire, Quebec).
2.2 Probe construction

A scanning endoscopic OCT probe was developed for 3-D imaging as shown in Figure 2.2. Probe rotation and pull back is accomplished by using a probe control unit. In order to achieve a high-rotation speed, the OCT probe is constructed using a stainless torque coil with an outer diameter (OD) of 0.965 mm (Asahi Intecc, Santa Ana, California) for translating torque from the proximal end of the probe to the distal end of the probe. In order to achieve long-range imaging, the probe is designed to have a working distance of 10 mm. From the most proximal portion of the probe tip, a single-mode fiber is fused to a precisely measured spacer made from no-core fiber whose length determines the working distance of the probe. In front of the no core fiber
portion, the diverging laser beam is focused by an angle cut gradient index (GRIN) lens (1 mm OD, GoFoton, Somerset, New Jersey) onto a 45-degree gold-coated rod mirror. The GRIN lens and fiber are attached by ultraviolet (UV) glue. The incident light on the mirror is then reflected at 45 degree relative to the incident beam creating a side-viewing probe. The optical assembly at the probe tip is protected by a metal housing. The metal housing is firmly soldered to the torque coil by inserting ~2 mm of coil inside the metal housing and applying a little lead-free solder to the junction between the housing and the coil, allowing the solder to seep in and fill the gap between the two components to increase the robustness of the entire probe assembly. To protect the probe during imaging, the probe is rotated within a fluorinated ethylene propylene (FEP) sheath.

Figure 2.2 Schematic of the long-range probe[16].
2.3 1300 nm MEMS VCSEL swept laser source

Thorlabs' MEMS-VCSEL laser source (Figure 2.3, Figure 2.4) is designed for high-speed (more than 100 KHz), long-range, swept-source optical coherence tomography (OCT) applications. This swept laser source is based on a patented Micro-Electro-Mechanical (MEMS)-tunable Vertical Cavity Surface Emitting Laser (VCSEL). With a record-breaking coherence length (more than 100 mm), this source provides single mode, mode-hop-free operation over a tuning range in excess of 100 nm (Figure 2.5). As a result, this laser source can provide a much longer imaging range (more than 10 mm) at 100 KHz sweep speed.
Figure 2.4 Rear Panel of 1300 nm MEMS VCSEL swept laser source

Figure 2.5 MEMS VCSEL Swept Laser Spectrum. The scanning trajectory of the MEMS mirror is optimized for linearity across the sweep. As a result, the MEMS mirror dwells at the edges of the sweep, thereby causing peaks in the emitted spectrum.
2.4 Imaging probe control unit

The imaging probe control unit is designed and constructed which is responsible for OCT imaging probe rotation and pull back (Figure 2.6). All the components are assembled on a linear motorized stage (Zaber Technologies Inc., Vancouver, British Columbia), which is used for linear pullback. The rotation of the motor (FAULHABER, San Diego, CA) is controlled by motion controller (FAULHABER, San Diego, CA). Torque from the motor is translated to the rotary joint by the gears and the belt. Then the probe, which is connected to the rotary joint, will rotate at the same speed. Commercial softwares are used to control the motor and the linear motorized stage.

Figure 2.6 Imaging probe control unit
2.5 Data acquisition and software

During the imaging experiment, the imaging probe control unit rotates the entire probe at 3000 rpm to achieve 50 frames/s (2000 A-lines per frame) with a pull back speed of 12.5 mm/s to minimize the time for future in vivo imaging. Data acquisition is achieved using a 12 bit, 1.8 GS/s digitizer (Alazar Technologies Inc., Pointe-Claire, Quebec). Our OCT software package is written entirely in C++ and features a multithreaded design for data acquisition, image processing and display, which allows for maximizing computational throughput. Due to the high-speed acquisition of LROCT data, the computer system is equipped with a commercial graphics-processing unit (GPU) for displaying real-time two-dimensional (2-D) cross sections imaging. Three-dimensional (3-D) reconstruction is then performed using the acquired 2-D images with the help of commercial software.

2.6 Imaging sample preparation

The trachea of New Zealand white rabbit from Dr. Wong’s lab was used as sample of in vitro OCT imaging. The rabbit trachea was stored in 10% formalin solution for 24 hours after extracted from the body and then was preserved in buffer solution at 4°C.
CHAPTER 3: Results and Discussion

3.1 Development of long-range OCT system

Following the schematic in figure 2.1, all the components were connected together to build up a long-range OCT system (shown in Figure 3.1). A 101.5 cm long, endoscopic OCT probe was made followed by the schematic in chapter 2 (shown in Figure 3.2).

Figure 3.1 Long-range OCT system
Figure 3.2 Endoscopic OCT probe

Extra fibers (103.5 mm) were used in the sample arm after measurement and calculation to make sure the reference arm and the sample arm have the same length approximately. An optical delay line (OZ Optics, Carp, Canada, shown in Figure 3.3) was used in the reference arm to make sure the reference arm and the sample arm have exactly the same length.
I used a superluminescent diode (SLD) light source (B&W TEK, Newark, DE) for the OCT system at that moment because it was easier to find the interference pattern compared to a swept laser source. I changed the length of optical delay line until the interference pattern shown as Figure 3.4 acquired by an oscilloscope came up, which means the reference arm and the sample arm have exactly the same length.
To deliver more laser from the laser source to the probe, I cleaned all the fiber tips and measured the power of laser after each fiber by power meters (Thorlabs, Newton, New Jersey, shown as Figure 3.5) shown as table 3.1.

![Figure 3.5 Fiber Optic Power Meters with Internal Sensor](image)

<table>
<thead>
<tr>
<th>Number of fibers by which laser travels</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power of laser</td>
<td>25.3 mW</td>
<td>24.8 mW</td>
<td>23.7 mW</td>
<td>23.0 mW</td>
</tr>
</tbody>
</table>

Table 3.1 Power of laser from the laser source to probe
3.2 Performance of the long-range OCT System

3.2.1 Lateral resolution and working distance measurement

From what we discussed in chapter 1, we know that the image lateral resolution is determined by the focused spot size of the OCT beam at working distance. I built up a 3-D stage to measure the lateral resolution and working distance of the probe. A beam profiler (DataRay, Redding, CA) was set up on the stage. The lateral resolution can be expressed by the FWHM (full width at half-maximum) of the beam, which can be measured by the beam profiler. In this experiment, the probe was put in front of the beam profiler (shown as figure 3.6).

![Figure 3.6 Experiment of measuring lateral resolution and working distance](image)

The 3-D stage was adjusted to make sure the light spot was detected by the beam profiler. The beam profiler was kept moving until we got the smallest FWHM in the beam profiler. From Figure 3.7 we can tell that the lateral resolution of the probe is 79.3 μm. From figure 3.8 we can
tell that the beam spot from the probe is very round, which means the probe was perfectly made. Working distance of the probe is 11.5 mm, which is the distance from the beam profiler to the probe.

Figure 3.7 Lateral resolution of the probe

Figure 3.8 Shape of the beam from the probe
3.2.2 Axial Resolution Measurement

Based on the theory of OCT, the axial resolution is determined by the point spread function (PSF) of the system. A mirror was used as sample to acquire an A-scan data in this experiment (shown in Figure 3.9). We put the mirror on a 3-D stage and adjusted the angle of the mirror to make sure all the light was reflected back to the probe. We measured the ratio of the spatial distance over the digital frequency at first by using the raw data at different imaging depth near zero delay. Then we got the axial resolution by calculating the product of FWHM of the peak in point spread function (PSF) and the ratio, which is 12.9 µm (shown in Figure 3.10 and 3.11).

![Figure 3.9 Experiment of measuring axial resolution and sensitivity of OCT system](image)
Figure 3.10 Point spread function of OCT system

Figure 3.11 Point spread function of OCT system (zoomed in)
3.2.3 Sensitivity Measurement

To measure the OCT system sensitivity, the interferometer should be constituted of a reference arm with higher optical power and a sample arm with lower optical power. So in our experiment, the optical sample arm was attenuated by 40 dB. In this condition, the SNR of PSF (also A-scan of a mirror) near the zero delay was measured, which is 56.8 dB (shown in Figure 3.12). The OCT sensitivity is expressed as

\[
Sensitivity[\text{dB}] = SNR[\text{dB}] + Sample\_Attenuation[\text{dB}]
\]

(22)

So the sensitivity of this OCT system is 98.8 dB.

Figure 3.12 Point spread function of OCT system
3.2.4 Sensitivity roll-off

For an OCT imaging system, an important performance parameter is the sensitivity roll-off as a function of the distance from the zero delay. The point spread function (PSF) versus distance from the zero delay graph shown in Figure 3.13 was acquired by moving the optical delay line at various distance. So the system features a 6 dB sensitivity roll off at 6 mm offset.

![Graph showing sensitivity roll-off](image)

Figure 3.13 Sensitivity of the OCT system with respect to distance from zero delay. The imaging ranges for 6 dB sensitivity roll off is 6 mm.
3.3 Test imaging of a human finger and tapes

To test the imaging capabilities of our system before in vitro experiment, a human finger and a roll of sealing tape (Intertape polymer group, Newport Beach, CA) were used as samples for OCT imaging. The OCT probe with a protective fluorinated ethylene propylene (FEP) sheath together was placed between the finger and tape closely. Next, the probe was rotated at a 3000 rpm to achieve 50 frames/s (2000 A-lines per frame) without pullback by the imaging probe control unit. Data was acquired by our system and the 2-D image is shown in Figure 3.14. Epidermis layer and dermis layer of finger are clearly differentiated as well. Layers of the tape are clearly differentiated. The depth of the tape imaging is 13 mm approximately.

![OCT image of a finger and sealing tape](image_url)

Figure 3.14 OCT image of a finger (left and right) and a roll of sealing tape (middle). Epidermis layer (E) and dermis layer (D) of the finger are clearly differentiated. Layers of the tape are clearly differentiated as well.
3.3 Rabbit Trachea *in vitro* OCT imaging

*In vitro* imaging was performed on a trachea of New Zealand white rabbit. The trachea is 5.2 cm in length with 7.8 mm OD approximately. The OCT probe with a protective fluorinated ethylene propylene (FEP) sheath together was inserted into the trachea (shown in Figure 3.15). Next, the probe was rotated at a 3000 rpm to achieve 50 frames/s (2000 A-lines per frame) with a pull back speed of 12.5 mm/s by the imaging probe control unit. Data were acquired and both circumferential and Cartesian 2-D images were generated by the OCT system at the same time (Figure 3.16, Figure 3.17). As the sensitivity of the system is very high, cartilage, mucosa layer and submucosa layer can be clearly differentiated.

![Figure 3.15 Experiment of rabbit trachea *in vitro* OCT imaging](image-url)
Figure 3.16 Cartesian OCT image of a rabbit trachea. Cartilage (C), mucosa (M) and submucosa (SM) can be clearly differentiated.

Figure 3.17 Circumferential OCT image of a rabbit trachea. Cartilage (C), mucosa (M) and submucosa (SM) can be clearly differentiated.
CHAPTER 4: Conclusion

A long-range OCT system, an imaging probe control unit and a long-range probe were constructed in this project. Performance of the system such as sensitivity and resolution were measured. *In vitro* endoscopic imaging experiment of rabbit trachea was conducted by the long-range OCT system. Cartilage, mucosa layer and submucosa layer of the rabbit trachea can be clearly differentiated in the 2-D OCT images.

In the future, 3-D model of rabbit trachea will be created from the acquired data sets. Also, we plan to assemble the OCT system, imaging probe control unit with power supply and the computer together in boxes to make it portable for *in vivo* imaging. This imaging technology will be applicable to a broad range of diagnostic conditions.
REFERENCES:


