INVESTIGATION OF LOW COHERENCE INTERFEROMETRY TECHNIQUES FOR BIOMEDICAL APPLICATIONS

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To my lovely wife, *Ng Hwan Teng* and my dear sons, *Qi Xuan* and

*Xuan Dong*. 
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Interferometric techniques have been used as imaging techniques in many applications. In using low coherence sources in interferometry, good axial resolution can be achieved. The objective of this thesis is to investigate the use low coherence interferometry in biomedical applications. Low coherence interferometry (LCI) was incorporated into an optical tweezers system to determine the trapping dynamics. An optical coherence tomography (OCT) system based on LCI principles was designed and implemented to detect virus infection in orchid leaves. The OCT system was then modified to realize a high-speed endoscopic OCT imaging system. A numerical method and a common-path probe design were investigated to address the issue of loss of axial resolution due to dispersion.

Optical tweezers typically use back-focal interferometry to determine the position of an object in the optical trap. Extensive initial calibration is needed before accurate positional measurements can be made. A LCI system, on the other hand, can provide accurate axial measurements with minimal calibration. A common-path LCI system was incorporated into an optical tweezers system. The light beams of LCI and optical tweezers are collinear, thereby ensuring a good axial accuracy. The trapping dynamics of a trapped microsphere under different trapping conditions were investigated. The results demonstrated that LCI is a viable alternative to back-focal plane interferometry in determining the trapping dynamics.

A spectral domain optical coherence tomography (SD-OCT) system was designed and built to identify virus infection in orchid plants. Besides revealing the cross-sectional
structure of orchid leaves, a highly scattering upper leaf epidermides were detected with OCT for virus-infected plants. This distinct feature is not observable under histological examination of the leaf samples. The results suggest that virus-infected orchid plants can be accurately identified by imaging the epidermal layers of its leaves with OCT, thereby potentially leading to a better control on the spread of viruses in the orchid industry.

*In vivo* imaging of human tissues has given valuable insights into the understanding of diseases and their management. Conventional *in vivo* imaging involves white-light endoscopy which relays the optical image of tissue surface back to the user. By contrast, OCT allows the imaging of tissue a few millimeters below the tissue surface which provides additional information on the tissue structure. A gradient-index (GRIN) rod probe was designed and incorporated into a nasopharyngeal rigid probe for both OCT and white-light imaging. To capture an image with minimal motion blur, a fast spectrometer was designed with high spectral resolution. The results showed that there is sufficient resolution in the overall endoscopic OCT probe system to pick out sweat ducts beneath a human skin.

The axial resolution of the OCT system depends on the bandwidth of the light source. However, dispersion mismatches between the sample and reference arms of the interferometer broadens the axial point spread function which worsens the axial resolution. A numerical technique was used to extract the phase of the interferogram which is then applied to offset any systemic dispersion mismatches in the system. The results showed that the numerical technique is effective in recovering the OCT image with dispersion. A common path probe with an axicon tip was also proposed to combat dispersion. The axicon tip allowed imaging of a few millimeters without significant
loss of resolution due to the diffraction-free properties. The common-path design
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<td>Back focal plane interferometry</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-coupled device</td>
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<tr>
<td>FWHM</td>
<td>Full-width at half-maximum</td>
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<tr>
<td>GRIN</td>
<td>Gradient index</td>
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<tr>
<td>LCI</td>
<td>Low coherence interferometry</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical aperture</td>
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<tr>
<td>NIR</td>
<td>Near infra-red</td>
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<tr>
<td>OCT</td>
<td>Optical coherence tomography</td>
</tr>
<tr>
<td>PSF</td>
<td>Point spread function</td>
</tr>
<tr>
<td>SLD</td>
<td>Super luminescence diode</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
</tr>
<tr>
<td>Ti:Sapphire</td>
<td>Titanium sapphire</td>
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Chapter 1  INTRODUCTION

1-1  Motivation

Interference effects in optics have been a source of intrigue since the time when Thomas Young conducted his famous double slit experiment. The results of the experiment swayed the scientific community from the corpuscular theory of light favored by Isaac Newton to the wave theory of light. The invention of the laser, with its temporal and spatial coherent properties, allowed interference effects to be studied more extensively. With more recent advances to produce single photon sources and detectors, interference patterns can still be observed in a double slit experiment. This was also observed for electrons when they passed through the equivalent of the Young’s double slit. These later experiments gave the corpuscular theory of light much more weight.

While interference as an effect has been the scope of extensive theoretical and experimental work, its application has grown widely as well. Astronomical interferometry provides very high resolution for observing stars and galaxies. Holography is an application of interferometry which is widespread in the financial (credit cards), the information technology (digital storage) and the entertainment industries. At the other end of the imaging scale, phase contrast and differential interference microscopy have become common tools in imaging small objects such as
biological cells and its subcellular components. A Nobel Prize was awarded to Zernike for the invention of phase contrast microscope which relies heavily on interference effects.

The phase contrast microscope is one of the earlier biological applications using interference effects. As biological cells are generally transparent, the contrast between them and the surrounding medium, e.g. water, under white light illumination using tungsten lamp or mercury-halogen lamp is poor. Phase contrast microscopy overcomes this by shifting the phase of the wave contributed by the surrounding medium. The delayed surrounding wave then interferes with the diffracted wave caused by the presence of a cell at the image plane to provide the necessary contrast.

Interference requires the phases of the light waves to be correlated. The concept of coherence length is used generally to describe how well the phases are correlated. Quasi-monochromatic sources such as the laser generally have very high coherence length. At the other extreme, sunlight or white light sources such as a tungsten filament lamp have low coherence lengths. While long coherence length properties are useful in many biological applications such as confocal microscopy, low coherence light sources are also useful in many other applications like optical coherence tomography (OCT) [1-3] and low coherence interferometry (LCI).

In LCI, interference effects are only observed when the optical path difference between the sample and a stable reference beam are within its short coherence length of the light source. Contrast is thus provided at a very precise location. This can be used to track small objects suspended in medium by tools such as optical tweezers,
which have been widely used to trap and manipulate small objects or biological cells using light [4, 5]. This allows the trapped object to be investigated without any physical contact. The dynamics of an optically trapped object reveal the size, shape and even its biological properties (e.g. state of health in red blood cells) [6]. Besides back focal plane interferometry used commonly in optical tweezers, LCI can provide an alternative method to track and measure the location of an object in an optical trap.

In OCT, interferometry is used to detect minute changes in the refractive indices in the axial direction of a sample. In conventional optical applications such as brightfield microscopy, the back-scattered light that is collected by a detector contains all information from the sample. The details of where groups of scattered photons originate from are lost in this type of configuration. OCT utilizes the low coherence length of the light source to provide the contrast and location of each back-scattered region to recover those details. A high coherence light source will not be able to pick out those details as no interferometric contrast will be available.

Compared to other biological imaging modalities such as ultrasound and confocal microscopy, OCT provides a niche area where imaging depth is sufficiently good to penetrate a few millimeters below a tissue surface and yet offers axial resolution in the region of a few micrometers. While ultrasound systems have penetration depths of up to a few centimeters, the resolution is not good enough to pick out tissue level morphologies. On the other hand, although confocal microscopy systems have the ability to resolve cellular level morphologies, the penetration depth is very shallow especially for samples with high scattering.
OCT is commonly used in ophthalmology applications [1, 7-12]. The ability to remotely image the internal layers of the retina without excision is one of the major strengths. With more recent advance developments in OCT, turbid tissues [13-15] can also be imaged. Being able to penetrate a few millimeters below the tissue surface, OCT is a good modality to image epithelial tissues. These are generally tissues lining the exterior of organs to provide protection from changes in environmental factors. As such, they are subjected to higher stresses which may lead to higher chances of inflammation or changes in tissue structure including acute or chronic inflammation, dysplasia and neoplasia. OCT has been shown to be capable of imaging epithelial tissues with good axial resolution [14, 16].

Much research of OCT has concentrated on animal and human tissue imaging. Less emphasis has been placed on plant imaging despite plants being an important source of food and medicine. Crop yields need to be closely monitored to ensure that there is enough food to feed the ever increasing world population. Virus infection is one of the serious conditions that can significantly affect crop yield. There is a need for a quick way to identify and isolate virus-infected plants thereby minimizing the impact on yields. Diagnosis can be performed on plant leaves to determine if they are infected and OCT can play a role in picking out inter-tissue structures that may indicate virus infection.

At the micro level, viruses and bacteria have been studied via optical trapping [17]. Due to its shape, composition and structure, different viruses and bacteria exhibit different behaviors when trapped. The optical spring constant is a metric to determine how the trapped object behaves. Optical trapping has conventionally use back focal
plane interferometry (BFP) [18, 19] to track trapped particles and thereby derive the trapping dynamics. However BFP has a limited axial tracking range [19]. LCI can potentially provide a longer tracking distance due to its coherence gating ability [20] resulting from a stable reference beam. The longer tracking distance will be useful in situations where multiple particles are trapped [21] or in optical chromatography [22]. In addition, the same OCT system used in the detection of plant viruses can be incorporated into an optical trapping system.

Very often, diseases occur due to viral or bacterial infection may display various symptoms at not easily accessible sites, such as roots in plants or tissue deep inside the body. To visually inspect such sites, endoscopy may be required. Biopsies are commonly taken at suspicious sites for further histopathological analysis. Depending on the size of the biopsy, typical analysis involves examining the epithelial layers down to the mucosa layers. White light endoscopy does not have the resolution or the penetration depth to examine these layers. By contrast OCT systems can be incorporated into endoscopic systems to extend its capabilities to reach deep inside the body. OCT can provide additional details by probing a few millimeters below the tissue surface to pick out early tell-tale signs of diseases. The incorporation of the OCT technology to endoscopy allowed cross-sectional imaging to be taken in areas such as the cardiovascular system, gastrointestinal, pulmonary, urinary and female reproductive tracts [23-30]. Besides medical imaging, such probes can be used for plant imaging in the field. This can provide real-time, mobile analysis that would mitigate the issue of false negative or false positive samples.
The use of low coherence sources in interferometry generally requires minimal dispersion mismatches between the two arms. These low coherence sources are required to have relatively large bandwidths, and consequently an axial point spread function (PSF) with a very small full-width at half-maximum (FWHM), in order to produce good axial resolution. Dispersion mismatches can cause broadening of the axial PSF which degrades the OCT image quality. The use of graded index (GRIN) rods in the endoscopy systems to deliver light at the sample can cause significant dispersion mismatches in the interferometry system. To mitigate the degradation of the image quality, it is therefore important to either physically or numerically limit the amount of dispersion mismatches.

1-2 Objectives

The objectives of this thesis are:

a) Investigation of LCI for localizing the position of optically trapped objects

LCI will be used to observe and locate optically trapped microspheres. The LCI system will be incorporated to an optical trapping system. Instead of using back focal plane interferometry to determine the trapping dynamics, LCI will be used to characterize them. The objective of this work is to provide an alternative approach to study trapping dynamics using LCI.

b) Detection of virus infected orchids using OCT

The current practice of identifying virus infected plants is by visual inspection or through the use of bio-chemistry methods (e.g. ELISA). In many cases,
virus infection takes place long before visible symptoms show up on the surface of leaves. By then, control methods to isolate the plants may be too late. A Fourier domain-OCT system will be developed to investigate its use in identifying virus infected plants before visible symptoms can be observed.

c) Design of an OCT probe and system for *in vivo* nasopharynx imaging

White light endoscopy is one of the common methods used to image the nasopharynx to screen for early stage nasopharyngeal carcinoma (NPC). Biopsies of suspicious sites (e.g. high density of blood vessels) are often taken and sent to the histologist to confirm the state of the tissue. To further enhance the endoscopy diagnostic ability of existing nasopharynx probe, an OCT system will be incorporated to a white light endoscopy system to image the nasopharynx. As the OCT system is capable of imaging a few millimeters below the tissue surface, the incorporation of OCT will provide additional depth information.

d) Optimization of OCT image quality through dispersion control mechanisms

The dispersion properties of the gradient index (GRIN) rod used in the OCT endoscopic system will be characterized and compare against the simulated model. The amount of dispersion mismatches in the system will be calibrated numerically so as to optimize the axial point spread function. An alternative probe design for *in-vivo* imaging based on a common path design to overcome dispersion and polarization mismatch will also be explored.
1-3 Originality of Thesis

The major contributions of the thesis are as follows:

a) An optical trapping system with LCI was designed and implemented to investigate the dynamics of a trapped object. LCI provided another mechanism to study the dynamics of an optical trapped object. Different numerical aperture microscope objectives were used in the system and the LCI system was used to derive the optical spring constant of the trap. This work was published in [31]. The co-authors in the published work were involved in reviewing the design and the experiment results. Lee, W.M. assisted in the setting up of the optical trapping portion of the system. A dual beam trap was also implemented and its optical spring constant was characterized using LCI.

b) A Fourier domain OCT system was developed to image the leaves of Oncidium orchids. The OCT system was able to discern virus infected orchids before symptoms were visible on the leaves. This was achieved by observing the presence of highly scattering upper leaf epidermides in the OCT image of virus-infected plants. A simple metric based on the average intensity values around the epidermal layer was used to classify virus-infected and virus-free leaves. Leaves that were stressed but not virus infected was also correctly diagnosed to be virus-free with the OCT technique. This work was published in [32]. Chia, T.F. provided the background to orchid diseases and the samples. The interpretation of the OCT scans and the subsequent link to virus detection were carried out in discussion with Tan, K. M.
c) A nasopharynx probe based on a gradient index (GRIN) rod was developed and incorporated into a white-light endoscopy system. A hand-held probe was designed to enable the clinician to use both OCT and white light imaging. The Ear, Nose and Throat (ENT) specialists at Changi General Hospital (CGH) provided design requirements on the use of the probe in a clinical setting. To achieve real-time OCT imaging, a high-speed spectrometer was designed. Coma aberration was minimized to achieve good spectral resolution which enabled high frequency spectral modulation to be detected. The high frequency spectral modulation translates to a larger imaging depth.

d) The dispersion of the GRIN rod used in the nasopharynx probe was characterized using numerical methods. This was used to calibrate the dispersion mismatches between the two arms of the OCT interferometer. An alternative probe design based on common-path OCT was also evaluated to physically minimize dispersion between the reference and sample signals. The common path design was published in [33]. Tan, K.M. fabricated the tip and Mazilu, M simulated the output optical profile of the conical fiber tip. The evaluation of the probe was conducted jointly with Tan, K.M.
1-4 Organization of Thesis

The thesis is organized into the following seven chapters:

Chapter 1 presents the motivation, objectives and major contribution of the thesis.

Chapter 2 provides a review of optical interference, LCI and OCT principles including both time-domain and spectral-domain variants. Comparisons with other imaging modalities in tissue imaging were also made in this chapter.

Chapter 3 describes a combined LCI and optical trapping system. The optical trapping spring constants were characterized using the combined system and objectives with different numerical apertures.

Chapter 4 presents the application of OCT in plant tissue imaging. OCT images of virus-infected and virus-free leaves are compared and discussed. The metric used to differentiate between them is also described.

Chapter 5 reports the design of a high-speed endoscopic OCT system for use in nasopharynx imaging. An OCT scanning probe based on an existing white-light endoscopy probe and a high speed spectrometer are designed, implemented and characterized to assess its use for tissue imaging.
Chapter 6 presents two methods to minimize the dispersion mismatches in the endoscopic OCT system developed in the previous chapter. The first method extracts the optical phase numerically to characterize systemic dispersion which can be used to enhance the axial PSF. An alternative probe design, as a physical method, is also presented to overcome certain shortcomings in the GRIN rod based probe design.

Chapter 7 presents the conclusion of the thesis and some recommendations for future work.
Chapter 2  BACKGROUND

2-1  Fundamentals of Interferometry

One of the earliest experimental works on wave theory of light started with the famous Young’s double slit experiment. Light was shown to exhibit wave-like behavior when bright and dark interference fringes form on a screen far behind two closely spaced narrow slits. These fringes can be explained by the principle of superposition where light waves originating from the two slits add constructively or destructively. Although the Young’s double slit experiment is commonly explained using classical physics, it can also be explained using quantum mechanics via the uncertainty principle. The large deviation of the horizontal momentum of the photon parallel to the plane of the screen is due to the narrow width of the slit.

A fundamental concept in interferometry is coherence. For Young's double slit experiment to work well, a small slit is generally placed between a light source and the screen containing the double slit. The small slit is effectively a small pin-hole which results in a secondary light source that is spatially coherent. In the wave-theory description, this implies that the phases of the electromagnetic wave passing through the small area at different points are correlated with one another. It should be noted that the phases across the area need not be exactly the same for interference to occur and it is the correlation between the phases that is more important. A spatially coherent wave arriving at the double slits will have phases that are correlated to one another.
such that interference fringes are formed at the screen on the far side (far field). If the phases are totally uncorrelated, such as illumination with an incoherent light source, no interference fringes will form.

The degree of correlation between the phases of two waves can be described by the interferometric visibility (or contrast) [34]

\[ V = \frac{I_{\text{max}} - I_{\text{min}}}{I_{\text{max}} + I_{\text{min}}} \]  

(2-1)

where \( I \) refers to the intensity of the fringes. For completely coherent, \( V = 1 \) (\( I_{\text{min}} = 0 \)) while \( V = 0 \) (\( I_{\text{max}} = I_{\text{min}} \)) when the interfering waves are completely incoherent. For \( 0 < V < 1 \), it is termed as partially coherent. The spatial coherence theory can also explain why distant stars flickers but nearby planets do not flicker in the night sky.

The atmosphere consists of air molecules that act as scattering zones or as “slits”. For the distant stars, the angle that subtends is very small making the light reaching the observer spatially coherent. The distant stars are effectively point sources relative to the air molecules. The superposed scattered waves from these slits coherently interfere to form a final image on the observer. However, the turbulent nature of the atmosphere shifts the position of the air molecules or slits randomly. The scattered waves constructively interfere and at other times, destructively interfere at the observer, which results in the twinkling of the stars. On the other hand, for nearby planets, the angle subtended is much larger (spatially incoherent) relative to the air molecules and as such, the scattered waves from them do not coherently interfere [35]. The brightness of such planets will appear relatively constant despite the turbulent atmosphere.
In addition to spatial coherence properties, light waves also exhibit temporal coherence which is dependent on the light source. It is a measure of the degree of correlation between the phases at the same location in space at different time points. To test for temporal coherence, an amplitude-splitting interferometer can be used.

2-1-1 Amplitude-splitting interferometer

One of the simplest amplitude-splitting interferometry setup is the Michelson interferometer (Figure 2-1). The basic components include a light source, a detector, two mirrors and a beam splitter. For simplicity, a monochromatic light source is considered in the setup. The E-field of the monochromatic light leaving the light source can be represented in complex notation as

$$\vec{E} = E_0 e^{-j(k \cdot r - \omega t)}$$

(2-2)

where $k$ is the wave-vector and $\omega$ is the frequency of the wave. The beam splitter divides the power of the incident light into the two paths with a pre-determined ratio (e.g. 50:50, 10:90). The mirrors reflect the light waves in each path and the detector is used to measure the interference. Depending on the relative distance or optical path difference between the two mirrors, the detector may measure light intensity of between zero and the full intensity of the light source.
Without loss of generality, the Michelson interferometer can also be represented by the folded form shown in Figure 2-2.
The reflected E-field from mirror $M_1$ can be written as

$$E_1(t) = E_1 e^{-j(k \cdot d_1 - \phi)}$$  \hspace{1cm} (2-3)$$

and the reflected E-field from mirror $M_2$ can be expressed as

$$E_2(t) = E_2 e^{-j(k \cdot d_2 - \phi)}$$  \hspace{1cm} (2-4)$$

$E_1$ and $E_2$ are the amplitudes of the reflected fields which are assumed to be time-independent and are a fraction of the original amplitude, $E_0$. $k$ is a scalar quantity as the propagation direction is along the axial direction of the light source where $k = \frac{2\pi}{\lambda}$.

The total E-field is given by the sum of the reflected fields from both mirrors ($M_1$ and $M_2$) and can be represented as

$$E'_t(t) = E_1(t) + E_2(t) = E_1 e^{-j(k \cdot d_1 - \phi)} + E_2 e^{-j(k \cdot d_2 - \phi)}$$  \hspace{1cm} (2-5)$$

The irradiance (in units of $W/m^2$) or commonly denoted as intensity, measured at the detector as a function of time is given by

$$I(t) \propto \Re \left( E'_t \cdot E'_t^* \right) = E_1^2 + E_2^2 + 2E_1E_2 \cos \left[ \frac{2\pi}{\lambda_0} \frac{\Delta}{t} \right]$$  \hspace{1cm} (2-6)$$

where $\nu = \frac{\Delta}{t}$, $\Delta = (d_1 - d_2)$ and $E'_t^*$ is the complex conjugate of $E'_t$.

It is worth noting that the cosine term in equation 2-6 is the interference term whereas the amplitude square term corresponds to a DC constant. The interference term provides the contrast. The visibility of the fringes can be re-written as
\[
V = \frac{\text{interference term}}{\text{d.c. terms}} = \frac{2E_1E_2}{E_1^2 + E_2^2}
\]  

(2-7)

where \( V \) is between 0 and 1. For the simple case of monochromatic light, maximum contrast (\( V = 1 \)) occurs when \( E_1 = E_2 \). No interference fringes are observed when either \( E_1 = 0 \) or \( E_2 = 0 \) (\( V = 0 \)) for the special case where there are no superimposition of the reflected waves.

The frequency of the interference term depends on the value of \( k_0 \) or \( \lambda_0 \) and the relative speed of the mirrors, \( v \). The interference term is essentially amplitude modulated with a carrier frequency of \( \frac{2v}{\lambda_0} \) and the modulation amplitude is determined by the product of \( E_1 \) and \( E_2 \). The sinusoidal variation of the interferometric intensity has a large range (optical path difference between the two mirrors) with little or no amplitude modulation. This implies that interference fringes will form regardless of the relative positions of the mirrors. As a result, interference fringes do not provide information on the relative distance between the two mirrors. This can be overcome when a low-coherence light source is used.

2-1-2 Low coherence interferometry

Low coherence interferometry (LCI) utilizes broad bandwidth light sources. Low coherence refers to the short coherence time that is a property of the broad bandwidth light sources. As interference fringes can only be observed when the optical path delay
between two light superposed paths is within the coherence time, interferometry using broadband light sources will have a very small optical path delay where these fringes are observed. A monochromatic light source has perfect temporal coherence as the phases of the electric field at the same spatial location are completely correlated in time. This allows interference fringes to be observed even if the waves are delayed infinitely. On the other hand, a low-coherence light source has low temporal coherence. The phases of the E-field at the same spatial location are only correlated within a short period of time. Beyond this coherence time, the phases from two superposed light paths are not correlated and interference fringes are not observable. The time averaged intensity of these superposed waves beyond the coherence time averages out to give just a constant value (i.e. the visibility of the fringes are zero).

It can also be shown that the coherence time is inversely proportional to the bandwidth of the light source [36]. For low coherent or partially coherent light sources, the degree of temporal coherence provides a measure of coherence time and is given by the autocorrelation function 

$$g_{12}(\tau) = \frac{\langle U_1^*(t)U_2(t+\tau) \rangle}{\langle U_1^*(t)U_2(t) \rangle}$$

(2-8)

where $U(t)$ is the scalar E-field, the angular brackets indicate time-averaging and the asterisk denotes the complex conjugate. The coherence time can be defined as the FWHM of the autocorrelation function. For monochromatic light sources, $g_{12} = 1$ for all $\tau$ (i.e. infinite coherence time). Consequently, equation 2-6 can be modified in the general case to cover different types of light sources as follows:
\[ I(z) \propto E_1^2 + E_2^2 + 2E_1E_2|g_{12}(\delta)|\cos[2k.(z - z_0)] \]  
\hspace{1cm} (2-9)

where \( \delta = c \times \tau \) and \( c \) being the speed of light, \( z_0 \) is the position of the reference mirror.

Figure 2-3 shows the plot of equation 2-9 with \( g_{12} \neq 1 \) and \( g_{12} \neq 0 \) (e.g. with a light source of 2 \( \mu \)m coherence length). It can be seen from this figure that interference will only occur when the relative distance between the two mirrors are small. The envelope of the interference fringes as defined by \( g_{12} \) provides a measure of the coherence length of the interferometry system. Thus, light sources with low coherence lengths are desired to accurately determine the relative position of the mirrors.

Figure 2-3: Interference fringes with partially coherent light with a Gaussian shaped spectrum and a coherence length of 2 \( \mu \)m.
The autocorrelation function (spatial or temporal domain) can be obtained mathematically by applying the inverse Fourier transform to the spectrum (in the wavenumber or angular frequency domain) of the light source as described by Wiener Khinchin theorem [36]. For a light source with a Gaussian shaped spectrum, the autocorrelation function is also Gaussian-shaped. The spectra of light sources such as the SLD and Ti:Sapphire lasers can also be approximated as Gaussian sources. The corresponding coherence length is then defined as the FWHM of the autocorrelation function, and is determined by the choice of the linewidth ($\Delta \lambda$) and center wavelength ($\lambda_0$) following

$$\Delta z = \frac{2 \ln 2 \lambda_0^2}{\pi \Delta \lambda} \quad (2-10)$$

It can be seen from equation 2-10 that the coherence length is inversely proportional to the spectral bandwidth.

The coherence length is an important system property in LCI which determines the ability of the system to resolve fine structures. The axial resolution in a LCI system can be defined by the FWHM of the inverse Fourier transform of the un-modulated light source spectrum. The axial resolution is therefore similar to the coherence length of the light source. If the sample is surrounded by a medium (e.g. water), the axial resolution is given by the coherence length divided by the refractive index of that medium. This implies that while the coherence length is a property of the light source, the axial resolution can be better than the coherence length if the sample has a higher refractive index than air.
2-2 Optical Coherence Tomography

Optical coherence tomography (OCT) is an extension of LCI. One main difference between them is that the axial depth profiles across a lateral scan obtained in OCT measurements are compiled into a tomogram [1, 3]. As such OCT is analogous to ultrasound imaging where the system detects echoes from discontinuities in a sample. In OCT, optical echoes are detected since light is being used as a source instead of sound. The discontinuity in the resulting OCT image is a result of changes in the sample refractive index. One key difference between ultrasound and OCT is in the detection method. The echoes are directly detected in ultrasound measurements. In the case of OCT, interferometry methods have to be employed to detect the back-reflected or back-scattered light as the frequency of light is extremely high.

It is desirable to use light sources with large bandwidths in OCT to achieve good axial resolution. Several types of broadband light sources have been used for OCT, namely SLDs, Ti:Sapphire lasers and supercontinuum light sources [37]. The typical optical bandwidth of SLD is 40 nm and can be increased to 95 nm at the expense of output optical power [7]. This corresponds to axial resolutions of between 11.1 µm to 27 µm at the center wavelength of 1550 nm. A few SLDs have been combined together to provide bandwidths as wide as 100 nm at the center wavelength of 800 nm [38]. The axial resolutions in such system can be as good as a few micrometers. By contrast, an optical bandwidth of 260 nm at a center wavelength of 800 nm can be achieved with a Ti:Sapphire laser yielding an axial resolution of 1.09 µm [39]. More recently the supercontinuum source, generated using photonic crystal fibers has the potential of achieving an axial resolution in the sub-micrometer range [40, 41].
Figure 2-4: Theoretical calculation of axial resolution as a function of optical bandwidth at center wavelengths of 800 nm and 1300 nm (from reference [3]).

Figure 2-4 depicts the theoretical calculations of the free space resolution as a function of optical bandwidth at center wavelengths of 800 nm and 1300 nm [3]. It can be seen from Figure 2-4 that the resolution improves with the bandwidth of the light source. Furthermore, lowering the center wavelength of the light source will also improve the axial resolution. The choice of light source also affects the depth penetration of the system. Two types of light source with different center wavelengths have been widely used. They are the 800 nm and 1300–1550 nm wavelength light sources. The imaging depth is limited predominantly by scattering in most biological tissues. Scattering is strongly dependent on wavelength and decreases for longer wavelengths. The optical diagnostic window where there are minimum scattering and absorption of light in biological tissues is between 800 and 1550 nm [42]. In this transmission window, an imaging depth of up to 3 mm can be achieved [3, 43]. Moreover, Rayleigh scattering
becomes more significant with lower wavelengths and may offset the gain in resolution with a lower back-reflected or backscattered signal.

In many microscopy modalities, the axial and lateral resolutions of the system are determined by the final imaging lenses. In a typical microscope system, the objective lens determines both the lateral resolution and depth of field. The lateral resolution is based on Rayleigh criteria and is given by

\[ \Delta x = \frac{0.61\lambda_0}{NA} \]  \hspace{1cm} (2-11)

where \( \lambda_0 \) is the center wavelength of the light source and NA is the numerical aperture of the lens. This is the smallest distance between two lateral points in the sample that can be resolved by an aberration-free objective lens. The depth of field is defined by

\[ \Delta z = \frac{\lambda_0 n}{NA^2} + \frac{n}{M \cdot NA} \cdot e \]  \hspace{1cm} (2-12)

where \( n \) is the refractive index of the medium between the objective and sample, \( e \) is the smallest lateral distance in the image plane in which the lens can resolve, sometimes referred to as the circle of least confusion, and \( M \) is the magnification of the lens. The first term has an inverse square term on the NA which dominates when the NA is low. This is also the region where light rays can be described using geometric optics. The second term, which accounts for wave optics effects such as diffraction, will have a greater effect on the axial resolution when objectives with larger NAs are used. For high NA objectives, the depth of field is extremely shallow but a good lateral resolution can be achieved. This is ideal for confocal microscopy where optical sectioning can be performed only at a desired axial location. An axial profile of the object can still be obtained by adjusting the relative positions of the
objective lens and sample so that the focal plane of the lens is scanned through it. However, with large NA lenses, the working distance limits the axial range that can be scanned. In applications such as tissue imaging where a relatively large depth of field is preferred while maintaining good lateral resolution, high NA objectives may not be suitable.

OCT circumvents the limited axial range when high NA objectives are used by decoupling of axial and lateral resolutions. In confocal microscopy, the out-of-focus light is rejected by a pin-hole placed at the detector. The optical sectioning capability and consequently the confocal image contrast are significantly improved. The method of rejecting the out-of-focus light is known as confocal gating. Confocal gating is also present in OCT by the use of single mode fibers. The standard single mode fiber has a core diameter of 9 µm while smaller diameter cores are available for cutoff wavelengths below 1200 nm. The small diameter of the single mode fibers effectively acts as a pinhole. One main advantage of OCT over confocal microscopy is that it further utilizes coherence gating. Due to the low coherence nature of the light source, there will only be contrast when optical path lengths of the reference and sample paths are within the coherence length. The additional gating mechanism allows OCT to achieve very good signal-to-noise ratio (SNR) [20]. The usable axial range in an OCT system is often larger than that of confocal system and it typically extends beyond the confocal parameter. The loss of contrast in OCT systems occurs when the axial PSF is near to the noise floor, which in most cases is determined by the detector shot noise [44-46]. Table 2-1 highlights the merits and drawbacks of OCT.
Table 2-1: Comparison of the merits and drawbacks of OCT [2, 3, 7].

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Axial resolution is independent of the imaging optics as long as the confocal parameter is large.</td>
<td>• Axial resolution limited by the optical bandwidth of the light source as large bandwidth ($\Delta\lambda &gt; 100$ nm) sources are relatively expensive.</td>
</tr>
<tr>
<td>• Compared to ultrasound, no transducer medium is needed; resolution is also better due to the high carrier frequency of the source.</td>
<td>• Penetration depth depends on the center wavelength of the light source.</td>
</tr>
<tr>
<td>• Good signal-to-noise ratio can be obtained due to heterodyne operation.</td>
<td>• As with all interferometry, a reference field is needed which involves additional hardware, the maximum amount of incident light at the sample is thus reduced.</td>
</tr>
<tr>
<td></td>
<td>• Proper management of dispersion and polarization matching is needed between the two arms of the interferometer for optimal axial point spread function.</td>
</tr>
</tbody>
</table>
2-2-1 Time-Domain OCT

One of the earliest implementations of OCT is an axial scanning type [1] which is now commonly known as the time-domain OCT (TD-OCT). A simplified schematic of the implementation is depicted in Figure 2-5, which essentially is a fiber-based Michelson interferometer. The broadband light source in the setup is a SLD. The output of the SLD is coupled to an optical fiber which is connected to a 2×2 fiber coupler. The coupler splits the light into two paths: the reference arm and the sample arm. The reference mirror may be mounted on a translation stage that moves in the axial direction. The sample arm typically consists of a collimated lens and an objective lens to focus the light beam on a sample. The sample may be placed on an x-y translation stage where the sample can be moved to vary the lateral scan position. The reflected light from both the reference and sample arms then pass through the coupler where it will be directed to a photodiode (PD).

The detected intensity as a function of time, \( t \) at a particular lateral position will take the form similar to equation 2-9 of

\[
I(t) = I_{\text{ref}}(t) + I_{\text{sample}}(t) + 2E_{\text{ref}}(t)E_{\text{sample}}(t)\cos\left(2\pi\frac{2v}{\lambda_0}t\right) \tag{2-13}
\]

where \( I \) and \( E \) represents the intensity and E-fields, respectively. The center wavelength of the source is denoted by \( \lambda_0 \) and the linear scanning speed of moving reference mirror is \( v \). The envelope of the amplitude modulated term contains information from both the sample and reference fields. However, as the reference field is reflected off a mirror and thus very stable, the envelope is just a scaled representation of the sample field. To recover the envelope from the signal, analog or
digital signal processing such as envelope detection will be needed. The signal processing will involve bandpass filtering, mixing and then low-pass filtering. A data acquisition card (DAQ) on the computer will digitize the recovered signal and store or display the tomogram of the sample.

Figure 2-6 shows the spectrum of a SLD source with a center wavelength of 1572.13 nm and a 3-dB bandwidth of 39.1 nm used in the fiber-based OCT setup of Figure 2-5. The expected coherence length is calculated to be 27.9 µm. The coherence length is measured by determining the FWHM of the envelope of the autocorrelation function. This function can be obtained by scanning the reference arm and in the fiber-based Michelson interferometer (Figure 2-5) with a fixed mirror placed on the sample arm and recording the resulting light intensity via a photodetector without any intrinsic
dispersion mismatches. Figure 2-7 shows the axial point spread function of the OCT system with the SLD source. The interference fringes shown in Figure 2-7a are spaced exactly one wavelength apart.

Figure 2-6: Measured spectrum of the SLD source used in the fiber-based OCT system.

Figure 2-7: (a) Measured and (b) filtered axial point spread function of the SLD source used in the fiber-based OCT system.

The axial resolution of an OCT system is defined as the FWHM of the point spread function which is equivalent to the coherence length of light sources with Gaussian-
shaped spectra when there is no dispersion or polarization mismatches. Figure 2-7b shows the envelope of the point spread function of the OCT system. From the figure, the axial resolution is determined to be about 31.3 μm in air and is 3.4 μm longer than the theoretical value of 27.9 μm. Factors such as fiber dispersion and instability in the scanning mechanism can cause the measured to increase.

2-2-2 Fourier Domain OCT

The analysis of the OCT system so far suggests that a movement of the reference mirror is necessary to observe interference fringes or to locate the position of the stationary mirror. Another approach of locating the mirror position is to use a coherence radar technique known as Fourier domain OCT (FD-OCT) [47, 48].

Rewriting equation 2-13 in the $k$ (wave number) domain yields

$$I(k) \propto E_1^2(k) + E_2^2(k) + 2E_1(k)E_2(k)\cos[2kΔ]$$

(2-14)

The signal obtained in equation 2-14 will contain information about the relative position of a reflecting surface. When an inverse Fourier transform is performed on equation 2-14 such that $f(z) = \mathfrak{F}^{-1}[I(k)]$, the equation becomes

$$f(z) = \int \left[ E_{\text{ref}}^2(k) + E_{\text{sample}}^2(k) \right] e^{-jkd} dk + \int E_{\text{ref}}E_{\text{sample}} \left( e^{-jkΔ} + e^{jkΔ} \right) dk$$

(2-15)

where

$$|f(z)| = S_{\text{ref}}(0) + S_{\text{sample}}(0) + S_{\text{ref-sample}}(Δ) + S_{\text{ref-sample}}(-Δ)$$

and

$S_{\text{ref}}(z) = \mathfrak{F}^{-1} \left[ E_{\text{ref}}^2(k) \right]$. Note that $E_{\text{ref}}^2(k)$ is a scaled representation of the light source spectrum. The magnitude of the Fourier transform has basically three profiles at different points on the horizontal axis; −Δ, 0 and Δ. Since the signal is symmetrical
about the vertical axis, the negative axis can be ignored with 
\[ |f(z)| = I(z) = S_{at}(0) + S_{ref, sample}(\Delta) \] containing all the necessary information. This is essentially the axial profile that will be measured by a TD-OCT system.

Figure 2-8 shows the schematic of a typical FD-OCT system, where the single photodetector is replaced by a diffraction grating and a CCD array. The diffraction grating disperses the combined light into its wavelength components and the CCD array records the intensity of evenly spaced wavelength points. Conversion of the measured spectrum from the wavelength domain to the wave-number domain is necessary prior to applying an inverse Fourier transform. The conversion between the two domains requires the use of a non-linear scaling algorithm [49] since \( k \) and \( \lambda \) has a non-linear relationship.

Comparing this technique to the TD-OCT, the axial profile is obtained without scanning or movement of the reference mirror. This results in a significant reduction
of imaging time. The interference term in equation 2-14 will be subjected to the convolution between the autocorrelation function of the source (inverse Fourier transform of the source spectrum) and the delta function $\delta(x-x_0)$, where $x_0$ is the optical path difference between the reference and sample arms. As with TD-OCT systems, the axial resolution of the system is still determined by the bandwidth of the light source. A light source with a large bandwidth will yield a system with high axial resolution. Figure 2-9 shows the corresponding relationship of the autocorrelation functions with broad-band sources and narrow-band sources for the same frequency modulation.

![Figure 2-9: Interferogram in spectral domain with (a) small and (b) large bandwidth light source. The axial PSFs of the respective light sources are shown in the inset.](image)

The inset shown in Figure 2-9a corresponds to the axial PSF of the smaller bandwidth source while the inset of Figure 2-9b corresponds to that of a larger bandwidth light source. The results clearly showed that the bandwidth of the light source determines the sharpness of the axial PSF. A larger bandwidth source is therefore desired for resolution improvement.
One of the main issues with the TD-OCT system is its slow-imaging process. Axial scans of the reference mirror were required to obtain the depth profile. On the other hand, FD-OCT does not require any axial scans and the depth profile can be obtained via a Fourier transform of the recorded spectrum. Figure 2-10 shows a schematic of the FD-OCT system. In this setup, a Ti:Sapphire laser is used as the light source [50].

![Figure 2-10: FD-OCT setup.](image)

Figure 2-11 shows the spectrum of the Ti:Sapphire laser captured using an optical spectrum analyzer. The center wavelength and bandwidth of the source were 774.7 nm and 107.3 nm, respectively. By approximating the spectrum to a Gaussian profile, the axial resolution in air is calculated to be 2.47 μm.
Figure 2-12 shows the depth profile of a mirror placed at the sample arm. There is only one peak in the profile and the location of the peak on the horizontal axis shows the distance between the two mirrors. The FWHM of the peak gives an axial resolution of 4.34 µm. The non-Gaussian shape of the peak (cross-correlation function) with side peaks may be attributed to the non-Gaussian spectrum of the light source. This may also account for the poorer axial resolution compared to the theoretical value of 2.47 µm. In addition, imbalance in dispersion between the two arms of the interferometer will also broaden the profile. Figure 2-13 shows the broadening of the autocorrelation function due to a mismatch in the second order dispersion simulated by placing one additional glass plate of 1 mm thickness in the reference arm. From the figure, the axial resolution increased by 32% to 5.72 µm. This demonstrates that a small imbalance between the two arms may also significantly worsen the axial resolution.

Figure 2-11: Measured spectrum of Ti:Sapphire laser.
The signal processing time in a FD-OCT system comprises of the integration time of the CCD, the transfer time between the CCD array to the computer (e.g. USB connection), and finally the processing speed of performing Fourier transform (processor dependent). This is the time taken for one axial scan. For instance, the
HR4000 spectrometer used in this work has adjustable integration time between 10 µs to 10s. The integration time can be adjusted to improve sensitivity or dynamic range. Typical integration time can range from 10 µs to 10ms. The HR4000 spectrometer used has a transfer rate 480 Mbps (USB2.0), with 3648 pixels read out in 15 packets of 512 bytes each. This translates to a transfer delay of at least 128 µs. For modern computers, the high speed parallel processors (> 2 GHz per processor core) allow quick computation of fast Fourier transform (FFT) in the order of 100 µs per computation.

Another common variant of FD-OCT is the swept-source OCT. In this setup, the light source is not a broadband source but a wavelength-tunable light source. The wavelength of the light source is swept across a relatively broad range while a photodetector collects a point interferogram. Figure 2-14 shows a typical swept source OCT setup which is similar to the FD-OCT except for the light source and detector. In place of a broadband source, it has a wavelength tunable source. The linear CCD array and diffraction grating is replaced by a balanced photodetector.
Instead of collecting the entire spectrum for an axial scan, the swept source system sweeps the spectrum of the laser over a short period of time. During this period, the balanced detector records the entire interferogram. The main difference between FD-OCT and swept source OCT is in the recording of the interferogram. In the former, the spectrum is recorded as a function of space (pixel location) while the swept source OCT records the spectrum in time as long as there is proper synchronization between the tunable laser and the data acquisition (DAQ) card. Once the interferogram is obtained in the swept source system, it is re-scaled into the $k$ domain via interpolation and inverse Fourier transformed to form the single point axial profile. The last few steps are identical to the conventional FD-OCT measurement. Table 2-2 highlights the merits and drawbacks of FD-OCT.
Table 2-2: Comparison of merits and drawbacks of FD-OCT [45, 51]

| Advantages | • Does not need a scanning optical delay line to generate an axial scan  
• Signal-to-noise ratio improvement over time-domain OCT  
• Real-time three dimensional scan is achievable with SD-OCT  |
| Disadvantages | • Imaging depth is limited by the number of pixels of the spectrometer  
• Aliasing effects is present  
• Dynamic range is compromised with the use of CCD arrays. CCD pixels can be saturated easily. However, a variant of SD-OCT, swept source overcomes this problem by utilizing balanced detector photo-diodes. |

2-3 OCT Key Parameters

There are basically four key parameters in an OCT system: axial resolution, lateral resolution, sensitivity and dynamic range.

2-3-1 Axial resolution

The axial resolution is mainly determined by the bandwidth of the light source. The ideal axial resolution is inversely proportional to the light source bandwidth in the absence of material dispersion or polarization mode dispersion mismatches [2, 3].

37
Effects of dispersion mismatches

In the presence of the dispersion mismatches between the sample and reference arms, the axial PSF will be broadened. As the axial PSF determines the axial resolution, a broad PSF worsens the resolution, and also lowers the signal-to-noise ratio. Material dispersion occurs when light waves of different frequency (colors) propagate at different speeds in a medium (e.g. glass, fiber). The use of broadband light sources in LCI or OCT is very susceptible to dispersion due to its broad spectrum. In most OCT applications, the light delivery method is via optical fiber. When light passes through a waveguide such as an optical fiber, it is subjected to waveguide dispersion. This occurs when light travels at different speeds between the core and cladding.

Another form of dispersion that may occur in an optical fiber is intermodal dispersion. This happens when the wavelength(s) of the light wave(s) is lower than the cutoff wavelength of the fiber, which depends on the dimensions of the core and cladding and their refractive indices. Intermodal dispersion occurs when each longitudinal mode in the waveguide has different optical pathlengths over a fixed physical length. Thus, each mode arrives at the distal end with different time delays. To minimize intermodal dispersion, the fiber is chosen such that its cutoff wavelength is smaller than the lower wavelength of the light’s source spectrum. This ensures that there is only a single mode operation. For some OCT applications, graded index rods may be used to deliver light to the sample. A graded index rod supports multiple longitudinal modes (equivalent to a multi-mode fiber). In this case, intermodal dispersion will be inherent in the interferometer system.
Despite all the various forms of dispersion which may broaden a temporal pulse being launched into the system, the shape of the axial PSF depends on the dispersion mismatch between the two arms of the interferometer. The axial PSF can be determined by observing the interference term (with two perfect reflecting surfaces for each of the interferometer arms) in equation 2-14,

\[
f(\Delta) = \int E_{\text{ref}}(k) E_{\text{sample}}(k) \left( e^{-jk\Delta} + e^{jk\Delta} \right) dk.
\]

In the absence of material dispersion, \( k \) is constant over all wavelengths. However, in the general case where material dispersion is present (e.g. the reflected light pass from each mirror through the two ends of a 2x2 fiber coupler, Figure 2-15), the interference term can be expressed as

\[
f(z) = 2 \int E_r(k) E_s(k) \left( \exp \left( j \left( k_j d_{r,f} + k_{\text{air}} d_{r,\text{air}} - k_j d_{s,f} - k_{\text{air}} d_{s,\text{air}} \right) \right) \right) dk \quad (2-16)
\]

![Figure 2-15: Interferometer with optical fiber as a dispersive material.](image)

For a dispersive material, the wavenumber \( k_{\text{fiber}} \) can be expanded as a Taylor series as a function of angular frequency about \( \omega_0 \),

\[
k \left( \omega \right) \bigg|_{\omega=\omega_0} = k_0 + \frac{dk}{d\omega} \bigg|_{\omega=\omega_0} (\omega - \omega_0) + \frac{1}{2} \frac{d^2k}{d\omega^2} \bigg|_{\omega=\omega_0} (\omega - \omega_0)^2 + \ldots \quad (2-17)
\]
where $\omega = ck$, $c$ be the speed of light.

The first term represents the center wavenumber. The second term

$$\frac{dk}{d\omega} = \frac{1}{v_g} = \frac{n(\omega_b)}{c} + \frac{\omega_b n'(\omega_b)}{c}$$

represents the inverse of the group velocity with which all wavelengths appear to travel. The third term is the GVD or second order dispersion. Non-zero second or higher order dispersion has the effect of broadening and distorting the axial point spread function. For the example shown in Figure 2-15, the lengths of the optical fiber in both arms are not equal ($\delta = d_{sam} - d_{ref}$) which implies that second (i.e. $(\omega - \omega_b)^2 \times \delta \neq 0$) or higher order dispersions are present.

It can be shown that the effect of second order dispersion broadens the axial point spread function (light source with a Gaussian spectrum) by [3]

$$\sigma_d' = \sigma_d \sqrt{1 + \left(\frac{1}{2} \left(\frac{d^2 k}{d\omega^2}\right)_{\omega=\omega_b} \delta \right)^4}, \quad (2-18)$$

where $\sigma_d$ is the standard deviation and the FWHM of the non-dispersed PSF is $\Delta d = 2\sigma_d \sqrt{2\ln 2}$.

2-3-1-2 Effects of polarization mismatches

Light, as a transverse electromagnetic wave, has polarization as one of the characteristic properties. The polarized state of light commonly refers to the direction of the $E$-field relative to the direction of propagation, although the $H$-field can also be used. A vector description (in Cartesian coordinates) of the $E$-field for light propagating in the z-direction can be expressed as:
\[ \tilde{E}(t,z) = \text{Re}\{\hat{x}E_{ao}(t)\exp[j(kz - \omega t + \epsilon_{x}(t))] + \hat{y}E_{oy}(t)\exp[j(kz - \omega t + \epsilon_{y}(t))]\} \]
\[ = \hat{x}E_{ao}(t)\cos(kz - \omega t + \epsilon_{x}(t)) + \hat{y}E_{oy}(t)\cos(kz - \omega t + \epsilon_{y}(t)) \] (2-19)

where \( \hat{x}, \hat{y} \) are unit vectors in the x and y directions, \( k \) is the wavenumber, \( \omega \) is the angular frequency, and \( \epsilon \) is the phase offset. In the above simplified description, the \( E \)-field comprises of components in the x and y directions. Depending on the ratio of \( \frac{E_{ao}(t)}{E_{oy}(t)} \) and the phase difference \( \epsilon(t) = \epsilon_{x}(t) - \epsilon_{y}(t) \), the polarization state can either be linear, circular (left or right) or elliptical. The irradiance can be described as

\[
I \propto \text{Re}\left( \begin{bmatrix} E_{x} \\ E_{y} \end{bmatrix} \cdot \begin{bmatrix} E_{x}^* \\ E_{y}^* \end{bmatrix} \right) \\
= \langle E_{x}(t) \rangle^2 + \langle E_{y}(t) \rangle^2 
\] (2-20)

where \( I \) describes the irradiance measured at the detector, \( E_{x}(t) = E_{ao}(t)\exp[j(kz - \omega t + \epsilon_{x}(t))] \), \( E_{y}(t) = E_{oy}(t)\exp[j(kz - \omega t + \epsilon_{y}(t))] \) are \( E \)-fields in the x and y direction respectively and the * represents the complex conjugate.

Thus, for a single wave, the total irradiance is the sum of the individual irradiances of orthogonal components.

In an interferometry system where two waves are imposed, the polarization states of the waves determine the contrast of interference fringes. The vector description of the superposition of two waves both propagating in the z-direction can be described as
The irradiance is then described as

\[ I \propto \text{Re} \left[ \bar{E}_{\text{sum}} \cdot \bar{E}_{\text{sum}}^* \right] \]

\[ = \text{Re} \left[ \begin{pmatrix} E_{1x} + E_{2x} \\ E_{1y} + E_{2y} \end{pmatrix} \cdot \begin{pmatrix} E_{1x} + E_{2x} \\ E_{1y} + E_{2y} \end{pmatrix}^* \right] \]

\[ = \langle E_{1ox} \rangle^2 + \langle E_{2ox} \rangle^2 + 2E_{1ox}E_{2ox}\cos(kd + \epsilon_{1x} - \epsilon_{2x}) + \]

\[ \langle E_{1oy} \rangle^2 + \langle E_{2oy} \rangle^2 + 2E_{1oy}E_{2oy}\cos(kd + \epsilon_{1x} - \epsilon_{2x}) \]

\[ = d.c.\text{ terms} + 2E_{1ox}E_{2ox}\cos(kd + \epsilon_{1x} - \epsilon_{2x}) + 2E_{1oy}E_{2oy}\cos(kd + \epsilon_{1x} - \epsilon_{2x}) \]

where \( k \) is the wavenumber and \( d = z_1 - z_2 \). The interference terms are those containing the cosine terms. The visibility \( V \) is again defined as \( V = \frac{\text{interference terms}}{\text{d.c. terms}} \).

Maximum contrast occurs when the polarization states of the two waves are the same, \( \frac{E_{1ox}(t)}{E_{1oy}(t)} = \frac{E_{2ox}(t)}{E_{2oy}(t)} \) and \( \epsilon_{1x}(t) - \epsilon_{2x}(t) = \epsilon_{1y}(t) - \epsilon_{2y}(t) \). In the earlier description of visibility fringes, the polarization states of the two waves were assumed to be same (i.e. exactly the same changes relative to the source’s original polarization state after reflection from the mirrors). At the other extreme, no contrast happens when the interference terms sum to zero. In this special case, the two waves are said to be orthogonally polarized to one another. For instance, \( E_1 \) has only a \( x \)-component while \( E_2 \) has only a \( y \)-component, in this case, the products \( E_{1ox}E_{2ox} = E_{1oy}E_{2oy} = 0 \). Orthogonal polarized states can be extended to non-linearly polarized fields such as
elliptic or circular polarized light, where the phase differences
\[
[\varepsilon_{1x}(t) - \varepsilon_{2x}(t)] = -[\varepsilon_{1y}(t) - \varepsilon_{2y}(t)].
\]

Between the maximum and zero contrast, the ratios of the \( E \)-fields
\[
\frac{E_{1ox}(t)}{E_{1oy}(t)} \quad \frac{E_{2ox}(t)}{E_{2oy}(t)}
\]
are not equal and/or the phase differences \( \varepsilon_{1x}(t) - \varepsilon_{2x}(t), \varepsilon_{1y}(t) - \varepsilon_{2y}(t) \) are not equal.

As a result, the contrast of the interference fringes is reduced. This can manifest as a form of polarization mismatch in an interferometer. For instance, the presence of birefringent materials in one of the interferometer arms changes the phase difference between the x- and y- components of the corresponding \( E \)-field (e.g. \( \vec{E}_2 \)). When the interferometer combines the two fields, the phase differences will be
\( \varepsilon_{1x}(t) - \varepsilon_{2x}(t) \neq \varepsilon_{1y}(t) - \varepsilon_{2y}(t) \). Mathematically, the amplitude of the interference term and be considered as the vector summation of the cosines of the phase differences (Figure 2-16).

![Figure 2-16: Illustration of polarization mismatch when the phase differences of the x- and y- components of the E-fields are not equal, with the resultant (bold arrow) showing the amplitude of the interference term.](image-url)
It is worth noting that the amplitude of the interference term does not depend on polarization state of the light source in an interferometer. The above analysis did not consider the inherent polarization state of the source. The interference contrast depends only on the *difference* in polarization state between the two light waves.

In typical OCT systems, a 2x2 fiber coupler serve to split the light from the source into two paths, reference and sample arms. The polarization states in these two arms may change with environment changes (e.g. temperature, pressure) or stress on the fiber. This is independent of the changes resulting from the sample. Mechanical stresses applied on the fiber (e.g. squeezing or bending the fiber) changes the refractive index in direction of the applied stress which causes birefringent effects. Bending the fiber is the simplest way of causing mechanical stress. If there are any systemic polarization mismatches between the two arms, the contrast of the detected signal may be reduced. One of the possible ways to mitigate any mismatches between the reference and sample arms may be to use long fibers and coil them around so that the bending is relatively fixed.

### 2-3-2 Lateral resolution

The ability to resolve a feature in an imaging plane perpendicular to the lens’ optical axis is determined by the lateral resolution of the system. In imaging systems, the lateral resolution is directly related to the resolving power of the imaging or objective lens prior to the sample. In typical OCT systems, the sample is illuminated by the same objective lens that collects the back-scattered/reflected light. In such scenario, it is equivalent to having a “virtual” condenser that has the same numerical aperture (NA)
as the objective lens. The lateral resolution can be shown to be \( \Delta x = \frac{0.61 \lambda}{NA_{\text{objective}}} \) using the Rayleigh criteria. This is also known as the diffraction limit of the system (in the absence of optical aberrations). In OCT systems where low NA objectives (0.1 to 0.3) are used to achieve a long imaging range (~1 to 3 mm), the typical lateral resolution is about 2.6 \( \mu \)m for light sources having 850 nm as their center wavelength.

The lateral resolution is not constant over the entire imaging depth in OCT systems. This is because even a gently focused beam (low NA) has different beam diameters along its axial direction. A convenient approximation of the beam profile is the Gaussian beam. The electric field is given by

\[
E(r, z) = E_0 \frac{\omega_0}{\omega(z)} \exp \left\{ -\frac{r^2}{\omega(z)^2} - jkz - i \frac{r^2}{2R(z)} + j \xi(z) \right\},
\]

where \( \omega(z) = \omega_0 \sqrt{1 + \left( \frac{z}{z_R} \right)^2} \) is the beam radius and \( \omega_0 \) is the minimum beam radius (at the focal plane). The Rayleigh range \( z_R = \frac{\pi \omega_0^2}{\lambda} \) is defined by the distance such that the beam radius is \( \sqrt{2} \omega_0 \). The imaging depth is mathematically defined as the confocal parameter which is twice the Rayleigh range. For instance, at a beam radius of 2.6 \( \mu \)m, the confocal parameter is about 50 \( \mu \)m (\( \lambda = 850 \) nm). At the edge of the imaging depth, the lateral resolution will be “degraded” (compared to the beam waist) by a factor of \( \sqrt{2} \). However, the actual imaging depth in OCT systems are longer (~1 to 3 mm) than the confocal parameter due to the effect of coherence gating [20]. At
such depths, the lateral resolution is degraded significantly (about 10 times worse, for an imaging depth of 1 mm, compared to the minimum beam radius).

The above analysis assumes that the Gaussian beam passes through material that does not distort an ideal spherical wavefront. However the presence of multiple-scatterers (e.g. cell nuclei, etc.) in biological tissue imaging can distort the spherical wavefront. This leads to optical aberrations which worsens the lateral resolution. Adaptive optics has been utilized to overcome these aberrations to achieve diffraction limited performance [52].

2-3-3 Sensitivity

Sensitivity in OCT systems is typically defined as the minimal sample reflectivity at which the signal-to-noise ratio (SNR) is 1 [45], i.e. $\frac{1}{R_s}$. The signal refers to the amplitude of the interference term which depends on the amount of back-scattered or back-reflected light from the sample. Noise, measured at the detector, can be classified into shot noise, excess noise and receiver noise. Shot noise is directly proportional to the average (or d.c.) current at the transistor junction of an electronic amplifier (transimpedance amplifier after the photodetector). Receiver noise is the equivalent noise of the system referred to the input. Excess noise, or 1/f noise is proportional to the square of the average current. Table 2-3 (from reference [45]) classifies the different signal and noise sources for FD-OCT and TD-OCT.
Table 2-3: Classification of signals and noise powers in FD-OCT and TD-OCT (adapted from reference [45])

<table>
<thead>
<tr>
<th></th>
<th>FD-OCT</th>
<th>TD-OCT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Signal power</strong></td>
<td>( \left( \frac{\rho \eta \tau P_0}{h \nu_o} \right)^2 \gamma R, \gamma R_i )</td>
<td>( 2S^2 P_o^2 \gamma R, \gamma R_i )</td>
</tr>
<tr>
<td>(Interference term)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average signal</strong></td>
<td>( \frac{\rho \eta \tau P_0}{h \nu_o} \frac{1}{N} (\gamma R_i + \gamma R_i) )</td>
<td>( I_{dc} = SP_0 (\gamma R_i + \gamma R_i) )</td>
</tr>
<tr>
<td><strong>Shot noise</strong></td>
<td>( \frac{\rho \eta \tau P_0}{h \nu_o} \frac{1}{N} (\gamma R_i + \gamma R_i) )</td>
<td>( 2q I_{d.c.} = 2q SP_0 (\gamma R_i + \gamma R_i) B )</td>
</tr>
<tr>
<td><strong>Receiver noise</strong></td>
<td>Readout noise + Dark noise</td>
<td>Noise equivalent current ( \times B )</td>
</tr>
<tr>
<td><strong>Excess noise</strong></td>
<td>( \frac{(1+\Pi^2)}{2} \left( \frac{\rho \eta}{h \nu_o} \right) \frac{P_0^2}{N^2} (\gamma R_i + \gamma R_i)^2 \frac{N}{\Delta v_{eff}} )</td>
<td>( \frac{(1+\Pi^2)}{\Delta v_{eff}} \left( \frac{\rho \eta}{h \nu_o} \right) SP_0^2 (\gamma R_i + \gamma R_i)^2 B )</td>
</tr>
</tbody>
</table>
Table 2-3a: Symbols of the terms in Table 2-3.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_0$</td>
<td>Average power of light source in mW</td>
</tr>
<tr>
<td>$S$</td>
<td>Responsivity in mA/mW</td>
</tr>
<tr>
<td>$\gamma_{s,r}$</td>
<td>Fraction of light source’s power received at the detector for sample or reference arm</td>
</tr>
<tr>
<td>$R_{s,r}$</td>
<td>Reflectivity from sample or reference arm</td>
</tr>
<tr>
<td>$q$</td>
<td>Electron charge ($1.6 \times 10^{-19}$ C)</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Spectrometer efficiency (includes losses from optical elements)</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Detector quantum efficiency</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck’s constant</td>
</tr>
<tr>
<td>$v_0$</td>
<td>Center frequency of light source</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Integration time of CCD in spectrometer</td>
</tr>
<tr>
<td>$N$</td>
<td>Number of pixels of CCD camera</td>
</tr>
<tr>
<td>$\Delta\nu_{\text{eff}}$</td>
<td>Effective spectral width of light source in Hz</td>
</tr>
<tr>
<td>$\Pi$</td>
<td>Degree of polarization</td>
</tr>
</tbody>
</table>

To simplify the sensitivity analysis, the noise sources are examined and typical operations are considered. In typical OCT systems where broadband light sources are used, the excess noise is negligible due to the large values of the spectral width ($> 10^{18}$ Hz). The shot noise in generally the most dominant form of the noise source in OCT.
systems due to the relatively high d.c. levels contributed by the reference reflectivity. By contrast, the receiver noise is generally much lower than shot noise (e.g. thermal noise at ambient temperatures is lower than shot noise for transimpedance amplified gain of 10 kohms by a factor of at least 1000). In most applications, the reflectivity of the sample is much smaller than the reference (i.e. $R_s \ll R_r$). The sensitivity at the shot noise limit for TD-OCT is:

$$Sensitivity_{TD-OCT} = \frac{2S^2P_0^2\gamma_s R_r}{2qSP_0\gamma R_r B} = \frac{SP_0\gamma_s}{qB}$$

(2-24)

For FD-OCT systems, the noise power is considered as the noise floor after Fourier transform. This implies that there is a scaling factor of $1/N$ for the total noise power (i.e. $\sigma_{noise}^2 = \frac{1}{N} (\sigma_{shot}^2 + \sigma_{excess}^2 + \sigma_{receiver}^2$). Thus the shot noise limit for FD-OCT is

$$Sensitivity_{FD-OCT} = \frac{\left(\frac{\rho_0\tau_0 P_0}{\hbar \nu_0} N\right)^2 \gamma_s \gamma R_r}{\frac{1}{N} \left(\frac{\rho_0\tau_0 P_0}{\hbar \nu_0} N\right) \gamma_s \gamma R_r} = \frac{\rho_0\tau_0 P_0}{\hbar \nu_0} \gamma_s$$

(2-25)

The shot noise limit represents the ideal situation where other noise sources are negligible [3, 44, 53]. However, in cases where the reflectivity of the reference arm is low, the receiver noise may dominate.
The sensitivity depends on the bandwidth of the detector, $B$, for TD-OCT and the integration time, $\tau$, for FD-OCT. The equivalent relationship between them is given in reference [45] as $\frac{\pi}{4 \ln 4} \frac{N}{m} \frac{1}{\tau}$, where $\frac{N}{m}$ represents the number pixels at FWHM of the source spectrum. The bandwidth also determines the maximum lateral scan rate. In typical OCT systems, each axial scan is processed individually before moving to the next lateral point. As such, the maximum lateral scan rate is limited by the bandwidth of the detector. In TD-OCT systems, this bandwidth is the same as the bandwidth of the detector. In FD-OCT systems, the bandwidth is the aggregate effect of the integration and read-out time of the spectrometer. For more advanced systems, an on-board buffer may be present so that bandwidth is not limited by the data transfer rate to the computer.

One common attributes of modern OCT systems is the fast scanning speeds supported by the high bandwidths of the detectors or fast swept source lasers. The sample can typically be scanned at video rates of 30 frames per second for display in 2D tomogram. Rollins demonstrated a TD-OCT system that can achieve very fast scan rates with the use of a rapid scanning optical delay line (RSOD) [25, 54]. With the advent of FD-OCT, scanning rates have increased significantly [51, 55, 56]. In swept source OCT systems, very fast scanning Fourier domain mode-locked (FDML) lasers can achieve up to 312,000 axial lines per second [57-60].
2-3-4 Dynamic range

While it is important to achieve good sensitivity, dynamic range is also critical in some systems. In biological applications, the tissue-air surface generally reflects a significant portion of the signal back into the system. If the dynamic range of the system is low, the reflection from the tissue-air interface will saturate the image which may obscure important details below the tissue surface. While gels and other index matching fluids may be applied to mitigate this reflective interface, the dynamic range of the system should not be significantly compromised [46].

2-4 Optical Techniques for Tissue Imaging

There are two main types of tissue imaging; excisional and non-excisional. In excisional imaging, the biological tissue is excised and viewed under a microscope. Common techniques include brightfield microscopy, fluorescence microscopy, confocal microscopy and multi-photon imaging. These techniques require tissues to be stained or tagged with fluorophores. Tissues normally need to be histological processed and stained. The preparation, mounting, slicing and staining of the tissue add variables and delay to the analysis process. Any contamination of the sample, even extremely low with well-controlled procedures, will introduce artifacts into the image.

The main advantage of using the histological process is that with good sample preparation, the image resolution (lateral) can be in the orders of sub-micrometers (approaching the diffraction limit). The resolution of these microscopic techniques depends greatly on the microscope objective lenses, as described in equation 2-11.
Recent advances in techniques such as 4-PI confocal microscopy [61] and stimulated emission depletion microscopy [62, 63] allow the diffraction limit to be attained or even surpassed.

In non-excisional imaging, tissues are imaged *in-vivo*. These techniques may offer real-time study of biological behavior and less discomfort for patients. However, *in-vivo* techniques typically lack the optical resolution achievable by *ex vivo* methods. Common *in vivo* techniques include magnetic resonance imaging (MRI), computed X-ray tomography (CT scans), positron emission tomography (PET) and ultrasound.

Optical contrasts are provided by staining or through fluorescence tagging in most *ex vivo* tissue imaging. For instance, iodine staining in conventional brightfield microscopy allows the user to identify the nucleus of the cells. In fluorescence confocal microscopy, the tissue is tagged with fluorophores. These fluorophores are tagged to specific parts of a tissue (antigen-antibody selective recognition) and these parts will fluoresce when an appropriate excitation light is incident on them. On the other hand, *in vivo* imaging requires a change in an optical property of the tissue to provide contrast. For instance, in ultrasound imaging, the density of different parts of an organ can be obtained due to different arrival times of back-reflected echoes. Similarly, in MRI, the relaxation properties of excited hydrogen nuclei in water and lipids are most frequently relied upon to provide contrast.
The description of the OCT systems in the earlier section focused on treating the sample with known reflectivities. However, light-matter interaction in biological tissues is more complex than simple specular reflections. Biological tissues consist of many cells and within the cells, many smaller components (e.g. cell nucleus, mitochondria, etc.). A typical cell has dimensions of between 10 to 100 µm. The cellular components are even smaller at about 1 to 5 µm. At this size, typical OCT systems do not have the resolution to resolve individual components. However, these components are important in OCT imaging as they can scatter and/or absorb the incident light. Light can be scattered multiple times before being collected by the system’s optics. In addition, within a small volume, there exist many scatterers which results in many back-scattered wavelets where they can coherently self-interfere and also interfere with the reference field. The random nature of the position of the scatterers gives rise to speckle in the OCT image [2]. This is manifested as a random grainy image with variations in the detected intensity.

Recall in the equation (8) that a single axial profile of a sample modeled as a single mirror is given by

$$I(z) \propto E_1^2 + E_2^2 + 2E_1E_2|g_{12}(\delta)| \cos[2k.(z - z_0)],$$

where $E_2$ is the amplitude of the sample field (which is a constant for a mirror or a specular reflected object). However, in the case of a biological sample, the sample field is now an aggregate of many scattered fields at different depths ($z_n$) [2, 53], i.e.

$$E_{\text{sample}}(t) = \exp(-j\omega t) \sum_{n=0}^{\infty} a(z_n) \exp\left(jk(z_n + n\Delta z)\right), \quad (2-26)$$

where $a(z_n)$ is the amplitude of the scattered field at depth $z_n$. 

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When considering the interference with the reference field, a localized distribution of scatterers within the coherent volume can interfere with the reference field. This volume is determined by coherence length of the OCT system. In TD-OCT systems, as the reference mirror is scanned, the system effectively “samples” individual coherent volumes of scatterers. Figure 2-17 shows a biological tissue (sample) illuminated by a focused beam. At a particular mirror position (e.g. P1), the scatterers within the coherence length will be “sampled” and coherently interfere with the reference field. As the spatial distribution of the scatterers is random at different depths, the resultant interference intensity at a particular depth will be random. This give rise to speckle effects seen in the tomogram.

Scattering in tissue can be classified as single or multi-scattering events. These depend on the number of scatterers per unit volume in the tissue which can be translated to the scattering coefficient. More scatterers per unit volume translate to higher scattering coefficient. At shallower depths, the scatterers will generally scatter the incident once before being collected by the objective lens. As such, the coherent volume will be
small. At deeper depths, multiple scattering events from shallower scatterers “interfere” with single-scattering events at deeper depths. This will result in a larger coherent volume. In addition, the volume will be slightly skewed towards the shallower depth.

The amount of change in the refractive index is detected by the intensity of the interference fringes. Factors affecting this intensity include scattering and absorption in the tissue. Various studies on tissue optics have been conducted to characterize these properties [64-67]. Both scattering and absorption cause the incident intensity to decrease as light propagates deeper into the tissue. As a first order approximation, intensity versus depth follows an exponential relationship (Beer-Lambert law). Thus, tissues with high absorption or scattering will have attenuated OCT signals at greater depths. In particular, for tissues with high scattering, another factor called anisotropy affects the intensity of light. Anisotropy is a unit-less parameter, with values between $-1$ and 1, that describes the direction of pre-dominant scattering. As OCT detects back-reflected or back-scattered light, this parameter has a significant influence on the detected signal. For example, a tissue that has high anisotropy implies that a significant incident optical power is maintained at deeper parts of the tissue. However the improved penetration depth is obtained at the expense of a reduced back-scattered component that is collected by the OCT system. Thus, a good understanding of the tissue properties is necessary to interpret the OCT image.

One of the pioneering applications of OCT in bio-imaging is in the field of ophthalmology [1, 8-12]. Both the anterior and posterior of the eyes have been imaged using OCT. The main advantage of OCT is the posterior imaging of the eye, especially
in the retina area. The ultra-high axial resolution of the OCT system provides a very convenient method to study the retina without excising it. The transparent nature of the tissues in the retina makes it relatively easier to design and optimize the OCT system. OCT has been proven to be useful in the study of macular degeneration, glaucoma and other forms of retina disease [7]. With more advanced developments of OCT, turbid tissues were being imaged in the skin [13-15] and the cardiovascular system [23]. The incorporation of the OCT technology to endoscopy allowed cross-sectional imaging to be possible in areas such as gastrointestinal, pulmonary, urinary and female reproductive tracts [24-30]. Imaging of neoplasia has also been performed on patients with relatively good resolution [68]. Recent applications of OCT have progressed to include the larynx and nasal passageway [69-73]. The resolution obtained from these images showed that identification of different morphological changes using OCT is relatively sufficient.

OCT can be either be ex-vivo or in-vivo [2]. One form of ex-vivo OCT is also known as OCM. The excised tissue is placed on a microscope stage where it will be scanned. Two or three-dimensional images of the tissue can be constructed depending on the scanning mechanisms. In general, OCM uses high NA objective lens to achieve good lateral resolution at the expense of a smaller focal volume. However, this is compensated generally by en-face scanning which conducts optical sectioning via coherence gating. In vivo OCT, on the other hand, generally requires a probe to be inserted to an imaging area. These probes typically contain a single-mode fiber that serves as both light delivery and collection mechanisms. For example, in gastrointestinal imaging [24], an optical fiber which forms part of the OCT system is
placed in a gastrointestinal probe. This allows the clinician to see the typical brightfield image of the gastrointestinal walls and the OCT image at the same time.

2-5 Summary

Coherence is an important parameter in interferometric applications. For low coherence sources, interference can only occur when the light waves are within the coherence length. Contrast is thus provided within a relatively small selected region. OCT is a form of LCI that uses low coherence sources to detect weak back-reflection or back-scattered light from a sample. The TD-OCT involves scanning the reference arm in the axial direction. Fourier or spectral domain OCT does not require any scanning in the axial direction as the detected spectrum will be inverse Fourier transform to obtain the axial profile. Key parameters for a good OCT system include axial resolution, lateral resolution, sensitivity and dynamic range. Common applications of OCT include ophthalmology and in-vivo tissue imaging.
Chapter 3 INVESTIGATION OF LCI FOR LOCALIZING THE POSITION OF OPTICALLY TRAPPED OBJECTS

3-1 Introduction

Ashkin pioneered the use of optical radiation for trapping microscopic particles [4] in 1970, and the optical trapping technique subsequently expanded its application to various fields in biology [5, 17] and atom cooling [74]. In optical trapping applications, the force and position of the trapped object can provide valuable information about the interaction of the object and its surroundings [75]. Various methods have been used to determine the position of a single trapped object [76, 77] and one of the more reliable methods is back focal plane (BFP) interferometry [18, 78]. A BFP system can provide position sensing down to the sub-nanometer range and is the most commonly used high resolution interferometric tracking method in optical trapping [18, 19, 79]. The high sensitivity of the BFP technique comes with the trade-off of a limited axial tracking range of about a few micrometers [19]. In the BFP technique, scattering of light from the trapped object interferes with the surround light and the interference pattern is imaged at the back focal plane of the objective onto a quadrant photodiode. For a single trapped particle, the interference pattern is relatively simple and the trapped positions can easily be calibrated. However, this technique will not work well if there is more than one trapped object as multi-interference between the objects and the surround light will complicate the interference pattern. Consequently the BFP

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technique is unable to discern two or more particles residing along the axial direction of a single optical trap [80], a requirement that is necessary for the detection and quantification of multiple particle dynamics in applications such as optical binding [81], optical assembly structures [82] and optical chromatography [83].

Grier and co-workers [84] developed an alternative approach for monitoring multiple optically trapped particles over tens of micrometers using the interference pattern from the far-field scattering of the trapped particles [84]. Although this approach overcomes the limitations of the BFP, one of its caveats is the need for an accurate model, such as the Rayleigh-Sommerfield or Lorenz-Mie model, to describe the interference of the scattered light fields and effects like light re-focusing between the trapped particles. Interference of highly coherent light sources is also susceptible to subtle noise in the form speckle (from scattering from impurities in the medium, e.g. water) introduced along the interference path. Consequently there will be some level of uncertainty in obtaining a direct measure of the optical centers of multiple particles residing in the optical trap using this holographic particle tracking technique.

By monitoring the position of a trapped particle over a period of time, the trapping dynamics can be examined. This in turn provides information about the optical forces which aids the study of the light-matter interaction between the incident light and trapped object [5]. With multiple trapped particles in the case of optical binding, measuring weak, sub-piconewton interaction forces have revealed intricate non-linear dynamics [85] due to the re-scattering of light among the particles. Understanding the
dynamics between multiple trapped particles is also important for the development of complex light-induced self assembled structures [86].

Reflectance mode LCI for microscopy imaging have provided high resolution tomographic images of cells [87] and is capable of measuring very subtle changes in the refractive indices [88]. As also observed in functional OCT applications such as Doppler OCT [89, 90], the speed and localization of blood flow can be monitored via interferometric means. Through coherence gating, particle displacements can be resolved with high signal-to-noise ratio (SNR) due to the rejection of photons whose optical path differences are beyond the coherence length of the light source. With this approach, displacement down to picometers has been demonstrated [91].

In this chapter, the use of coherence gating or LCI to determine the positions and trapping forces of optically confined particles is investigated. A common path system was integrated with a single beam optical trapping system to determine inter-particle separation with accuracy limited by the coherence length of the light source. The optical centers of two particles axially aligned in a single optical trap can clearly be discerned in the integrated LCI-trapping system. The optical trapping forces were inferred from the time-varying displacement of the particle optical center from its mean equilibrium.
3-2 Principles of Optical Tweezers

3-2-1 Momentum Transfer

In a typical optical tweezers, a coherent laser beam is focused via a high numerical aperture (NA) microscope objective lens to form an optical trap. A small object near the vicinity of the trap experiences optical forces due to its interaction with the optical light field. The momentum of a photon, $p$, is given by

$$p = n_1 \frac{h}{\lambda}$$  \hspace{1cm} (3-1)

where $h$ is the Planck’s constant, $\lambda$ is its freespace wavelength and $n_1$ is the refractive index in the incident medium. If $\frac{N}{\Delta t}$ is the number of photons interacting with the object per unit time, the optical power of the laser trapping beam can be expressed as:

$$P = \frac{N h \nu}{\Delta t}$$  \hspace{1cm} (3-2)

where $h \nu$ is the energy of a single photon and $\nu$ is the light frequency. Each photon traversing an object will experience a change in its momentum. The resulting rate of change of photon momentum will exert a reaction force, $F$, on the object:

$$F = \frac{\Delta p}{\Delta t} = \frac{\Delta N \times h \nu \times n_1}{c \Delta t} = \frac{\Delta N \ n_1 P}{N \ c}$$  \hspace{1cm} (3-3)

The force on the object at a particular direction can be estimated by computing the change in the number of photons in that direction. For example, a mirror totally reflecting off photons with an angle of incidence of $\theta$ will experience a force of
magnitude \( \frac{2\pi P \cos \theta}{c} \) parallel to its normal. There will not be any perpendicular component in this case as the photons did not lose any momentum in that direction.

The amount of reflection, transmission, scattering and absorption of a photon depends on the optical properties, shape and size of the object, which in turns determines the force acting on the object. It is therefore possible to determine the force on the object by analyzing the initial (before) and final (after interaction) momentum of the photons. The problem is thus reduced to examining the path of the photons.

**3-2-2 Ray Optics**

For objects whose dimension is much larger than the wavelength of the incident laser beam, the optical forces and thereby trapping dynamics (e.g. trap stiffness) can be determined through the use of ray optics. Light-matter interaction can then be considered broadly as transmission, reflection or absorption by the object. Figure 3-1 shows a basic ray diagram where a microsphere is trapped via a focused beam modeled as optical rays. Each optical ray represents possible paths a photon may take under the ray optics approximation. Although this is physically incorrect as individual photons cannot be distinguished, it is a useful approximation for estimating the optical forces when the object is much larger than the wavelength of the incident beam. With every interaction between the photon and object, the photon can be reflected, transmitted and absorbed. This results in forces on the object due to the reflection \( (F_r) \), transmission \( (F_t) \) and absorption \( (F_a) \) of the photon. The photon can also interact with
the object several times before leaving the object. By considering all the photon-object interactions, the optical forces due to a particular ray can be computed [92].

By defining the scattering (gradient) force to be parallel (perpendicular) to a particular incident ray, the scattering and gradient forces can be written as [92]:

\[ F_s = \frac{n_p}{c} Q_s \]  \hspace{1cm} (3-4)

\[ F_g = \frac{n_p}{c} Q_g \]  \hspace{1cm} (3-5)

Here, \( Q_s \) and \( Q_g \) are the normalized scattering and gradient forces, respectively, defined as
\[ Q_s = 1 + R \cos 2\theta - \frac{T^2 \left[ \cos(2\theta - 2r) + R \cos 2\theta \right]}{1 + R^2 + 2R \cos 2r} \]  

\[ Q_g = R \sin 2\theta - \frac{T^2 \left[ \sin(2\theta - 2r) + R \sin 2\theta \right]}{1 + R^2 + 2R \cos 2r} \]  

(3-6)  

(3-7)

where \( \theta \) and \( r \) are the angle of incidence and refraction, respectively. The reflectivity \( R \) and transmittivity \( T \) can be computed based on the Fresnel equations.

For a spherical object with the focal plane at its optical center, the angle of incidence is zero for all rays. Under this condition, there will be no gradient force but a non-zero scattering component is present. If the object is trapped along the optical axis, the scattering force in the z-direction is \( Q_s \cos \phi \), where \( \phi \) is the angle between the incident ray and the optical axis. Similarly, the gradient force from the same ray will result in net axial force of \( -Q_g \sin \phi \). Due to symmetry, there will be no net lateral forces in this case.

The optical forces defined in (3-4) and (3-5) do not account for photon absorption. This is a valid approximation especially within the diagnostic window of biological samples [42]. Similarly, polystyrene spheres used in this work are not very absorptive in the near infra-red region [93]. To compute the total force from all rays, the contribution of individual scattering and gradient forces can be summed. In addition, the forces can be weighted accordingly via a suitable probability distribution to account for non-uniform optical intensity profile (e.g. Gaussian) at the focal plane.
For microscope objectives with a low NA, the scattering force due to the peripheral ray is almost parallel to the optical axis of the objectives. The scattering force will act predominantly in the direction parallel to the optical axis. It is therefore easier to perform optical levitation with the object suspended above the microscopic objective in such weak traps.

![Diagram](image)

**Figure 3-2:** (a) Schematic of an optical trap with normalized $z = 0$ corresponding to the position where the microsphere center coincides with the focus of the objective lens. Normalized scattering and gradient forces (summed over the plane of incidence) for objective lens with (b) NA = 0.4 and (c) NA = 0.9.
Figure 3-2 shows the normalized scattering and gradient forces for a 1 unit radius microsphere trapped along the optical axis of the objective lens. The horizontal axes in Figure 3-2b and 3-2c depict the position of the focal point with respect to the microsphere center in the z-direction. The distance of the focal point from the microsphere center is normalized to the focal length of the lens, where a positive value indicates a microsphere center positioned beyond the focal length of the lens. The normalized forces on the vertical axis of Figure 3-2b and 3-2c are positive when they are in the same direction as the beam. The gradient force is therefore negative when the sphere is before the focal point. This implies that the gradient force acting on the microsphere is opposite to the beam direction which gives rise to the tweezing effect. For weak traps arising from small numerical apertures, the dominant scattering force thus results in a net forward force. The magnitude of the actual force is computed by multiplying $\frac{n_1 P_1}{c}$ to the normalized scattering and gradient forces. A higher NA lens generally produces stronger optical forces both in the axial and lateral directions compared to a lower NA lens. However, due to the more complex construction of high NA lenses, they typically suffer from higher optical losses which negate some of the high NA effects.

### 3-2-3 Restoration to Equilibrium Position

Figure 3-3 shows the axial forces acting on a microsphere when it is trapped (in a medium such as water) along the optical axis. At equilibrium, there is no net force acting on the microsphere. The optical force is balanced by the buoyancy force.
(weight of water displaced) and the weight of the microsphere. Small particles, such as microspheres, are highly susceptible to thermal forces. Thermal forces are caused by microscopic local variations of medium temperature. The thermal forces act to push it out of equilibrium. At the same time, the optical force will pull it back to equilibrium due to a change in the relative position between the beam and the microsphere. The weight and the buoyancy forces remain unchanged as long as the microsphere stays completely submerged.

![Figure 3-3: Vertical forces due to gravity, radiation pressure and buoyancy at equilibrium. The optical force $F_o$ is the vector sum of the scattering and gradient force.](image)

### 3-2-4 Damped Harmonic Oscillator

The optical forces act as a restoration force on the microsphere when it drifts out of its equilibrium position due to thermal forces. The system can be modeled as a harmonic oscillator with optical force behaving like a spring force, with a net downward force contributed by the weight and buoyancy. As water is relatively viscous, friction has to be considered in the harmonic oscillator model. Consequently the microsphere in an
optical trap can be treated as a damped harmonic oscillator system. The damped harmonic oscillator is described by [94]

\[ -kz - c \frac{dz}{dt} = m \frac{d^2z}{dt^2} \]  

(3-8)

where the second term on the left hand side represents the drag force, \( k \) is the spring constant, \( z \) is the displacement, \( c \) is the damping coefficient, \( m \) is the mass of the object. The modeling of the trapped system as a harmonic oscillator facilitates the determination of the optical system’s spring constant.

The drag force based on Stokes’ law is expressed as

\[ F = -6\pi \mu Rv_s^2 \]  

(3-9)

where \( \mu \) is the dynamic viscosity and \( R \) is the radius of the microsphere. The corresponding terminal velocity, \( v_s \), is given by

\[ v_s = \frac{2}{9} \frac{(\rho_p - \rho_f)}{\mu} gR^2 \]  

(3-10)

where \( g \) is the acceleration of free fall, \( \rho_p \) is the density of the particle (microsphere) and \( \rho_f \) is the density of the fluid (water).

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\(^2\) The dynamic viscosity of water at 25 °C is about 8.90×10^{-4} Pa·s. As a result, \( c = 6\pi \mu R, c = 8.388\times10^{-8} \) N s m⁻¹. Density of polystyrene = 1.05 g.cm⁻³. Net downward force = 250 fN and equivalent mass = 25×10⁻¹⁵ kg.
For a 10 \( \mu m \) diameter microsphere, the terminal velocity is calculated to be \( v_t = 3.06 \mu m/s \). Such terminal velocity is not achieved for most experimental situations. However, it is generally assumed that Stoke’s Law applies for small objects that do not experience any turbulence and the drag force is directly proportional to velocity. Since these objects do not experience turbulence, the linear relationship between velocity and drag force holds until the objects reach terminal velocity.

In a more general representation, the equation can be written as

\[
\frac{d^2 z}{dt^2} + 2\zeta \omega_0 \frac{dz}{dt} + \omega_0^2 z = 0
\]  

(3-11)

where \( \zeta \) is the damping ratio which accounts for friction and \( \omega_0 \) is the natural frequency of the harmonic oscillator system. Rearranging the terms in (3-8) and comparing the coefficients gives

\[
\zeta = \frac{c}{2ma_0} \quad \text{and} \quad \omega_0 = \sqrt{\frac{k}{m}}
\]  

(3-12)

The value for \( k \) in weak optical traps is generally less than 100 fN,\( \mu m^{-1} \). This represents a very slow response to a step input resulting in an overdamped oscillation. The damped oscillator equation essentially models the motion of an optically trapped microsphere and the trap stiffness can be obtained by examining the optical potential energy of the system.

### 3-2-5 Determination of Optical Trap Stiffness

While it is possible to numerically compute the optical forces based on geometry, it is more practical to first determine the spring constant (or trap stiffness), \( k \), of the
damped harmonic oscillator model and subsequently inferring the optical forces in the system. For a spring-mass system, the potential energy of the mass is $\frac{1}{2} kx^2$. The probability of the particle in a particular position, $P(x)$, in the spring-mass system follows a Boltzmann distribution given by

$$P(x) \propto \exp \left( -\frac{U(x)}{k_B T} \right) = \exp \left( -\frac{kx^2}{2k_B T} \right)$$

(3-13)

where $U(x)$ is the potential energy, $k_B$ is the Boltzmann constant and $T$ the absolute temperature in units of Kelvin. The potential energy of the spring in this case is the optical potential energy. The trapped microsphere at a particular position will have an optical potential energy analogous to the elastic potential energy in a spring-mass system. This is independent of mass since the extension from the equilibrium position is considered.

When the position of a trapped microsphere is measured, the potential energy can be obtained. Although the microsphere may be moving at a particular instant thereby possessing kinetic energy, it is not included in the analysis as the damping forces will convert the kinetic energy to other forms. By sampling the axial position of the trapped microsphere over a sufficiently long time period, a distribution of its axial position can be obtained. The probability $P(x)$ is estimated by forming a histogram and normalizing it to the sample size. To determine the trap stiffness, the logarithm of the probability function is fitted to a quadratic function and the coefficient of the quadratic term gives the trap stiffness.
The power spectrum method can also be used to determine the trap stiffness [76]. The random positions of the trapped object due to thermal fluctuations are monitored. The time series is then Fourier transformed to provide the power spectrum which is Lorentzian in nature when there is Brownian motion in a harmonic potential. The trap stiffness can then be obtained by examining the roll-off frequency expressed as:

\[ f_0 = \frac{k}{2\pi(6\pi\mu R)} \]  

(3-14)

### 3-3 Position Detection Mechanisms

The scattering forces from the trapping beam guide the particles in the longitudinal direction and the corresponding gradient forces draw the particle into the beam. A weak equilibrium position of the microsphere is obtained when the upward forces due to scattering forces and buoyancy balances the weight of the microsphere. At its equilibrium position, the trapped particle can be considered a harmonic oscillator in an over-damped system with an associated stiffness. The axial trapping forces and therefore the axial trap stiffness can be increased by increasing the NA of the trapping optics (Figure 3-2). The value of the trap stiffness is sensitive to changes in the geometrical intensity distribution in the optical trap.
Figure 3-4: Comparison between two position detection mechanisms: (a) BFP interferometry and (b) LCI.
In conventional optical tweezers using back-focal plane (BFP) interferometry [18], the forward scattered light is used to determine the position of the trapped particle. The BFP of the detection objective is imaged to a quadrant photodetector (QPD), as shown in Figure 3-4a. Variations in the position of the trapped particle changes the interference pattern at the BFP, which corresponds to the optical Fourier-transform of the light fields at the sample plane. The QPD is configured to provide the sum and differences between the light irradiances in different quadrants. With proper calibration of known positional shifts of the trapped particle to the variation in the voltages of sum and differences, the BFP technique can achieve resolution down to the nanometer region [18].

By contrast, LCI uses back-scattered light from the sample and a reference beam to determine the position of a particle (Figure 3-4b). The phase of the reference beam can be controlled accurately via the position of the mirror, thereby giving very good positional accuracy. In typical LCI applications, a key performance metric is axial resolution which is the ability to differentiate two back-scattering points. This is different from the positional resolution which only depends on the stability and accuracy of the mirror’s position, i.e. phase stability and accuracy of the reference beam.

As illustrated in Figure 3-5, both the reference and sample signals in common-path LCI share the same optical path (i.e. passes through the same optics). The reference beam in a conventional setup is normally provided by a mirror while a partially reflecting surface provides the reference beam in common-path LCI. Although the
strength of the reflected beam in the common path is weaker which may reduce the heterodyne gain, this is compensated by the reduction dispersion and polarization mismatches [95].

![Figure 3-5: Common path LCI with a coverslip providing the reflected reference beam. The incident beam is focused on the coverslip. The reflected reference wavefronts are shown in black, sample wavefronts reflected are shown in red.](image)

In spectral domain optical coherence tomography (SD-OCT), a spectrometer is used to detect the spectrum as a function of wavelength which essentially discretizes it. To obtain the axial profile, the measured spectrum has to be converted to $k$-space or the wavenumber domain before inverse Fourier transforming it to the spatial domain. In continuous Fourier transform, the limits of integration spans from negative to positive infinity. This implies that an infinite $k$-space translates to infinitesimal resolution in the spatial domain. It is, however, not possible to have infinite $k$-space with practical spectrometers as its finite wavelength range will limit the resolution in the spatial
domain. A wider span in the wavenumber domain will result in a better spatial resolution and vice versa.

The positional resolution in LCI is defined as

$$\Delta z = \frac{\pi}{\Delta k_{\text{range}}} = \frac{\lambda_{\text{y}}^2}{2\Delta \lambda_{\text{range}}}$$  (3-15)

where $\Delta k_{\text{range}} = \frac{2\pi \Delta \lambda_{\text{range}}}{\lambda_{\text{y}}^2}$ and $\Delta \lambda_{\text{range}}$ is the range of the spectrometer. The positional resolution can be improved by increasing the spectral range. This range can also be artificially increased by zero-padding the data points beyond the spectrometer range. For instance, a spectrometer with a wavelength range of 700–900 nm and 3,648 pixels gives a positional resolution of 1.6 $\mu$m. Expanding the wavelength range to 600–1,000 nm by zero-padding the 600–700 nm and 900–1,000 nm range will improve the positional resolution to 0.8 $\mu$m. Zero-padding does not affect the axial resolution since it is a function of the full-width, half maximum bandwidth of the light source. The light source does not have spectral intensity that covers the zero-padded regions and this method does not affect the signal content that is already embedded in the modulated spectrum.

While zero-padding can significantly improve the positional resolution, it is not extensively used as it is computationally intensive. Furthermore, if the positional fluctuation is large, improved positional resolution does not provide any newer information. For example, a system with a positional fluctuation of 1 $\mu$m will limit the positional resolution to 1 $\mu$m. Moreover, zero-padding is not going to help much if the
objective is to image objects with high axial resolution. This is similar to the microscopy analogy of digital magnification without improvement in resolution.

The maximum measurable optical depth of 3.3 mm, \( \Delta z = \frac{1}{4} \frac{\lambda_0^2}{\delta \lambda} \), where \( \lambda_0 \) is the center wavelength at 850 nm and \( \delta \lambda \) is the pixel resolution of the spectrometer at 0.05 nm (200 nm/3648 pixels) [51]. In spectrometer based LCI systems, the sensitivity rolls off with axial depth due to the finite pixel dimensions of linear CCD detectors (\(~4\,\text{dB at Nyquist depth}) [45]. However, the impact of such roll-off is mitigated in this system. As the cavity is only about 1 mm thick, the maximum optical path length that the system can measure is 1.33 mm, given that the particles are in water. In addition, the axial height of the trapped particles seldom exceeds 500 \( \mu \text{m} \) above the coverslip with the optical powers used and this furthers limits the effects of the sensitivity roll-off.
3-4 LCI-Trapping Experimental Setup

Figure 3-6: Schematic diagram of collinear LCI sensing system consisting of the following components: MO: microscope objective, BS: beamsplitter, VA: variable attenuator, DM: dichroic mirror, BE: Beam expansion optics, CL: collimating lens, FC: fiber coupler (2x2), SLED: Super-luminescence diode ($\lambda_{\text{center}} = 820 \text{ nm}, \Delta\lambda = 100 \text{ nm}, \text{theoretical axial resolution of } 2.8 \mu\text{m}$), CCD camera, fiber illuminator, diode laser ($\lambda = 532 \text{ nm, } P_{\text{max}} = 50 \text{ mW}$). Laser power fluctuation is less than 1% during measurements.

A weak optical trap is formed using a 0.4 NA objective lens (Edmund Optics).

Figure 3-6 shows the schematic of the experiment implementation of an integrated LCI-trapping system sharing the same optical path through a 0.4 NA microscope objective. The trapping beam is telecentric expanded to ensure that the back aperture of the objective lens is overfilled. The low coherence beam is aligned collinearly with the trapping beam. A custom-made cavity, using coverslips and glass spacers, of 1 mm in depth is filled with water and polystyrene microspheres (PolySciences Inc.) of 10 $\mu$m and 50 $\mu$m diameters. The bottom surface of the cavity acts as a reflecting surface to produce the reference beam in the common-path LCI. A spectrometer (Ocean Optics, HR4000) with 3,648 pixels covering a wavelength range of 759–961 nm gave...
an imaging depth of 3.3 mm in air. The measured spectrum is zero-padded to 16,384 points to minimize discretization errors, and a positional resolution of ±217 nm is achieved. The sample signal, arising from reflecting surfaces of microspheres in the optical trap, interferes with the reference signal from the first coverslip to give a modulated SLD spectrum. The positions of the two reflecting surfaces of the microsphere are determined by taking the inverse Fourier transform of the measured spectrum.

Due to the weak optical trap and relatively weak incident laser power, only the 10 µm microspheres can be levitated while the 50 µm microspheres (125 times more massive than the former) are positioned at the base of the cavity to serve as secondary stationary spheres, and refocusing micro-lenses in the experiments. The lateral positions of the microspheres were monitored with the aid of a CCD camera.
3-5  Results

3-5-1  Long Range Tracking

Figure 3-7: (a) Optical setup of a levitating 10 µm microsphere, (b) Low coherence image of a levitating 10 µm microsphere (dashed line shows the position of the optical center), (c) depth-resolved intensity profile at time point 1 in (b), (d) brightfield images with the in-focus image showing the microsphere at the bottom of the cavity. The position of the optical center is marked as OC. The dashed arrows mark the location of the microsphere reflecting surfaces, (ii) and (iii), relative to the bottom surface of the coverslip.
Figure 3-7 shows the LCI imaging results of a levitating 10 µm microsphere as a function of time. The optical power of the diode laser after the microscope objective was found to be 10 mW. The microsphere is levitated over a detection range of 100 µm, which is much longer than that achieved with the BFP interferometry method. As depicted in Figure 3-2a, the optical forces are strongest when the focus is near the edge of the microsphere. In the experiment, the focus of the objective lens is placed above the bottom coverslip in the cavity (Figure 3-7a) by adjusting the position of the z-stage. Due to the strong forward optical forces, the microsphere is accelerated along the z-axis. As the microsphere rises, the optical force starts to decrease resulting in a reduction in acceleration. In addition, while there is initially significant acceleration and therefore significant linear momentum, there is also a drag force to slow down the microsphere. The microsphere will stop moving when the optical force balances the net downward forces arising from its own weight and buoyancy.

As shown in Figure 3-7b, the surfaces of the 10 µm microsphere ($n_{\text{microsphere}} = 1.59$) along the axial direction is represented by two successive bright lines separated by an optical path difference of about 16 µm as the microsphere rises in the cavity. The optical center (OC) of the trapped particle is determined from the mean physical positions of the microsphere surfaces. The microsphere shows effects of defocusing in the brightfield images (Figure 3-7d) as it rises in the cavity but the microsphere surfaces can still be observed at these time points in the LCI image. Background blurring from confocal effects is mitigated by coherence gating which serves to improve contrast [20]. This illustrates the long range tracking capability of LCI.
Figure 3-8: (a) Schematic diagram and (b) depth-resolved intensity profile of the 10 and 50 µm microspheres in a weak optical trap. The dashed arrows marked the optical centers of the microspheres. The axial positions of the four peaks are (i) 217.2 µm, (ii) 299.1 µm, (iii) 324.7 µm, and (iv) 340.7 µm.

Figure 3-8a shows the surfaces of two microspheres imaged by LCI. The depth-resolved intensity profile (Figure 3-8b) of the optically trapped 10 µm microsphere positioned above a 50 µm microsphere situated on a coverslip demonstrates the capability of the system to image more than one particle in an optical trap. This potentially allows the capability to study optical binding effects where there is optical interaction between more than one trapped object [21]. The optical path difference between peaks (ii) and (iii) corresponding the edges of the two spheres was 25.3 µm. Accounting for the refractive index of water (1.33), the physical distance (gap) between the particles was about 19 µm at an optical trapping power of about 2.3 mW. The physical distance between the particles is obtained by dividing the optical path
difference by the refractive index of water. In determining the physical axial position of the 10 µm sphere, the refractive indices of the 50 µm sphere and the surrounding medium (water) has to be accounted. The measurement uncertainty in the axial position of the particles include the uncertainties in the determination of the refractive indices of the microspheres and the surrounding medium (e.g. water), and also the positional resolution (217 nm for the LCI-trapping system).

The effective focal length \((EFL)\) and NA the of a ball lens with an incident collimated beam is given by

\[
EFL = \frac{nD}{4(n-1)}
\]

and

\[
NA = \frac{2d(n-1)}{nD}
\]

respectively, where \(D\) is the diameter of the microsphere, \(n\) is the refractive index of the microsphere (surround medium is air) and \(d\) is the beam diameter of a collimated beam incident on the ball lens. For a ball lens in a surround medium of water, the effective refractive index is given by the ratio of the lens material to the surround medium. For a polystyrene microsphere \((n = 1.59)\) and a surround medium of water \((n = 1.33)\), the effective refractive index about 1.2. Consequently the effective focal length of a 50 µm microsphere is 76.44 µm in water and the back focal length is 51.44 µm. Compared to 19 µm of separation between the two microspheres, the back focal length seems excessively large. Three factors can account for the deviation from the expected value of the back focal length. The first is the assumption of an incident
collimate beam on the ball lens. The experiment uses a microscope objective to focus the trapping beam into the cavity. The beam is no longer collimated after the microscope objective. Secondly, the effects of optical binding have been ignored. The presence of a 10 \( \mu \text{m} \) after the 50 \( \mu \text{m} \) microsphere alters the incident beam shape which violates the collimated beam assumption. Lastly, the upward optical force is proportional to the incident power. The equilibrium position will thus depend significantly on the incident power.

**3-5-3 Axial Resolution Measurement**

The ability to resolve the two surfaces is limited by the axial resolution of the system, which is inversely proportional to the bandwidth of the light source. The center wavelength and bandwidth of the SLD used were 820 nm and 100 nm, respectively. This gives a theoretical axial resolution in air of 2.8 \( \mu \text{m} \). The measured axial resolution was closer to 4 \( \mu \text{m} \) and the 10 \( \mu \text{m} \) microsphere can drop as near as 4 \( \mu \text{m} \) above the coverslip before it cannot be resolved. The poorer achieved resolution can be attributed to the bandwidth limitation of the optical fibers and optics. The finite pixel number in the spectrometer (i.e. finite spectral range) also contributes to the poorer resolution.
Figure 3-9: (a) Schematic diagram illustrating the arrangement of an optically trapped 10 µm microsphere used to evaluate the resolution limit of the LCI system. (b) shows the axial profile of the microsphere and coverslip measured with the LCI system at Rayleigh criterion and (c) clearly resolvable surfaces between microsphere and coverslip.

The individual microspheres of different sizes dwelling in the optical trap can potentially be differentiated and resolved with LCI, which is important for applications such as optical chromatography. To quantify the limits of the LCI system in resolving microstructures, the axial Rayleigh criterion was used to determine the axial resolution using an optically levitated 10 µm microsphere and the top surface of the coverslip, as illustrated in Figure 3-9a. The 50 µm microsphere was not used to minimize optical binding effects which may change the profile of the incident fields. Moreover, optical interference effects with the two microspheres changes the phase of reflected beam and can affect the accuracy of the axial resolution measurements. The longitudinal separation between the 10 µm microsphere and the coverslip was monitored with the LCI system. A typical axial profile of the microsphere measured with the LCI system is depicted in Figure 3-9b. The axial resolution limit of the LCI system was determined from the microsphere-coverslip separation such that the surfaces are barely
resolvable (Figure 3-9c). This is found to be 4.9 µm and is ~10% higher than the full-width at half-maximum (FWHM) resolution of 4.3 µm. The discrepancy is attributed to interference effects when the surfaces are near to one another [96].

3-5-4 Microsphere Dynamics

The microsphere dynamics is determined by monitoring its optical center with LCI. The dynamics of a microsphere without being optically trapped is Brownian motion in nature and can be characterized by observing its mean-square position as described by

\[ \langle x^2 \rangle = 2Dt \]  

(3-18)

where the diffusion constant \( D = k_BT/6\pi nr \), \( n \) is the viscosity, \( r \) is the microsphere radius and \( t \) is the duration of observation (\( k_BT = 4.1fN.\mu m, T = 300 \) K). This gives a free diffusion constant and a mean square displacement of 0.826 µm².s⁻¹ and 1.65 µm², respectively for a 10 µm microsphere over a period of 1 s. The LCI implemented in this work is capable of measuring the Brownian motion of the 10 µm microsphere since the position displacement is within the linear detection range of the system (positional resolution of 0.217 µm and linearity of >100 µm). In the presence of a weak optical trap, the stiffness, \( k_{trap} \), of a trapped 10 µm microsphere can be computed from equipartition theorem where

\[ k_{trap} = \frac{k_BT}{\langle x^2 \rangle} \]  

(3-19)

\[ \text{Density of polystyrene} = 1.05 \text{ g cm}^{-3}, \text{ weight of a 10 µm sphere} = \frac{4}{3}\pi r^3 \rho_{\text{polystyrene}}g = 5.5 \text{ pN. Buoyancy force} = \frac{4}{3}\pi r^3 \rho_{\text{water}}g = 5.24 \text{ pN. Net downward force} \approx 260 \text{ fN. Optical power after microscope} = 2.3 \text{ mW, since optical force} = \frac{n_{water}P}{c}Q_{total} \text{ which has to be equal to 260 fN at equilibrium. Based on the values shown in Figure 3-2 for NA = 0.4, } Q_{total} \approx 0.027 \text{ which places the center of the sphere slightly below the focal point of the objective lens.} \]
To determine $<x^2>$ with respect to the equilibrium position, the optical center of the 10 µm microsphere is monitored using the LCI system over a period of 10 minutes. With a sampling time of 100 ms, 6,000 data points were obtained. The probability of the microsphere displacement in a potential well, modeled as a Boltzmann distribution [19], is obtained from the histogram of these data points. The trapping potential energy, $U(x)$, is determined by taking the logarithm of the probability [19]. The potential energy, having a quadratic dependence on the displacement, is then fitted to a quadratic function to determine the trap stiffness. Figure 3-10a shows the optical potential energy of a trapped 10 µm microsphere obtained from the LCI system using two different NA objectives but with the same optical power of 2.3 mW. When the NA of the objective is increased from 0.4 to 0.7, the trap stiffness is increased by almost 60 times from 1.46 fN·µm$^{-1}$ to 86.4 fN·µm$^{-1}$. This is in agreement with the higher axial intensity gradient expected in the optical trap from the use of a larger NA objective.

![Figure 3-10: Optical potential profiles of a 10 µm microsphere measured with (a) objective lenses of 0.4 (○, $k_{0.4NA} = 1.46$ fN·µm$^{-1}$) and 0.7 (○, $k_{0.7NA} = 86.4$ fN·µm$^{-1}$) NA and (b) with (○, $k_{50um} = 11.24$ fN·µm$^{-1}$) and without (×, $k_{50um} = 1.57$ fN·µm$^{-1}$) a 50 µm microsphere in the optical trap. The corresponding fitted optical potential profiles (lines) are also shown in the figure. The fitting accuracy as quantified by R-square is at least 0.82 in the experiments.](image-url)
Besides changing the NA of the objective, the effect of microsphere lensing on the axial trap stiffness was also investigated. The re-focusing of an initial trapping beam through a microsphere can strongly influence the dynamics of the trapped particle through longitudinal optical binding [97]. A single stationary 50 µm microsphere, as illustrated in Figure 3-8a, is used as the refocusing element. The 50 µm microsphere acts as a ball lens which re-focuses the trapping beam to yield a shorter focal length and increases the NA of the trapping system. Both the equilibrium axial position and the associated trap stiffness are monitored with LCI. The trapping position of the 10 µm microsphere changed from 65 µm to 170 µm above the cavity floor when the 50 µm microsphere is removed. Figure 3-10b shows the optical potential profiles of the 10 µm microsphere with and without the 50 µm microsphere. The measured trap stiffness is observed to increase by 7 times from 1.57 to 11.24 fN.µm\(^{-1}\) in the presence of the 50 µm microsphere. This is in good agreement with the expected result of increasing the NA of the optical trap. Based on the values of the trap stiffness, the effective NA of the 50 µm microsphere is inferred to be between 0.4 and 0.7. The measurements of both the absolute positional shift of the 10 µm microsphere and the increased trap stiffness due to the re-focusing of light by the 50 µm microsphere highlights the strength of LCI in the direct visualization of inter-particle optical mechanical interactions. The results in Figure 3-10b elucidate the use of LCI in the measurement of inter-particle dynamics.

### 3-5-5 Dual Counter-Propagating Beam Trap

A dual beam trap was implemented to examine the effects of an additional trap on the overall trap stiffness. The dual beam trap has the advantage of more degrees of
freedom in controlling the trapped object. Figure 3-11 shows the schematic of the axial dual beam trap. Due to the presence of a forward optical force from the top beam, the trapped object is pushed down to a lower position of the bottom beam. As a result, it will experience a stronger upward force from the bottom beam. Thus, it is expected that the trap stiffness will increase significantly compared to a single trap.

The low coherence beam monitoring the position of the trapped particle is collinear with the trapping beams. The two trapping beams have different wavelengths to ensure that no interference patterns form near the trap. The dual beam trapping is equivalent to a spring-mass system with a constant force pushing against the spring. This will result in a stiffer trap, as the mass experiences a stronger spring force at equilibrium. In the optical force equivalent, the second beam pushes the trapped object back into
the first beam. This results in a stronger forward force from the first beam which makes the trap stronger. Thus, with a second beam in the system, the trap stiffness can be controlled by adjusting its power.

(a) \( \mu_{\text{position}} = 500.5 \, \mu m, \sigma = 2.1 \, \mu m \)  
(b) \( \mu_{\text{position}} = 434.0 \, \mu m, \sigma = 1.1 \, \mu m \)

Figure 3-12: Histograms of a single microsphere trapped in (a) single and (b) dual beam trap.

Figure 3-12 shows the histogram of the positions of a trapped microsphere of 10 \( \mu m \) diameter monitored using LCI over a period of 2,000 s in both the single and dual beam traps. The optical powers at the trap position were about 5.0 mW and 1.0 mW for the red and green lasers, respectively. The mean positions of the trapped particle are 500.5 \( \mu m \) and 434.0 \( \mu m \) for single and dual beam traps, respectively. This shows that the dual beam trap has the effect of pushing the microsphere to a lower equilibrium position. In addition, the standard deviation of the dual beam trap histogram is significantly lower indicating that a stiffer trap is formed. The addition of the second beam therefore allows more degrees of freedom to control the forces acting on the trapped particle. The dual beam trap is very useful in trapping multiple particles.
due to its flexibility in varying its optical power and focal position of the second beam. LCI can be used to monitor multiple microspheres in such situations.

3-6 Discussion

The accuracy of the LCI measurements depends on the relative phase of the scattered or reflected waves from objects to a reference plane. For objects with planar surfaces, such as flat mirrors, the phase is very well defined upon reflection. However, for spherical objects, besides the straightforward reflection off the edge nearest to the source, surface waves can occur.

![LCI Image](image)

Figure 3-13: LCI image of a single microsphere levitated over a distance of 150 µm.

Figure 3-13 shows a LCI image of a 10 µm sphere levitated over a distance of 150 µm using the setup shown in Figure 3-6. As the refractive index of polystyrene is about
1.59, the optical path difference between the top and bottom points should be around 15.9 µm. However, at positions (i) and (iii) seen in Figure 3-13, the difference between the peaks in the axial profiles at these positions are greater than 15.9 µm which implies that the LCI system is not detecting reflection or scattering from the top and bottom points. Some other modes, such as surface modes, may have been excited and thus shown up on the axial profiles.

![Figure 3-14: Axial profiles of the levitated 10 µm microsphere at positions (a) (i), (b) (ii) and (c) (iii) as indicated in Figure 3-13.](image)

Figure 3-14 shows the axial profiles obtained at the three different positions marked in Figure 3-13. In these diagrams, the first peak around the optical pathlength of 200 µm represents the top surface of the bottom coverslip. The distance between the second and third peaks is 15.9 µm (Figure 3-14b). This is also the region corresponding to the confocal parameter of the microscope objective as shown in Figure 3-13. Beyond these regions, the distance between the peaks diverged from 15.9 µm. If surface modes are responsible for these peaks, tracking the microsphere beyond the confocal parameter of the lens may result in erroneous results.
The effects of surface waves are more pronounced when the object is far from the focal plane. This is the region where the wavefront of the incident beam is more spherical than planar. As a result, this promotes a better chance of exciting a particular surface mode which will manifest itself as an additional reflecting surface. These modes can distort the accuracy of the position of the microsphere if the aim is to track it over long distances. Moreover, if two trapped objects are close to one another, then the presence of these modes may also result in misinterpreting the separation between the trapped objects. On the other hand, if the intention is to monitor the forces on a single trapped particle over a relative short distance, then it is important to ensure that the measurement distance does not transit between the regions where surface modes start to appear. The Rayleigh length ($\pi w_0^2/\lambda$) of the LCI beam was optimized for the particle diameter to accurately determine the reflecting surfaces of the microsphere.

3-7 Summary

The capability of coherence gating in a common-path LCI-trapping system for characterizing the positional fluctuations of microspheres with 0.2 µm accuracy over a long axial range of 3 mm is demonstrated in this chapter. Inter-particle optical forces down to the femtonewtons range were also measured using the same approach. The technique of coherence gating provides a tool to investigate inter-particle dynamics with low trapping forces over longer axial distances. The functionality of LCI in optical trapping is especially useful for studying multi-particle interactions in optical
binding [85], *in-situ* measurement of different particle separation in optical chromatography [83] and cell deformation in an optical cell stretcher [97]. In these applications, the measurement of the minute trapping force provides a description to the dynamics of the trapped system which in turn determines the physical properties of the trapped object(s) (e.g. shape/size). The long working distance in which LCI operates is suitable as the trapped particles in these applications are generally located beyond the distance in which BFP can accurately compute. The common-path approach can easily be incorporated into any optical trapping platform, especially in optical fiber trapping systems [21] with minimized dispersion and polarization mismatches.
4-1 Introduction

4-1-1 Virus Infection in Orchids

Orchid culture and export is an important business in many countries. Singapore, for example, exported S$26 million worth of fresh orchids, orchid plants and cuttings in 2006 [99]. Viral diseases are a major problem in the orchid industry due to their detrimental effects on the quality of the flowers and plants, which directly affects their exportability. Two most prevalent and economically important orchid viruses are the Cymbidium Mosaic Virus (CymMV) and the Odontoglossum Ringspot Virus (ORSV) [100, 101]. They are known to cause chlorosis (Figure 4-1) and deformation in the leaves, color break in the flowers and a reduction in flower yield. The main source of virus transmission is the horticultural tools used to divide plants and harvest flowers. There is presently no cure once a plant is infected, although some plants may develop resistances to the virus. The only effective control or mitigation measure is to isolate and destroy the infected plants.
Figure 4-1: (a) Chlorosis in an orchid leaf, (b) deformation in an orchid leaf and (c) color break in an orchid flower.
The current gold standard test for detecting virus infection in orchid plants is the Enzyme-Linked Immunosorbent Assay (ELISA) [102], a serological detection technique that requires plant samples to be sent to a commercial laboratory. Another method utilizing polymerase chain reaction (PCR) to amplify the viral nucleic acids for electrophoresis analysis is reported to be more sensitive and reliable than ELISA [103]. However, both methods have to be performed in the laboratory, and are time consuming and destructive. Moreover, decision on selecting orchid plants for virus infection tests is mainly based on visually obvious color breaks in flowers or chlorosis in leaves. This method of visual inspection has several limitations. Firstly, young orchid plants suffering from virus infection may not show visible symptoms. Furthermore, factors such as abnormal nutrition and environmental conditions can also produce virus-like symptoms. Lastly, it is often too late when visual symptoms appear as this usually implies that the plant is already badly infected.

4-1-2 Motivation

Light microscopic methods [104] such as confocal microscopy [105]; and electron microscopy [106] have been proposed to detect virus. These methods generally require preparation of samples which may be more straightforward compared to serological techniques. CymMV has been reported to show banding inclusions in which the virus aggregates and band together locally within the infected cell [105]. Rather than sampling different parts of the plant (e.g. leaves, roots or stems), light microscopic techniques can be more localized in diagnosing virus infections. This provides a useful way to determine the extent of virus infection within a plant tissue, i.e. whether it is restricted to the epidermis or is a systemic infection.
The efficient control of orchid virus diseases requires rapid and accurate diagnosis. While existing techniques provide an accurate way of determining the extent and seriousness of virus infection, these techniques are heavily based in the laboratory. A cheap and portable mechanism can be useful in providing rapid and accurate diagnosis of virus detection. OCT is a non-invasive, low coherence imaging method that can potentially fulfill the need for a fast and non-destructive diagnostic tool [1, 107]. The imaging depth of OCT is primarily limited by optical absorption and scattering, and morphologic features as deep as 3 mm can be imaged in tissues. With down to micrometer axial resolution, the technique is capable of producing high resolution images of tissue structure beneath the surface layer that is comparable to histology [107] and has potential applications in the in situ, real time diagnosis of early neoplasias [8].

OCT has recently been used in the area of botany to visualize the inner structures of botanical subjects [108, 109] and to study the early physiological changes in plants suffering from a pathogen attack [110]. Due to the high density of small organelles in a typical animal cell, OCT generally cannot differentiate individual cells in animal tissues. However for a plant cell with large vacuoles making up to 60% of its volume, it is generally much easier to discern individual cells [37] for different tissue layers. The differences in plant structures due to variations in aridity have been investigated using OCT [111, 112]. In addition to water stress, ozone stress in plants has been monitored using OCT [113]. OCM, with its high lateral resolution and the good SNR
due to coherence gating, has also been used to observed morphological changes in plant tissues [114].

This chapter investigates the use of a high resolution OCT method to non-destructively examine leaf samples from both healthy and virus-infected orchid plants. The study focuses on CymMV-infected plants since its incidence is significantly higher than those of the ORSV-type [100]. The OCT images of the leaf samples were compared with histological analysis and correlated to standard ELISA test. The OCT images of leaf samples from stressed but healthy plants exhibiting virus-like visual symptoms were also obtained for comparison. Following the work reported in this chapter, virus infection in seeds and subsequent study using OCT has also been reported [115].

4-2  Background

4-2-1  Structure of Orchid Leaves

![Figure 4-2: Structure of a typical orchid leaf.](image)

Figure 4-2: Structure of a typical orchid leaf.
The health of a plant may be reflected by the state of its leaves, stems or roots. However, the leaf of a plant is generally the most easily accessible part. To better understand how the virus affects the orchid plant, a brief description on the structure of the leaves is presented in this section. Orchid leaves can generally be classified as either the thin or thick type [116]. The thickness of thin leaves ranges between 0.3–0.5 mm while thicker leaves have more layers and its thickness is between 1–2 mm. Regardless of the thickness of the leaf, the general structure of the leaf remains largely similar as shown in Figure 4-2.

A typical leaf consists of three layers: the upper epidermis, the mesophyll layer and the lower epidermis. The upper epidermis consists of a thin layer of epidermal cells which is typically transparent due to a lack of chlorophyll for efficient light penetration. The epidermal cells are closely-spaced to provide protection from insects and foreign materials. The top of the leaf has a thin layer of wax known as the cuticle which acts to minimize water loss. The middle layer consists of mesophyll cells where the bulk of the leaf’s chlorophyll is contained. This is the layer in which photosynthesis mainly occurs. The first layer of mesophyll cells is most tightly packed to maximize light collection. Deeper layers have more air space for oxygen and carbon dioxide to mix with the cells to increase the efficiency of photosynthesis. Both the xylem and phloem can be found in this layer that transport water and food to other parts of the plant. The last layer is the lower epidermis. This layer has guard cells around the stomata that open up during photosynthesis, to allow gaseous exchange and water evaporation in the form of transpiration to cool the plant. In other periods, the guard cells remained close so as to minimize water loss [116, 117]. One of the most common symptoms of
an unhealthy plant is chlorosis in which certain parts of the leaf or flower lose their colors. Other common symptoms include ringspots and mosaic patterns on the leaves or flowers. The causes of these symptoms may range from malnutrition, lack of water (stressed) to bacteria, fungus or virus infection.

4-2-2  Plant Viruses

Plant diseases can be caused by bacteria, fungi or viruses. While the first two are generally mobile, viruses need a vector to be transmitted to a host. In addition, viruses are made up of tens to hundreds of proteins and are much smaller than bacteria or fungi. A plant virus generally has three parts, a deoxy-nucleic acid (DNA) or ribo-nucleic acid (RNA), movement protein(s) and coat protein. Although small and generally non-fatal to the plant, the effects of viruses cannot be underestimated. An estimated economic loss of up to hundreds of millions of dollars due to loss in crop yields and reduction in market value has been attributed to virus infections [118].

One common vector for virus transmission is insects such as bees and aphids. Another method is mechanical when there is human intervention. This typically involves harvesting tools being shared between infected and non-infected plants. Once the virus is inside a plant cell, it makes use of the cell protein synthesizing machinery to replicate. The next step is to infect the rest of the plant to facilitate further replication. Although plant cells have cell walls which serve as a physical barrier to prevent neighboring cell infection, viruses have evolved to move through them via the plasmodesmata. Plasmodesmata is a small channel (50-60 nm) [119] that allows inter-cell interaction (e.g. transfer of proteins, signaling pathways) in plants. Recent studies
have indicated that movement proteins are responsible for the inter-cellular movement of viruses [120]. Besides inter-cellular movement, viruses also require another mechanism through the endoplasmic reticulum or other membranes to move within the cell [121].

The two most common orchid viruses are CymMV and ORSV [101] with a higher incidence of CymMV than ORSV [100]. The genus of CymMV is the potexvirus (Potato X Virus) which has a general molecular structure of a positive sense single-stranded RNA, a triple gene block and a coat protein [101]. The virus particles is 480 nm in length and 13 nm wide [101]. Under light microscopy, banding inclusions are commonly observed for CymMV-infected plant with the width of banding about 0.5–1 \( \mu \)m. The banding inclusion occurs very close to the cell walls and is proposed to be virion aggregates packed side by side in orderly arrays separated by a narrow margin [104, 105]. These inclusion can be observed in both epidermal and mesophyll cells. Orchids infected with CymMV may experience chlorosis or leaf necrosis. The lack of leaves after virus infection will result in poorer growth which in turn affects their quality and yield.

As a potexvirus, CymMV spreads through the mechanical transfer between an infected and a non-infected plant. Once the virus is inside the plant, its movement between cells occurs through the plasmodesmata. Although the size of the viruses are relatively small (10 to 80 nm), the size limit of plasmodesmata is even smaller (~ 1 nm) [120]. Thus, the cell-to-cell spreading and movement requires more than just diffusion of virus particles. Generally, the virus unfolds with the aid of a coat protein and/or
movement proteins during the cell-to-cell movement process. The movement proteins target localized regions or channels in the plasmodesmata for transporting to another cell. Once the unfolded virus and the movement protein are near the cell wall, the movement proteins interact with the cell wall to increase the exclusion size limit. Finally, the virus moves across the cell to infect another cell. Potexviruses such as CymMV have a triple gene block (TGB1, TGB2 and TGB3) which serves as a movement protein [120, 122]. TGB 1 is responsible for increasing the permeability of the plasmodesmata (size exclusion limit, SEL). TGB2 and TGB3 are membrane binding which facilitate the process by binding to the endoplasmic reticulum (ER) and aid intracellular and intercellular transfer [122].

4-3 Materials and Methods

4-3-1 Optical Coherence Tomography

A list of design targets for the OCT system (Table 4-1) were determined to ensure that the imaging of the orchid leaf was feasible. Considerations such as the leaf structure were taken to estimate the necessary resolutions required to obtain an image with good signal-to-noise ratio. These design targets were used to guide the optimization of design parameters so that trade-offs can be properly managed (e.g. imaging speed vs sensitivity).
Table 4-1: Design targets of OCT system for orchid leaf imaging

<table>
<thead>
<tr>
<th>S/N</th>
<th>Parameter</th>
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<th>Min</th>
<th>Typ</th>
<th>Max</th>
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<td>Imaging speed(^5)</td>
<td>frames/s</td>
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<td>µm</td>
<td></td>
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<td>1.5</td>
<td></td>
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</tr>
<tr>
<td>6</td>
<td>Lateral image span</td>
<td>mm</td>
<td></td>
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<tr>
<td>7</td>
<td>Average illumination intensity(^7)</td>
<td>W/cm(^2)</td>
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<td>3 \times 10(^6)</td>
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</tr>
<tr>
<td>8</td>
<td>Average energy per lateral point</td>
<td>nJ</td>
<td></td>
<td>54</td>
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<tr>
<td>9</td>
<td>Center wavelength(^8)</td>
<td>nm</td>
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</table>

A Fourier domain OCT (FD-OCT) system [56] similar to that presented in the previous chapter was set up in this work to achieve high resolution OCT imaging. The schematic diagram of the FD-OCT system used for this study is shown in Figure 4-3.

The light source used in the system is an Integral OCT femtosecond laser (Femtolasers

\(^5\) Constrained by the average power on the leaf to prevent burnout (i.e. dwell time)
\(^6\) Minimum to identify different layers of the orchid leaf cells
Produktions GmbH) with a center wavelength of 820 nm and an optical bandwidth of about 120 nm. Identical microscope objective lenses (NA = 0.3) were placed in the sample and reference arms to mitigate dispersion mismatch in the two arms. The laser light is coupled into a 2×2 fiber coupler, which splits it equally into the reference and sample arms. Light reflected from the reference arm and the sample arm is combined in the interferometer and measured in the frequency domain using the Ocean Optics HR4000 spectrometer. The original optical spectrum is modulated at a frequency directly proportional to the optical pathlength between the reference mirror and the sample. The spatial reflectance profile of the tissue is obtained from an inverse Fourier transform of the optical spectrum.

Figure 4-3: Experimental setup of the FD-OCT system used in this study.

The axial resolution of the OCT setup was measured to be 4 µm in free space. The lateral resolution was 9 µm, as determined with a USAF target card. A sensitivity of 93 dB was achieved with this setup. The scan rate was about 1 A-line/s and is limited
by the acquisition time of the spectrometer and the signal processing time for data scaling and Fourier transforms. The power delivered to the leaf sample was about 5 mW which, together with the objective lens, gave an irradiance \((7.9 \times 10^3 \text{ W/cm}^2)\) that was lower than the damage threshold of plant leaves [123]. Furthermore, the laser pulse width was broadened significantly by dispersion after passing through various optical components. Consequently, the peak power was also not sufficient to damage the leaf samples. Based on the above measured parameters, the OCT setup met the design targets.

4-3-2 Plant Materials

Leaf samples from Oncidium orchid plants were used in this study. Leaf samples with differing symptoms were collected from both healthy and CymMV-infected plants. These leaf samples were first imaged using the FD-OCT system to obtain cross-sectional OCT images. After OCT imaging, part of the leaf samples were subjected to standard histological preparation: fixation, sectioning and haematoxylin-eosin staining. Some of the leaf samples underwent cryohistology to study its optical properties without staining. The sections were examined under a bright-field or phase-contrast microscope. The remaining leaf samples were sent for ELISA tests to positively identify virus-infected samples. It is noted that ELISA tests are sensitive enough to detect the presence of virus due to the high-titer nature of the CymMV in Oncidium orchids [125-127].
4-4  Results

4-4-1  OCT Analysis

The OCT and bright-field histological images of a leaf sample from a healthy plant in Figure 4-4 were compared. The OCT tomogram is a raw false-color image depicting the interferometrically detected optical intensity, which is due to the combination of the backscattered E-fields from different depths in the leaf sample and the reference E-field. The backscattered intensity is associated to the differences in the refractive index between the cellular components and its surrounding medium in the plant tissue. As the axial information entails both the refractive index and the physical depth, the axial depth from the surface of the leaf sample shown in Figure 4-4 has been corrected using the average refractive index of plant tissues of about 1.43 [128].

Comparing with the histological section of the leaf sample in Figure 4-4b, the upper epidermis, vascular bundle and mesophyll cells are clearly evident in the OCT image. The distinctive horizontally elongated shape of epidermal cells and the more rounded shape of mesophyll cells are readily visible in the OCT image. The nuclei of the plant cells are barely discernible due to the resolution limits of the system. The OCT image is also “darker” at lower depths with less discernible features. However, as the leaf structure remains largely unchanged, the weaker OCT signal implies higher attenuation at increasing depths into the leaf sample. A penetration depth of approximately 400 µm in the leaf sample was achieved with the OCT system. The limited measurement depth is attributed to the attenuation of the incident light via scattering in the plant tissue [124], the finite Rayleigh range of the microscope
objective and the sensitivity of the spectrometer. A notable missing component in the OCT images is the lower epidermis. In addition, the vascular bundle is not as detailed compared to the histological section.

Figure 4-4: Comparison between the (a) OCT image and (b) histological section of an orchid leaf sample. Scale bar corresponds to 100 µm. \( ue \) – upper epidermis, \( vb \) – vascular bundle, \( st \) – stoma, \( mc \) – mesophyll cell.

The OCT images of leaf samples from both healthy and virus-infected plants were compared as shown in Figure 4-5. Three CymMV-infected leaf samples with differing
symptoms were imaged with the OCT system. One of the virus-infected leaf samples has visible chlorosis while the remaining samples have no visible symptoms. One of the virus-infected samples with no visible symptoms is a small young leaf. ELISA test performed on these samples confirmed that they are infected with CymMV. The ELISA tests yielded positive CymMV infection with results of approximately 104 µg/g fresh weight for all three leaf samples.

Figure 4-5: OCT images of (a) a healthy leaf, (b) a virus-infected leaf with chlorosis, (c) a virus-infected leaf without visible symptoms and (d) a small young leaf with virus infection but no visible symptoms. Inset shows the magnified view of the upper epidermal layers of the leaf samples.
The OCT images in Figure 4-5 clearly revealed the epidermal layer and the underlying mesophyll cells in all the leaf samples. It is noted that the mesophyll cells in two of the virus-infected samples (Figure 4-5b and c) are larger as compared to those of the healthy sample. This can be explained by the larger size of the two virus-infected leaf samples in Figure 4-5b and c as compared to the healthy sample. The mesophyll layer of the young leaf sample in Figure 4-5d appears to be highly scattering, although this is not seen in OCT images of other virus-infected young leaf samples. The origin of the highly scattering region throughout the young leaf sample is presently unclear, but it could be due to structural damage in the mesophyll cells as a result of the virus infection.

A clear difference between the OCT images of healthy and virus-infected leaf samples becomes apparent when epidermal layer was examined. The epidermal layers of virus-infected samples are found to be highly scattering compared to a healthy sample. In particular, the horizontally elongated structure of the epidermal cells is no longer visible in the virus-infected leaf samples. Optical sections from the bottom surface of the leaf samples were also obtained with the OCT system. The lower epidermal layers of virus-infected samples exhibit the same highly scattering characteristics as the upper epidermal layer (Figure 4-6). Furthermore the lower epidermal layers of healthy leaf samples appeared transparent and the epidermal cells can clearly be differentiated from each other. To verify the results, several other healthy leaves and virus-infected leaves which are visually symptom-free were imaged. The OCT images of all the leaf samples consistently showed a highly scattering epidermal layer in virus-infected leaf samples and a clear epidermal layer in healthy leaf samples.
Figure 4-6: OCT image of the lower epidermis of a virus-infected leaf.

As a quantitative measure, the average intensity value of the epidermis excluding cell walls in the OCT images was used to compare between the healthy and virus-infected leaves. This value is proportional to the intensity of the incident light and the reflectivity of the tissue components in the epidermis. The average intensity value over five healthy samples is 0.16±0.09 (arbitrary units). By contrast, the average intensity value is 0.38±0.22 for the five virus-infected samples. The average intensity values in the epidermis of healthy leaves are close to the measurement noise floor of 0.15, indicating that there is little or no scattering in the volume of healthy epidermal cells. The virus-infected samples have significantly larger average intensity values due to increased scattering in the epidermal cell volumes.

Abnormal nutrition and environmental conditions such as nutritional imbalance, excess salts, high light intensity and genetic disorders can result in virus-like
symptoms on orchid plants. A stressed plant can easily be mistaken to be virus-infected with just visual inspection. To investigate if stressed plants without virus infection exhibit the same high scattering feature in its leaves as virus-infected plants, leaf samples of several stressed plants that suffered from a lack of water over a prolong period were imaged. Figure 4-7 shows the typical OCT image of leaf samples from stressed plants without virus infection. The optical sections of these leaf samples are found to be similar to those of healthy leaves in Figure 4-5a. The stressed leaf samples did not exhibit the highly scattering epidermal layer observed in the OCT images of virus-infected samples.

![OCT image](image)

Figure 4-7: Typical OCT image of leaf sample from virus-free but stressed plants.

### 4-4-2 Histological Analysis

Histological sections of healthy and virus-infected leaf samples were examined under the microscope with a 10× objective. Figure 4-8a and 8b show the typical histological cross-sections stained with haematoxylin and eosin of healthy and virus-infected
leaves, respectively. This is a standard stain procedure to highlight the nucleus and cytoplasm. Morphological features such as the epidermal layers and vascular bundles are observed to be similar in appearance between the healthy and virus-infected samples. Although the bright-field images showed considerable details of the cellular structures, there appears to be no discernible difference between the histological images of healthy and virus-infected samples. In particular, the highly scattering epidermal layers seen in the OCT images of virus-infected sample is not observed in the corresponding histological sections. One possible explanation is that the content within the leaf cells are altered or removed by the histological preparation process. To examine the plant cells in its original form, cryosections of the leaf samples were also taken without any fixation and staining. These cryosections were then mounted on microscope slides and observed under both bright-field and phase-contrast microscopes. There were no significant differences in the epidermal layers between the healthy and virus-infected leaf samples.
Figure 4-8: Histological sections of (a) healthy and (b) virus-infected leaf samples. Scale bar corresponds to 100 µm.
4-5 Discussion

The optical scattering signal is based on the scattering coefficient of the leaf sample, where the back-scattering signal is stronger when the scattering coefficient is higher. This can be attributed to either increase in the scattering cross-section or the concentration of scattering particles. The scattering cross-section can be increased by changes in the shape and size of the scattering object. While the CymMV particle is too small to be imaged directly, the banding inclusion [104, 105] may enhance the scattering signal to improve the OCT contrast. Moreover, the presence of banding inclusion may turn out to be the dominant scattering component among other organelles. In addition, due to virus replication, the number of sites for banding inclusion increases which increases the concentration of scattering particles. The net effect is an increase in the scattering coefficient which can account for the higher scattering observed in the epidermis of virus-infected cells as compared to the healthy ones.

The OCT images of Oncidium orchid leaves revealed a characteristic highly scattering epidermal layer when the plant is infected with CymMV. The significant increase in scattering in the epidermis of the virus-infected samples may be associated to cell death processes such as autophagy, which plays a protective role against external infection [129]. The autophagy process involves the degradation of intracellular components which can lead to changes in the optical scattering characteristics of the epidermal cells. The reason for the general lack of significant scattering signals from the intracellular region of mesophyll cells in virus-infected leaf samples is unknown and is a subject of future study. The highly scattering feature in the epidermal layers of
virus-infected leaf samples is not observed in the leaves of stressed plants, despite showing similar visual symptoms as virus-infected plants. This suggests that the highly scattering feature in the epidermis is related to the CymMV infection and can be used to accurately identify virus-infected orchid plants. Histological analysis of the leaf samples showed that the highly scattering feature in virus-infected samples cannot be observed under bright-field microscopic observation. This is possibly due to the relatively low scattering coefficients of plant tissues [124] and the small difference between the scattering coefficients of healthy and virus-infected epidermal tissues. By contrast, the high sensitivity of OCT ensures that subtle differences in the scattering properties of the epidermal layers can be detected.

Besides giving the optical cross-sectional images of orchid leaves, OCT can detect a distinctive highly scattering epidermal layer in the leaves of virus-infected plant. This highly scattering feature can be used to quickly diagnose virus infection in orchid plants by imaging its leaves with an OCT system. The OCT system can be made portable and compact by replacing the femtosecond laser employed in this work with a broadband SLD source [130]. Real time OCT imaging can be achieved through the use of fast CCD arrays [56]. A probe-based sample arm in the OCT system will allow in-situ diagnosis of the orchid plants. Such a tool will enable orchid growers and import authorities to carry out non-destructive, real time screening of orchid plants for virus infection.
4-6  Summary

The use of OCT for diagnosing virus infection in orchid plants at the tissue level is explored. The morphological structure of cells within the orchid leaves can clearly be seen in the high resolution OCT images. Highly scattering upper and lower epidermal layers in the leaves of virus-infected plants, which were not visible under histological observation, were detected with the OCT technique. This highly scattering feature is not present in the leaf epidermides of stressed but healthy plants exhibiting similar visual symptoms as virus-infected plants. Consequently virus-infected orchid plants can be accurately identified by imaging the epidermal layer of its leaves with OCT. The OCT modality is found to be suitable for fast, non-destructive diagnosis of orchid virus infection, which can potentially lead to significant cost savings and a better control on the spread of viruses in the orchid industry.
Chapter 5  DESIGN OF AN OCT PROBE AND SYSTEM FOR IN VIVO NASOPHARYNX IMAGING

5-1  Introduction

5-1-1  Screening for Nasopharyngeal Carcinoma

Globally, approximately 10 million new cases of cancer are detected each year. The incidence and mortality of cancer are highest amongst the richest and most affluent countries. With improved early cancer detection and better cancer treatment, the mortality rate has been slowly declining in these countries [131]. As a developed country, Singapore is also burdened by cancer. The mortality age-standardized (ASR) rates for males and females are 144.2 and 90 per 100,000, respectively, between 1998 and 2002 [132]. In particular, nasopharyngeal cancer (NPC) is the most common head and neck cancer in Singapore with an incidence ASR of 10.8 per 100,000 in males and 4.2 per 100,000 in females. It is ranked the 6th and 12th most common cancer males and females, respectively, in Singapore [132]. In addition, it has one of the highest incidence among Chinese males from China, Hong Kong and Singapore compared to other parts of the world [133].

The nasopharynx lies in an area just behind the nose and just above the back of the throat (Figure 5-1). Up to 90% of the posterior wall of the nasopharynx is lined up with squamous epithelium. Near the anterior wall, the lining changes to stratified squamous epithelium [134]. These epithelial cells lining the nasopharynx can become
cancerous and give rise to NPC [135]. As NPC commonly starts from epithelial (squamous) tissues [2], it is also known as nasopharyngeal carcinoma. A comprehensive study on the histology of the nasopharynx can be found in reference [134].

![Image of the anatomy of the head and neck](image)

**Figure 5-1: Anatomy of the head and neck**

The current practice of diagnosis for NPC is through examining patients with a nasopharyngoscope under local anesthesia. This is a fiber-based endoscope that allows clinicians to view the surface of the nasopharynx and look for abnormal growths, bleeding or other signs of disease. The main challenge is the identification of early NPC especially if the cancer starts from the epithelial layers below the tissue surface.
The nasopharyngoscope system which uses white light imaging is a very useful tool for clinicians to observe the nasopharynx. However, visual inspection is often not sufficient to determine the condition of the nasopharynx. Upon detection of suspicious lesions or abnormalities in the nasopharyngeal tissue, a biopsy will be performed to excise some tissue under mild local anesthesia. The excised tissue is subsequently examined under a microscope by a pathologist. A report describing the type and extent of the cancer is then sent to the clinician. This technique is currently the gold standard in staging NPC. There are a number of potential problems with this process. First, the selection of the site where the tissue is to be excised is generally based on its morphology. The decision in performing a biopsy is highly dependent on the skills and experience of the clinician in identifying the precancerous or cancerous lesions. For late stage cancer where the cancerous cells have spread to large area, the selection of the excision site may be rather obvious. However, in cases where the clinicians suspect that there is a potential cancer risk, the choice of excision sites will be generally based on their experience. This may result in false negatives which run the risk of delayed treatment. Secondly, the histological process generally takes time. It may take up to three days from the time a tissue is excised to the completion of the histopathological report [136].

There exist non-invasive imaging techniques that have deep tissue penetration and have improved the process in cancer diagnosis and management. X-ray computer tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) scans are some common imaging modalities that can be used. When applied specifically for NPC, these imaging modalities build three-dimensional
(3D) image of the region where the cancer is located. However, these techniques suffer from a lack of resolution to identify early stage cancer where there are minuscule changes in the tissue structure. There is therefore a need for a minimally invasive technique that offers good resolution and significant penetration depth to image below the surface of the nasopharyngeal tissue to assist in detecting early NPC or to streamline cases that should warrant a biopsy.

5-1-2 Motivation

Endoscopes provide a useful way to explore the functions, physiology of internal organs with minimal invasive procedures. One of the earliest use of endoscopy dates back to 1806 for inspecting internal organs. Besides being able to examine and diagnose the condition of an organ, endoscopes can be used to guide surgery and assist in obtaining a biopsy for further investigation. Most endoscopes are white-light imaging devices where reflected or scattered light from an illuminated sample surface are captured for analysis. Two major components are necessary for white-light endoscopy: light delivery and light collection. Endoscopes usually use fiber optic bundles to deliver white light to the sample location [137]. Typical illumination sources include halogen bulbs or LED sources to provide the desired color temperature. The light collection mechanism depends on the application of the endoscope. In rigid endoscope applications where the access to the tissue-of-interest is more straightforward (e.g. oesophagus, nasopharynx, larynx, etc), the imaging plane is located at the proximal end. Light can be collected through a series of graded index lenses or Hopkin lenses and the capturing device such as a CCD camera is placed at the proximal end [138]. For applications in the colon or lungs which require more maneuver, flexible probes are usually needed. It may not be practical to place the CCD
camera at the proximal end with many optical elements to direct the light back to the camera. As such, the CCD camera is mounted at the distal end and the image is transmitted back to the proximal end by electrical signals [139].

Other imaging modalities such as confocal [140], fluorescence imaging [141], multiphoton technique [142], and ultrasonography [143] have also been integrated into endoscopes to enhance its capabilities. These modalities can give better measurement contrast and consequently improve the specificity of the endoscopic diagnosis. The performances of these modalities generally fall into the same category as that of the confocal microscopy or the ultrasound technique. These modalities have either very good resolution (~1 μm) with relatively shallow penetration depth (~1 μm) or good tissue penetration (>3 mm) but with low resolution (~15 μm) [144]. Optical coherence tomography (OCT) provides a good balance between resolution (<5 μm axial and lateral resolution) and penetration depth (~2 mm) [3, 145]. OCT is an imaging technique which relies on a broadband light source to provide good axial resolution [1, 2]. With coherence gating mechanisms, the penetration depth can be a few millimeters below the epithelial tissue surface. Many early stage carcinomas occur within a few millimeters of the internal and external body layers [131]. This is due to the abnormal growth of epithelial cells occurring typically on the outermost layer of an organ. The achievable imaging depth of OCT enables tissue abnormalities such as dyplasia and neoplasia to be observed with good resolution [146]. Minimally invasive techniques coupled with an endoscopic OCT probe can further allow internal organs or intravascular vessels to be observed with high resolution [27, 147].
An extensive review of the different methods and applications of endoscopy with OCT is provided by Yaqoob et al [137]. Endoscopic OCT can generally be implemented using either the side-imaging or forward-imaging method. The side-imaging technique is suitable for imaging the walls of organs (e.g. colon) [148]. In side-imaging OCT endoscopes, a window at the side of the endoscope allows light to be delivered and collected. One of the common configurations of side-imaging OCT involves the use of a rotating prism driven by micro-motors [149-151] in the distal end, in which circumferential scans can be obtained to form a two dimensional (2D) tomogram. To form a 3D tomogram, the endoscope can be moved linearly along the tissue surface while the prism is being rotated [148]. Forward imaging techniques, on the other hand, deliver and collect light via the front of the endoscope [68]. This technique provides useful structural information about the tissue and its topology as the endoscope does not usually make contact with the tissue surface. It is, however, more difficult to incorporate minute beam scanning mechanisms at the tip of the endoscope while maintaining a small diameter with forward imaging techniques.

This chapter investigates the design and implementation of an OCT probe and high speed spectrometer system for nasopharyngeal imaging. The OCT probe was designed around a rigid nasopharyngoscope and optimized to give good dispersion and polarization matches between the reference and sample arms. A high speed spectrometer with good spectral resolution, reasonable spectral range and good signal-to-noise ratio (SNR) for measuring the interferometric signals from the OCT probe was also designed, implemented and characterized. The completed system was also tested with \textit{ex vivo} samples to evaluate its performance.
5-2 Design Considerations and Implementation

Nasopharyngeal carcinoma generally starts at depth ranging between 1 to 2 mm below the epithelium surface [134]. Clinicians generally look for abnormal tissue growth in the nasopharynx surface using white-light endoscopy before deciding whether a biopsy is required to confirm the abnormality. There are generally two types of white-light endoscopy probes used for nasopharynx imaging; a rigid probe and a flexible probe with a mounted CCD camera. To facilitate the use of OCT, the modality has to be designed around existing endoscopy probes. A forward-imaging OCT device is more suited for this application to provide additional tissue structural information.

The requirements of OCT endoscopes generally include: size, resolution, working distance, angular/lateral scan range and speed. The size of the endoscopes depends on the application and endoscopes for the nasal cavity will require a smaller diameter compared to that used in colonoscopy. Axial resolution is typically dependent on the broadband light source used. Dispersion and polarization mismatches between sample (delivery and collection optics), and the reference arm will worsen the axial resolution of the OCT images. Lateral resolution is a function of the numerical aperture (NA) of the final optical element. A high NA system will have good lateral resolution at the expense of a reduced lateral scan range. To achieve high speed imaging, it is necessary to have very fast beam steering and axial scanning mechanisms. The former may be achieved through MEMS or very high speed galvanometers while the latter could be achieved using spectral domain techniques employing Fourier domain mode-locked lasers [152] or high-speed spectrometers [58].
5-2-1 Forward Imaging Probe

A proposed OCT scanning endoscope is conceptualized as shown in Figure 5-2. This endoscope design is similar to the forward imaging probe design [153] where the white light path is collinear with the OCT beam.

![Figure 5-2: Schematic drawing of the operation of the OCT endoscope.](image)

The endoscope comprises a white light and an OCT imaging path. In the white light path, light from a halogen source illuminates the tissue-of-interest via a graded index (GRIN) rod incorporated into an existing rigid endoscopy probe used by clinicians. The returning light from the tissue is relayed back to the viewing eye-piece through the same GRIN rod, a scan lens, a dichroic mirror and a relay lens. The dichroic mirror allows light of wavelength between 400–550 nm to be transmitted.
The OCT section in the endoscopic system consists of a single mode fiber which delivers the broadband, near infra-red (NIR) light from either a SLD or a Ti:Sapphire laser to a mirror on a two dimensional (2D) scanner. The incident NIR light is directed to the dichroic mirror which then reflects it into a scan lens with a focal length of ~40 mm. The scan lens focuses the broadband NIR light onto the proximal end of the GRIN rod with a pitch length of two, which acts as relay lens. To achieve a working distance (distance between the distal end and the tissue-of-interest) of about 5 mm, the scan lens is positioned such that its back focal plane is about 5 mm into the GRIN rod. The 2D scanner raster scans the OCT light across the proximal end of the GRIN rod which relays the scanning beam on the tissue-of-interest. The backscattered NIR light is collected by the reverse path which goes back to the single mode fiber. Figure 5-3 shows the OCT endoscopic prototype integrated with the existing rigid probe. The following section describes the operation of each part in more detail.

Figure 5-3: Photograph depicting the prototype of the OCT endoscope implemented in this work.
The depth-of-field of the proposed probe design is limited by the aperture and focal length of the scan lens (Figure 5-2), since there are no further lens elements at the distal end. A scan lens with a focal length of 40 mm (Edmund Optics) was chosen to optimize the lateral resolution. With a full aperture limited only by the scan lens diameter, the depth-of-field is relatively shallow (~ 1 mm). Consequently the image will only be sharp when the probe is within a narrow range of distance from the object. This is in contrast to the standard clinical nasopharyngoscope which has a wide field-of-view and long depth-of-field and the image is almost always sharp at any distance. The combination of field-of-view and depth-of-field is also known as the focal volume. A possible way to overcome the small focal volume with the GRIN rod design is to add an objective lens at the distal end. However, the presence of the objective lens causes problems with the scanning OCT beam. The backscattered or reflected light cannot be effectively collected by the GRIN rod if an objective lens is included.

To maintain a proper scanning beam while achieving a larger focal volume, an alternative solution is to place an iris in-between the two imaging lens in the white light imaging path, as illustrated in Figure 5-4. The iris acts as a pupil stop that allows adjustment of the depth of field. The optimal position of the iris (in the endoscopy path) which maximizes the depth-of-field is located at the focal lengths of the scan lens and the eye-piece optics. In addition, the scan lens and the eye piece optics are placed in a telescopic lens arrangement to relay and magnify the object. The depth-of-field can be inferred by analyzing the through focus modulation transfer function (MTF) of the entire system, which is a useful measure of image resolution and contrast.
Figure 5-4: Proposed white-light imaging path with the inclusion of an iris/diaphragm.

Figure 5-5 shows the through focus MTF for on-axis illumination simulated using the Zemax software. With an iris diameter of 1 mm, the modulus of the MTF at 10 mm from the focal plane tails off to a value of 0.4. By contrast, the modulus of the MTF at the same position drops to a value of 0.7 when the iris diameter is reduced to 0.5 mm. As the modulus of the MTF for a particular spatial frequency relates to the contrast of the image, the simulation result indicates that there is a drop of about 30% in the contrast at 10 mm away from the focus where the diameter of the iris is reduced from 1 mm to 0.5 mm.
An USAF target glass chart was used to check the resolving power of the optical system in the probe. Group 4, element 1 (16 line-pair/mm pattern and a resolution of 62.5 µm) of the USAF chart was chosen to evaluate the imaging resolution and contrast of the probe design. The depth-of-field was determined by moving the target chart away from the focal plane until the element can no longer be resolved. The probe design was first characterized without the iris and the results are shown in Figure 5-6. Degradation in image sharpness was observed with small axial shifts from the probe tip and a depth-of-field of about 1.3 mm was estimated.

Figure 5-7 shows images of the same element obtained from the probe with an iris of about 0.5 mm diameter. The depth-of-field is increased by 492% to approximately 7.7 mm. This is, however, achieved at the expense of reduced image brightness (6 times weaker intensity) and would require the exposure time of the CCD camera to be increased to compensate for the reduced intensity. Furthermore it is also important to
accurately position the iris laterally and axially at the foci of both lenses in the white-light path to avoid additional vignette effects. With a larger focal volume in the white-light path, the clinician will be able to discern more features in the nasopharynx and guide the probe to a desired location for OCT scans. The images in Figure 5-6 and Figure 5-7 show some vignette effects. The vignette effect seen in the images is due to the reduced light collection near the peripherals of the GRIN rod. At the edges of the rod, light collected will be reduced as high angled rays cannot propagate down the GRIN rod as they are outside the NA of the GRIN rod.

Figure 5-6: Images of group 4, element 1 of the USAF target chart obtained at various distances from the probe tip without an iris. Scale bar represents 500 µm
Figure 5-7: Images of group 4, element 1 of the USAF target chart obtained at various distances from the probe tip with a 0.5 mm diameter iris. Scale bar represents 500 µm

5-2-1-2 Optical coherence tomography path

The broadband light source (Ti:Sapphire) used for orchid leaf imaging in the previous chapter is employed in the OCT modality of the nasopharyngeal probe design. A list of design targets for the OCT system (Table 4-1) were determined to guide the probe design.
Table 5-1: Design targets for OCT imaging of the nasopharynx

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<td>frames/s</td>
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</table>

In most OCT systems, a NIR beam is scanned across the sample. The backscattered or back-reflected light is collected by the same imaging optics for interference with a reference light. The interference pattern is then digitally processed to give a cross-section image of the sample. In this work, a scanning and focusing mechanism is used to map out the cross-sectional image of the sample.

<sup>9</sup> Minimum to identify different layers of the nasopharynx tissue cells

<sup>10</sup> Constrained by the movement of the patient, operator
In this work, a 2×2 fiber coupler is used to split the NIR light from a Ti:Sapphire source (Femtolasers) into a reference and sample path in the fiber coupler. In the sample path, the NIR beam is steered two-dimensionally by a piezo-scanner (S-334, Physik Instrument). A dichroic mirror after the piezo-scanner reflects the NIR light into the GRIN rod. The GRIN rod essentially serves as a relay lens to focus the focal plane after the scan lens on the sample. The reflected or backscattered light is then collected by the same GRIN rod and scan lens, and re-enters the optical fiber. A reference beam from the reference optics will then interfere with the backscattered light. The resulting interference pattern is detected by a high-speed spectrometer. The optics in the reference arm is almost matched to that in the sample arm (inclusive of GRIN rod and scan lens) in order to minimize dispersion mismatch.

In a laser scanning microscopy system, a scan lens is placed after a galvanometer or PZT scanner to focus the collimated beam from the laser source at the object plane. By placing the galvanometer at the back-focal plane of the scan lens, the object can be scanned by varying the tilt of the mirror on the galvanometer. As illustrated in Figure 5-8, any change in direction of the laser beam will result in a displaced beam parallel to the optical axis. This arrangement allows the object to be uniformly scanned with a flat field.
Figure 5-8: Schematic illustrating flat field scanning when the galvanometer mirror is situated at the back focal plane.

In a GRIN rod system, the GRIN rod acts a relay lens to transfer the image from the proximal to the distal end. Typical GRIN rod endoscopic systems employs a GRIN rod with integer multiples of pitch length and an objective GRIN lens to relay the image at the sample plane. The purpose of the objective lens is to enlarge the field-of-view under white light endoscopy. The combination of the objective lens and the GRIN rod is to relay the sample plane to a proximal end of the rod as shown in design A of Figure 5-9.

Figure 5-9: GRIN lens system for endoscopy applications.
While the design is useful for white light endoscopy, it is difficult to obtain a proper scanning field in the sample plane for OCT applications. Figure 5-10 illustrates effect of adding a scan lens to a GRIN rod. For both cases, the scan lens focuses the collimated beam on the plane just outside the proximal end of the GRIN rod. The image of the diffraction limited spot (Airy disk) will reside at the distal face of the GRIN rod and have the same size as the focused spot at the proximal end. This effectively relays the beam profile at the proximal to the distal end. Thus, by scanning the beam at the proximal end, the sample will also be scanned at the distal end. A working distance of a few millimeters would also be necessary to ensure that the distal end of the probe does not make contact with the tissue surface. The position of the focused spot at the distal end can be shifted out by reducing the distance between the proximal end of the GRIN rod and the scan lens. On the other hand, with the presence of a GRIN objective, an enlarged image of the Airy disk will be formed at the distal end. The focused spot at the proximal end is relayed as a defocused spot. This will result in poor illumination at the sample which also translates into inefficient collection of back-scattered light.

![Figure 5-10: Scan lens (a) without and (b) with GRIN objective at the distal end.](image-url)
The rigid probe comes in two versions which differ in the window configuration on the distal end. One version has a flat-faced window terminating the distal end and is generally used for direct, frontal viewing. The other type has a 30° angled window mounted on the distal end which provides a side-imaging field-of-view combined with an appropriate Hopkin objective lens. The rigid probe with the 30° angled window and the Hopkin lens removed is more suitable for the design implemented in this work. An advantage of this choice is that the Fresnel reflection from the angled window on the distal end is not collected into the GRIN rod. With a flat-faced window, the Fresnel reflection will show up as unwanted peaks in the OCT axial profile and the resulting OCT image will become noisier. Figure 5-11 shows the comparison between a probe with a flat-faced and an angled window. The light source, with a bandwidth of about 120 nm, used in the measurement was the broadband Ti:Sapphire laser used in the previous chapter for orchid leaf imaging. The high frequency modulation observed in the spectrum obtained from a probe with a flat-faced window clearly illustrates the effects of Fresnel reflection on the OCT measurements. The dip around pixel number 1400 (as measured with a custom-made spectrometer with high-speed line-scan CCD camera) shown in Figure 5-11b is attributed to the non-uniform reflectivity (across different wavelengths) of the 45° dichroic mirror (see inset).
Figure 5-11: OCT spectra measured with a mirror placed in the sample arm of a (a) flat-faced and an (b) angled window probe. Inset shows the reflectivity of the dichroic mirror.

5-2-1-3 Characterization

As point-by-point OCT scanning in the lateral direction is required to form 2- or 3-D tomograms, the objective lens cannot be used. Consequently the OCT scanning capability is realized at the expense of the enlarged field-of-view offered by conventional white light imaging system design (with objective lens). The field-of-view is therefore limited to the cross-sectional area of the 2 mm diameter GRIN rod and the maximum lateral scan range is 2 mm. To achieve a working distance of about 5 mm, the back focal point of the scan lens was positioned 5 mm into the GRIN rod.
To minimize vignetting effects of the optics, the effective lateral scan range is reduced to about 1.1 mm. Figure 5-12 shows the image of a transmission USAF target chart captured by the CCD camera through the white light endoscopic path of the probe. The vertical pattern observed in the figure belongs to group 4, element 6 of the USAF chart. Although the dichroic mirror reflects most of the NIR light back to the single-mode fiber for interference, the scan line due to the NIR laser is still visible in the captured CCD image. Due to the relatively low NA of the scan lens, the lateral resolution achieved is about 30 µm which is comparable to the forward imaging technique by Boppart et al [153].

With no GRIN rod in the reference arm for physical dispersion compensation, the axial PSF was extremely broad with a SNR of almost 0 dB. With the placement of the GRIN rod in the reference arm, the dispersion mismatch was significantly corrected to
yield a FWHM of 6.7 \mu m (Figure 5-13). A SNR of about 60 dB is achieved using the HR4000 spectrometer from the previous chapter.

![Figure 5-13: Axial PSF when GRIN rod placed in reference arm to minimize dispersion mismatch.](image)

In the actual implementation, there are additional fibers placed after the 2x2 fiber couplers to facilitate the delivery of the OCT beam to the target tissue. To minimize dispersion mismatch, a 2 m fiber was placed in each of the reference and sample arms. Due to variation in the fibers, perfect dispersion compensation of the fibers was not achieved. Figure 5-14 shows the OCT axial profile of a coverslip measured without any dispersion or polarization compensation and a glass slide measured with dispersion compensation and polarization adjustment. The axial profile of the coverslip (Figure 5-14a), measured with a mirror placed in the reference arm, comprises of the autocorrelation term which is due to self-interference between the top and bottom glass-air interfaces of the coverslip and the cross-correlation terms. The dispersion and polarization of the common-path in the sample arm is almost matched.
as both fields travel the same path. The cross-correlation feature in the axial profile results from the interference between the sample and reference arm signals, which is very different from the self-interference feature. First, the widths of the peaks of the self-interference and cross-correlation features differ by about 500% (FWHM of 6 µm vs 30 µm). Secondly, the peak of the cross-correlation feature is an order of magnitude lower (51.36 vs 5.135 in arbitrary units) which implies a loss in SNR.

Figure 5-14: OCT axial profile of a (a) coverslip measured without dispersion compensation or polarization adjustment, and a (b) glass slide measured with dispersion compensation and polarization adjustment.

Figure 5-14b is obtained by imaging a 1 mm thick glass slide with dispersion compensation and polarization adjustment of the light source. The dispersion compensation was introduced by placing additional glass-slides in the reference arm. The FWHM width has improved to 4 µm, but this comes at the expense of a long tail in the resulting axial profile which is not present in the pre-compensated case. The adjustment of the polarization controller is highly dependent on the existing conditions.
of the fibers. Any motion in the fiber during imaging will cause the axial point spread function to be distorted. This can potentially be a problem since it is unlikely that the patient or clinician can remain perfectly still during imaging. A more practical solution has to be implemented to improve the resolution, possibly in the form of post-signal processing [154]. The widths of the base of the features near the noise floor of the autocorrelation term, the pre-compensated cross-correlation term and the post-compensated term are 80 µm, 60 µm and 150 µm, respectively. This indicates that the compensation method may have introduced higher order dispersions which serve to widen the width of the base.

5-2-2 High Speed Spectrometer

5-2-2-1 Review

OCT has evolved over the past 30 years from a system showing static retina images [1] to real-time 3D tissue imaging [155]. Improvement in imaging speed has allowed the study of tissue dynamics upon drug delivery [156] and surgical guidance [157]. In addition, image blurring resulting from patient movement can be significantly reduced with high speed imaging. Real-time comparison with white light endoscopy is also critical in improving the accuracy of a clinical diagnosis [7, 158]. While high-speed imaging is desired, there is often a traded-off with SNR. Hence, it is important to maintain a reasonable image quality while maximizing the imaging speed.

The speed of OCT imaging is generally dependent on the number of axial scans required and the rate of each axial scan [159]. Depending on whether the OCT system
is based on time or spectral domain, the challenge either lies in having a fast scan rate of the reference arm or a high speed data generation and/or collection of the spectrum. The conventional time-domain OCT is generally limited to relatively slow imaging speeds in the kHz range due to a need to scan the reference arm in a linear fashion [1]. Axial scan rates as high as 4 to 8 kHz can be achieved with rapid scanning optical delay lines (RSOD) in the reference arm [54, 160]. This is, however, insufficient for achieving real-time imaging with significant lateral range. For example, scanning 512 lateral points at an axial rate of 8 kHz will only give a frame rate of 15 per second, assuming no further delays due to data acquisition or computation complexity. Moreover, the main issue with time domain systems is the significant trade-off between the scan rate or bandwidth of the system and SNR of the image [2]. The SNR of the image is compromised with increasing imaging speed due to the limited bandwidth of the photo-detector.

For a similar imaging speed, the spectral-domain OCT system has better bandwidth performance [45, 161]. Moreover, there is no need for movable optics in the reference arm of the system and this also improves the reliability of the system. Spectral domain OCT systems can be implemented based on either a swept-source or the Fourier domain technique. The imaging speed of swept-source OCT system is limited by the speed of the tunable filter in the laser and the photodetector. On the other hand, the imaging speed of Fourier domain OCT systems is limited by the bandwidth of the line scan CCD camera (e.g. 29 kHz for a Basler L104k camera) in the spectrometer.
Fourier domain mode-locked (FDML) laser is a form of tunable laser used in swept source OCT where the line scan rates can be as high as 20 MHz [155]. A narrowband optical filter is swept in time corresponding to the optical round trip time of the laser cavity [60]. Instead of amplitude modulation in a standard mode-locked laser, the FDML modulates the spectrum of the cavity that is tuned to the round-trip time of the cavity. Using long cavities and modulating the spectrum at an integer multiple of the frequency of the round-trip time, very fast tuning of the wavelengths of the broadband gain medium can be achieved. Coupled with a high speed detector and fast computer, real-time 3D imaging of tissues can be achieved with 4.5 Gvoxels (giga-volumetric pixels) per second. One critical parameter for a tunable laser configuration is the need for long coherence length [162]. Each tunable wavelength has a narrow bandwidth (or long coherence length) to reduce aliasing effects in the tomogram.

The Fourier domain technique requires the use of a high speed spectrometer to collect the resulting interference signals. Using femtosecond lasers with bandwidths in excess of 120 nm [50, 163], ultra-high axial resolution images can be obtained. In this configuration, the spectrometer bandwidth limits the usable imaging speed. To achieve high speed imaging, the spectrometer must be able to refresh and send the pixel data quickly to the computer. The fastest system currently available has an axial scan rate of 312,000 lines per second [58]. This is achieved by using a very high speed linear CCD camera with a line scan rate of 140 kHz, albeit at the expense of sensitivity and axial resolution. The higher axial scan rate compared to the line scan rate is achieved by defining a smaller area of interest compared to the maximum CCD size.
In spectral domain OCT systems, the axial information at a particular scan point is obtained by inverse Fourier transforming the measured interference spectrum. As the spectrum is pixelized, the inverse Fourier transform is carried out using digital fast Fourier transform algorithms. The number of axial pixels in an OCT image is determined by the number of pixels in the spectrometer. A 2D image is constructed from the inverse Fourier transforms of interference spectra measured in the entire lateral scan range.

While the line rate of the system due to axial scan rates or generation/collection of the spectrum is critical to real-time imaging, the high speed lateral scan on the beam is just as important. Small beam steering mirrors can achieve high scan rates of 1 kHz over a small lateral range. The scan area at the sample can be adjusted with suitable optical design, such as using a series of optical elements to magnify the scan area. The scanning speed can be further improved with the use of a resonant scanner in one direction and a relatively slower scanner in the orthogonal direction. Thus, a typical 2D scan rate can be as high as 8 kHz using a single scanning spot. To realize higher scanning speeds, multiple spots over different areas have also been proposed. This allows scanning speed as high as 3.6 kHz to be achieved using standard galvanometer scanning mirrors [155]. Similarly, the scan rate can be increased by utilizing both the forward and reverse scans of the galvanometer and using duty cycles that are larger than 50%. To minimize lateral scanning, full-field OCT where en-face images were obtained [164] has also been proposed.
One of the speed bottlenecks of OCT systems is the Fourier transform computation time. A dedicated digital signal processing (DSP) chip may be useful to perform all the necessary digital Fourier transforms and band-pass filtering. Alternatively, with multi-core processors, it is possible to speed up the digital signal processing by assigning successive line spectral to each processor. For swept-source or time-domain OCT, external electrical components such as band-pass filters can be added to the system to speed up the signal-processing process. Given the high-speed requirements of the real-time display, high-speed data acquisition of > 1 GSamples/s is also necessary.

5-2-2-2 Proposed Design

A high-speed spectrometer was constructed (Figure 5-15) in order to implement real-time OCT imaging within the constraints of utilizing existing equipment. To achieve real-time imaging, a frame rate of 30 frames per second is generally needed. For a 2D image with 512 lateral points, a CCD line rate of at least 15.4 kHz (30×512) would be needed, assuming that the scanning mechanism can at least match the frame rate. The Basler CCD camera (L104k) has a maximum line rate of 29 kHz which is sufficient to meet the requirement of real-time imaging capability. This was the fastest line-scan camera in the market at the time of this work.
The range and spectral resolution of the spectrometer affects the imaging depth and SNR of an OCT system. As the axial profile is obtained from the inverse Fourier transform of the spectrum in $k$-space, the span (or range) of the $k$-space determines positional resolution. On the other hand, the spectral resolution of the spectrometer determines the largest imaging depth achievable for the system. While it is desirable to achieve both high positional resolution and large imaging depth, there is usually a tradeoff between these two parameters. Since the number of CCD pixels is fixed, the spectral range determines the spectral resolution (wavelength range divided by pixel number). The positional resolution can be improved by zero-padding the measured spectrum. This involves assigning zeros, or some infinitesimal small values to avoid signal processing problems, to the extra pixels added to the measured spectrum. This effectively extends the spectrum beyond the initial pixel number in the spectrometer,
although computation time is increased and real-time imaging performance can be affected. As discussed in the former chapter, zero-padding does not improve the axial resolution as this is solely dependent on the light source spectrum.

In this work, the wavelength range of the spectrometer is designed to be about 190 nm with a starting wavelength of 742.9 nm. This is to ensure that the CCD can capture the entire spectrum of the broadband light sources (> 100 nm bandwidth, center wavelength of ~830 nm). With 2,048 pixels in the L104k Basler CCD camera, a theoretical maximum imaging depth and positional resolution of ~1.9 mm and 1.83 µm, respectively, can be achieved before zero-padding the measured spectrum. As the L104k has a pixel size of 10 µm × 10 µm, the spot size of any individual wavelength should be less than 10 µm to prevent aliasing or cross-talk effects. The entire wavelength range of 190 nm should also fit into the spatial range of 20.48 mm (2,048 pixels). With these constraints, the other optical components in the light path can be determined in the reverse order to the fiber.

The L104k camera housing extends about 46.5 mm from the CCD surface, which limits the minimum focal length of the focusing lens. In addition, the size of the lens has to be larger than 20.48 mm to ensure that the edges of the spectrum can be captured by the CCD camera. Thus a good starting point for the focusing lens is a 1” diameter lens with a back focal length of 50 mm. However, a problem with a 1” diameter lens is that the edges of the spectrum near the marginal areas of the lens are prone to spherical aberrations. The spot sizes near the lens edges may therefore be larger than those at the lens center and may give rise to cross-talk effects. To maintain
the paraxial condition in which most lenses are optimized, a larger diameter lens can be chosen. Figure 5-16 compares the spot diagrams obtained with Zemax simulation of 1” and 2” diameter lenses (Thorlabs) with effective focal lengths of 75 mm. The field angle was set at ±10° which represents the worst case diffraction from the grating. The geometric radius of the focus spot from the 2” lens is 40% smaller than that of the 1” lens (211 µm vs 355 µm) for an incident beam spot of 2 mm. This is attributed to a lesser spherical aberration for the 2” lens. When the field angle is reduced to ±5°, the geometric radius of the focus spot from the 1” lens reduces to 89 µm, giving an improvement of 75%. The field angle can be reduced by using a lens with a longer focal length so that the CCD detector area is covered. However, this will come at the expense of a very large overall size of the spectrometer which may not be practical.

![Figure 5-16: Spot diagrams for a (a) 1” and (b) 2” lens with effective focal length of 75 mm.](image-url)
The diffraction limited spot size is given by

\[ \phi = \frac{0.61 \lambda}{\text{NA}} \]  \hspace{1cm} (5-1)

where \( \lambda \) is the incident wavelength and NA is the numerical aperture of the lens when the incident beam uniformly illuminates the lens. Given that the final spot size at the CCD camera should be less than 10 \( \mu \text{m} \) and using the longest wavelength of 932 nm, the NA should be at least 0.06. For a long focal length lens with a focal length of \( f_1 \), the entrance pupil radius should be 0.06\( f_1 \). For example, selecting a focal length of 70 mm, the minimum radius should be 4.2 mm. A lens with a diameter of 2” can then be selected to mitigate the effects of spherical aberration.

The following constraint is placed on the focal length of the collimation lens. As the single mode fiber (SM600) has a mode-field diameter of about 4.6 \( \mu \text{m} \) and a NA of 0.1, the focal length of the collimation lens should be at least \( f_2 = \frac{0.06f_1}{0.1} = 0.6f_1 \). To ensure that this criterion is well met, the focal length of the collimation lens is designed to be similar to the focal lengths. This results in an image mapping of the fiber core at unity magnification at the CCD camera under aberration-free conditions. Since the SM600 fiber has a mode-field diameter of 4.6 \( \mu \text{m} \), this ensures that the spot size on the CCD camera is below its pixel size of 10 \( \mu \text{m} \).

5-2-2-3 \hspace{1cm} Transmission Grating

The transmission grating was selected based on the center wavelength of the light source. For both the Ti:Sapphire laser (Femtolaser) the center wavelength is around
820 nm. A transmission grating with high efficiencies near the 830 nm wavelength was therefore chosen. For efficient first order diffraction, a grating with 1200 lines/mm with a blazing angle of 29.8° was selected. The design equation of a grating is given by

\[ a \sin \theta_i + a \sin \theta_t = n \lambda \]  

(5-2)

where \( a \) is the spacing (in nanometers) between the grooves, \( \theta_i \) and \( \theta_t \) are the angle of incidence and transmission, respectively, \( \lambda \) is the wavelength in units of nanometers and \( n \) is an integer indicating the diffraction order. The first diffraction order was chosen to keep the dimensions of the spectrometer to a practical size as large diffraction angles from the center wavelength will result in a significantly large spectrometer width when a 70 mm focusing lens is used.

The incident angle \( \theta_i \) is selected to be equal to the blazing angle of 29.8° to minimize optical losses when the incident beam strikes the grating. With a starting wavelength of 742 nm and a wavelength span of 190 nm, the minimum and maximum diffraction angles are 23.2° and 38.5° respectively. The center wavelength of the spectrometer corresponds to a diffraction angle between these diffraction angles, i.e. 30.8° at a wavelength of 841.5 nm. Since the optical axis of the focusing lens is coaxial with the direction of the diffracted beam at the center wavelength, the focusing lens is placed at 30.8° with respect to the transmission grating. The extreme wavelengths therefore diffract at an angle of 7.6° from the optical axis.
The focusing lens is placed at one focal length away from the transmission grating so that individual wavelengths are focused on the line-scan CCD camera. As the width of the CCD array is 20.48 mm and considering the maximum subtend of each extreme diffracted wavelength of 7.6°, the focal length of the lens should be around

\[
\frac{10.2}{\tan 7.6°} = 76.4 \text{ mm}
\]

The same focal length was selected for the collimation lens to maintain unity magnification of the fiber beam spot.

5-2-2-4 Alignment Process

The alignment process starts with laying out the optical components as shown in Figure 5-15. The CCD camera is then replaced with a mirror to measure the reflected power indirectly via the 2×2 fiber coupler. The positions of the source, grating and lens are adjusted to maximize the reflected power. This includes tilting and shifting individual components. In addition, a beam alignment plate or iris can be used to ensure that the beam diameter is relatively small so that spectral crosstalk is minimized.

Once the maximum reflected power is obtained, the mirror was replaced by the CCD camera. A calibration source (Avantes, Hg-Ar lamp) was used to map the pixel position to the corresponding wavelength. The spacing between the peaks (in pixel number and wavelength) can then be obtained. A calibrated commercial spectrometer (HR4000, Ocean Optics) was used to validate the alignment of various optical components. The step-by-step alignment process is detailed in Appendix A.
The choice of the collimation lens at the front end affects the spectral resolution. The NA of the SM600 single mode fiber is about 0.1. For a collimation lens with focal length of 45 mm which meets the minimum criteria of $0.6f_1$ with $f_1 = 76 mm$, the incident beam diameter at the grating is about 9 mm. Since the diffraction limited spot size is a function of wavelength and NA of the focusing lens, the largest spot size is defined by the longest wavelength in the system. The resolvable longest wavelength is approximately 984 nm for a diffraction spot size of the same order as the CCD pixel size (10 µm). Wavelengths longer than this will result in aliasing or crosstalk problems.
Figure 5-17: Reference spectral lines from a Hg-Ar calibration lamp measured with the custom spectrometer implemented in this work using collimation lens of focal length of (a) 10 mm, (b) 45 mm and (c) 75 mm. The reference spectral lines are also measured with a commercial spectrometer (HR4000) for comparison.
Figure 5-17 shows the measured spectrum of the Hg-Ar calibration lamp using various configurations of the custom spectrometer in comparison with that obtained from the commercial spectrometer. It is noted that as the focal length of the collimation lens increases, the spectral lines sharpens. This is attributed to the larger beam diameters prior to the focusing lens which results in tighter focus spots. This in turn provided better spectral resolution. The spectral lines near the edges were also observed to be poorer in resolution compared to those situated in the center of the lens. This is due to coma aberration from the focusing lens originating from light rays entering the lens at oblique angles.

5-2-2-6 Discussion

The initial design of the spectrometer did not result in a satisfactory OCT imaging depth. This is due to poor spectral resolution. In spectral domain OCT, reflective surfaces at large distances relative to the reference plane generates high frequency modulations on the source’s spectrum. If the spectrometer cannot resolve these high frequency modulations, the imaging depth of the system will become limited. The spectral resolution of the spectrometer is generally limited by either the spot size of the diffracted beam or the pixel size, whichever is larger.

The spectral resolution is poorer nearer the edges of the CCD camera representing minimum and maximum wavelengths. To investigate the loss of resolution, the line-scan CCD camera was replaced with a 2D CCD camera at the back focal plane of the focusing lens to image the spectral emission from the reference Hg-Ar calibration
lamp. The image obtained from the 2D CCD camera revealed comet-shaped beam spots with the “comet tail” pointing towards the optical axis and large beam spots nearer the edges of the camera (Figure 5-18). The resulting coma aberration as wavelengths other than the center wavelength are incident to the focusing lens at non-zero angles lead to poor spectral resolution near the start and end wavelengths of the measurements.

![Towards the optical axis of the focusing lens](image)

Figure 5-18: 2D CCD image showing coma aberration from a 1” achromat with a focal length of 75 mm.

![Spot diagram for a 1” achromat lens at wavelengths of (a) 742 nm and (b) 827 nm.](image)

Figure 5-19: Spot diagram for a 1” achromat lens at wavelengths of (a) 742 nm and (b) 827 nm.
Figure 5-19 shows the simulated beam shape at the focal plane of a 1” diameter, 75 mm focal length Thorlabs lens (AC254-075-B) at two different incident angles and an incident beam width of 15 mm. At an incident angle of 7.6° corresponding to the diffracted angle of the 742 nm wavelength, the coma aberration resulted in a spot size of about 400 µm. When the source is at normal incidence (λ = 827 nm), the spot size reduces to 8.4 µm.

To minimize coma aberration, a lens with a larger diameter can be used. This is to ensure that marginal rays do not pass near the lens edge thereby reducing coma aberration. Figure 5-20 shows the spot diagrams from a 2” lens with 75 mm focal length (AC508-075-B) at 2 different wavelengths. The coma aberration is observed to be reduced and the shape of the spot becomes more symmetrical. In addition, the spot size for the wavelength at 742 nm is also smaller compared to the 1” lens at 297 µm. However, the spot size at normal incidence is found to be larger at 52 µm due to the thicker optical elements.

Figure 5-20: Spot diagram for a 2” achromat lens at wavelengths of (a) 742 nm and (b) 827 nm.
Another method to minimize the coma aberration is to place an iris before the focusing lens to cut out stray light and to only allow a limited bundle of parallel rays for each incident angle to pass through. Figure 5-21 shows the effect of including an iris before the lens. Although coma aberration is significantly suppressed when an iris is used, the overall incident power on the image plane is also reduced. This solution is not desirable as the SNR will be compromised. Moreover, the introduction of a small aperture may increase the spot size of the diffraction limited beam which can degrade the resolution of the spectrometer.

![2D CCD images showing the calibration source spectral line spots (a) with and (b) without an iris placed before the focusing lens.](image)

Figure 5-21: 2D CCD images showing the calibration source spectral line spots (a) with and (b) without an iris placed before the focusing lens.

The two solutions discussed are not sufficient to tackle the coma aberration. The 2″ lens by itself gives a relatively large spot size of more than 10 µm while the placement of an iris to reduce the stray light will significantly reduce the incident power on the CCD. A compound lens design is proposed to solve this issue. In particular, two achromats can be placed next to one another to minimize coma aberration. Zemax simulation of the two lens system is performed to assess the degree of coma aberration.
and the final spot size. The simulation was setup with an incident beam diameter of 2 mm on the entrance pupil. The radial angles used are ±7.6° for the 742 and 932 nm wavelengths. Two 2″ achromats (AC508-150-B) were chosen to achieve an effective focal length of 70 mm. When the achromats are placed without any air gap between them, the effective focal length is approximately half the original focal length. Consequently two air-spaced achromat doublets of focal length 150 mm were chosen in the simulation and the resulting ray diagram is shown in Figure 5-22.

Figure 5-22: Ray diagram for two achromats (AC508-150-B) simulated with Zemax.

Figure 5-23: Spot diagrams of a (a) single 2″ achromat and (b) air-spaced 2″ achromat doublets simulated with a radial angles of 7.6° with respect to the optical axis.
From the simulated spot diagrams shown in Figure 5-23, the root mean square spot size is reduced by about 3 times (from 29.6 to 9.6 µm) when the air-spaced 2” doublets is used. Since the spherical aberration is no longer fully compensated in a compound system, the stop position is of utmost importance [165]. At a stop position of 70 mm, the coma aberration is almost removed. Appendix B quantifies the degree of coma aberration with different positions of the stop. With the improved spot size from reducing coma aberration, the achievable OCT imaging depth is observed to increase to at least 1.4 mm (see Figure 5-24) as measured with a mirror placed at that depth.
The sensitivity roll-off of the system is very much dependent on the spot size of the beam at the CCD and the pixel size of the CCD. In this system, the pixel size of the Basler CCD is 10 µm. With the improved coma aberration design, the spot size has been reduced to about 9.6 µm to match the pixel size of the Basler CCD. Due to the finite dimensions of the linear CCD pixel, the sensitivity rolls off with axial depth 4 dB lower at the Nyquist depth [45].

5-3 System Integration

The complete OCT system for in-vivo nasopharynx imaging implemented in this work is shown in Figure 5-25. The OCT light source used is a Ti: Sapphire laser from Femtolasers (center wavelength = 780 nm, bandwidth = 120 nm). The nasopharynx probe as described in section 5-2-1 is used for in vivo imaging. The output power at the distal end is 450 µW. The spectrometer comprises of a high-speed line scan camera (Basler L104K) and an efficient transmission grating (Wasatch Photonics) which is capable of line-rate of 29 kHz. Scanning of the sample for OCT imaging is provided by a galvanometer system (Physik Instrument PI-334). A computer was used to control the various instruments and collect data.
Figure 5-25: Schematic of the integrated OCT probe system for *in-vivo* nasopharynx imaging implemented in this work.

Figure 5-26: OCT image of a human finger obtained with the integrated OCT probe system.
Figure 5-26 and Figure 5-27 show the OCT image of a human finger and palm, respectively, obtained using the integrated OCT probe system. Due to the limitation of computing power, the 512 wavelengths profiles were first stored and processed later to construct the OCT image. The acquisition time for 1 frame is estimated to be 54 ms and the resulting 2D frame rate is 18 frames/s. The axial resolution of the image is about 4 µm and the lateral resolution is at least 30 µm with a lateral scan range of 1.1 mm. The usable imaging depth is found to be about 900 µm and the sensitivity is ~15 dB at this depth. Comparing to the design targets set out in Table 5-1, most of the parameters met the targets except for sensitivity and imaging depth. This can be attributed to the lower incident power (~450 µW) at the distal end of the probe. Despite these limitations, the presence of the sweat ducts can be clearly observed. The results demonstrated that salient features of the finger and palm could be imaged by the OCT system.
Two preliminary attempts were made to image the nasopharynx of two volunteers via the white light endoscopy path and the OCT path. In both attempts, the white light endoscopy did not manage to obtain any significant images despite the halogen lamp intensity being set to the maximum. This is in contrast with the standard rigid nasopharyngoscope which was very successful in picking up a clear image. The main reason for the lack of a bright image for the OCT nasopharynx probe is attributed to the limited field-of-view and the presence of an iris to improve the depth-of-view. In the case of the OCT signal, the trial measurement revealed that the uneven surface of the nasopharynx can result in very weak back-scattering and reflections. Consequently, no OCT image of the nasopharynx with significant SNR using the integrated OCT probe system was obtained. Further optimization and measurement of the OCT probe system with a larger nasopharynx sample size is required to investigate its potential use in early NPC diagnosis. Nonetheless this work established the knowledge base of rigid OCT nasopharyngeal probe design and identifies several important key areas for further optimization.

5-4 Recommendations

The limitations of the above probe design manifested with the lack of a good SNR OCT image. This was exacerbated by the lack of a brightfield image to “guide” the scanning of the relevant zones of the nasopharynx. One of the main issues for the existing design is that the brightfield image of the nasopharynx cannot be obtained. This was mainly due to a limited depth of field (~ 10 mm) in the imaging system.
Moreover, the field of view is fairly limited to the diameter of the GRIN rod (i.e. 2 mm). As a result, it is also challenging to guide the probe to the nasopharynx to obtain a reasonable OCT scan.

In conventional white light endoscopy used by the ENT clinicians, wide angle imaging was implemented to provide a field of view of about 60° (±30°). However, OCT imaging requires a focused beam to be laterally scanned at the sample. To achieve these two requirements, a single “scan” lens at the proximal end of the GRIN rod may not be sufficient. The imaging mode will only capture light within its entrance pupil diameter (2 mm). To circumvent these issues, variable focal lenses are proposed to be placed at the distal end. Electro-wetting technologies to adjust the curvature (and therefore focal lengths) can be utilized to achieve both requirements of the wide angle lens and scanning lens [166].

The proposed system will have two modes: imaging and OCT scanning modes. The system will alternate between both modes depending on the intended application. For instance, during the insertion of the probe through the nose, the probe will be in imaging mode while the OCT path is “switched” off. This allows the clinician to observe the surrounding tissue. When the probe is near the nasopharynx, the liquid lenses change their curvature to allow for OCT lateral scans. If the speed of curvature change is fast enough (e.g. > 30 Hz), the brightfield and OCT scanning be alternated so that clinician can identify and record the location of OCT scans. Two liquid lenses are needed to achieve both requirements. If there is only one lens, it does not provide enough surfaces to direct the light into the GRIN rod under the imaging mode.
A ray-tracing simulation of the proposed design was conducted using the Zemax software where the radii of curvature of the lens surfaces were optimized to provide both modes. In the imaging mode, the magnification is about -0.14 with the object at 10 mm from the distal end. The effective focal length is 1.733 mm with an equivalent F number of 6.9. The viewing angle at 10 mm object distance is +/- 30°. In the OCT scanning mode, the effective focal length adjusted to 21.5 mm with a F number of 21.4 yielding a lateral spot size of 9 µm. The lateral scan range is +/- 0.75 mm.

One of the main limitations of the proposed design is the actual diameter of a commercial available liquid lens is about 8 mm with an open aperture of only 2.25 mm. The reduction in usable aperture is due to the presence of electrical circuits and connections to control the surface tension of the lenses. To incorporate the liquid lens into a probe, the final diameter has to be even larger than 8 mm. Such large diameters may not feasible for nasopharynx application. However, it is highly possible that the size of these liquid lenses can be reduced further in the near future to be used in biomedical applications in small orifices.

5-5 Summary

This chapter described the design of a nasopharyngeal OCT probe and a high-speed spectrometer for in-vivo imaging. The key design considerations in the probe included the incorporation of a white-light imaging path, fast 2D scanning capability and good dispersion, polarization matches between the reference and sample arms into an
existing nasopharyngoscope. The white-light imaging path had a depth-of-field of about 7.7 mm obtained by adjusting entrance pupil diameter of the system. With good dispersion balance between the reference and sample arms, the axial resolution was about 4 \( \mu \)m which was close to the theoretical limit. For the high speed spectrometer, the key design considerations include good spectral resolution, reasonable spectral range and good SNR. A frame rate of 18 frames per second was achieved. A spectral range of 190 nm resulted in an axial imaging depth of at least 1.4 mm. Ex-vivo images of a finger and palm with good resolution and imaging speed were successfully obtained.

Following this work, additional OCT endoscopy has been proposed. In particular, to address the issue of large field of view for endoscopy and OCT scanning, Burkhardt et al has proposed a fan shape scanning pattern for the OCT beam [167]. For laryngeal imaging, Cernat et al has recently implemented a dual instrument for in vivo and ex vivo imaging based on a single 1300 nm swept source [168]. For in vivo imaging, an endoscope probe unit incorporates a fiber bundle for endoscopic viewing and rigid GRIN probe of 1.9 mm for OCT imaging. A magnetic scanner was implemented for OCT to provide scanning in the lateral direction. A very similar concept to the proposed design described in section 5-4 has been simulated by Choi et al [169]. In their proposed design, the focal length of the lenses could be adjusted via microelectrofluidics resulting in three different imaging modes (OCT, OCM and optical depth scanning, ODS). To address the need for high-speed lateral scanning, a resonant scanning cantilever design was implemented by Huo et al [170]. Their
proposed design achieved scan rates of 62.5 Hz which is faster than piezo-electric scanners or magnetic scanners used in miniature probes.
6-1 Introduction

One key advantage of OCT systems is that the axial resolution, defined by the FWHM of the axial point spread function (PSF), does not depend on the imaging optics. Under non-dispersive conditions, the axial PSF of the OCT system is simply given by the inverse Fourier transform of the light source spectrum. The axial resolution in this case will be the theoretical minimum and is transform limited. Therefore in a well-designed system, the axial resolution depends only on the bandwidth and center wavelength of the light source. However, the measured axial resolution often deviates from the theoretical value. One of the common causes for the degradation in the axial resolution is dispersion mismatches between the two arms in the OCT interferometer. A dispersive material in the OCT path will result in a wider axial PSF and a poorer resolution which will degrade image quality.

In the previous chapter, the use of a GRIN rod in the sample arm introduced severe dispersion mismatch thereby broadening the axial point spread function. The issue can be mitigated by placing an equivalent GRIN rod in the reference arm thereby reducing the mismatch. However, alternative methods are investigated here to minimize the complexity of the reference arm.
The speed of light in dispersive materials is dependent on the wavelength of the source. Dispersion can be broadly classified into chromatic and waveguide dispersion. In chromatic dispersion, light travels at different speed in a medium due to differences in the refractive index that varies with wavelength. For waveguide dispersion, the effective speed of light in a waveguide (e.g. optical fiber) varies with wavelength as well. In optical fibers, the mode field diameter (MFD), which describes the energy distribution between core and cladding, is a function of wavelength (higher wavelengths have larger MFDs). The effective refractive index then depends on this distribution.

The incident light from an OCT system is highly susceptible to dispersion effects since its light source is generally broadband. Compared to other modalities such as two-photon imaging, the dispersion effects in OCT measurements depend only on the relative mismatch between both arms of the interferometer. If the dispersions on both arms are matched, the absolute dispersion on either arm does not matter. This is not so for modalities like two-photon imaging which require extremely low dispersion to function optimally. Consequently the goal in OCT dispersion management is to match both the reference and sample arms. For applications in ophthalmology, the dispersive medium is generally the water (aqueous vitreous humor) in the eye. This can be compensated by placing an equivalent amount of water or dispersive material, such as BK7 glass, in the optical path to match the dispersion introduced by water in the sample [171]. As a result, light in both sample and reference arms pass through the same optical path length. Another approach to OCT dispersion management is to cancel out the dispersion on the sample arm itself. This will be similar to the technique
used in *in-vivo* two-photon excitation microscopy where a pulse compressor is placed before the *in-vivo* probe to introduce negative dispersion. The input pulse from the mode-locked laser will be compressed (negatively dispersed) prior to launching into the probe. At the focal point of the probe end, the pulse width can be restored to its theoretical minimum and become transform-limited.

In OCT bio-imaging applications, the biological sample may have complex optical properties. It may not be convenient or easy to place a similar material in the sample arm to compensate for dispersion mismatches. Furthermore, normal dispersive materials will only provide positive group velocity dispersion (GVD). An adjustable dispersive optical device may be needed to compensate the dispersion under different imaging conditions in the sample. One suitable device is the rapid scanning optical delay line (RSOD) [172]. RSODs are used in the early days of time-domain OCT for rapidly translating the group delay in the reference arm [54, 160]. The grating and mirror are positioned such that they are around one focal length on either side of the focusing lens. By adjusting the positions of the focusing lens and mirror relative to the grating, positive or negative GVD can be achieved [173]. This is especially useful when there are systemic dispersion mismatches that can either be positive or negative. However, the drawback of RSOD is that the higher order dispersions such as third order dispersion (TOD) compensation cannot be adjusted for both positive and negative values. The RSOD has only positive TOD regardless of the amount of adjustment [174].
Dispersion compensation can also be achieved through numerical methods [154, 175]. The main advantage in utilizing this method is its versatility as it can be applied to different biological samples. Moreover, numerical methods can also compensate for higher order dispersions. With the availability of fast, powerful and cheap computers, more complex algorithms can be dynamically applied to the OCT signal providing real-time, sharp and high-resolution images.

This chapter therefore investigates the optimization of the OCT axial PSF to mitigate the effects of dispersion using the method proposed by Wojtkowski et al. [154]. Numerical dispersion compensation on a system with a GRIN rod is performed and the resulting improvement in image quality is evaluated. The amount of GVD and TOD of the GRIN rod is also estimated based on the amount of the compensation and compared to simulation. In addition, a common path probe designed to mitigate dispersion mismatches in the OCT system is also proposed and studied.

6-2 Mathematical Description of Dispersion in OCT

The intensity of an OCT axial profile with a single reflecting surface at the sample can be obtained by performing an inverse Fourier transform on the intensity spectrum as

\[
I(z) = \mathcal{F}^{-1}[I(k)] = 3^{-1/2} \left[ |E_{\text{sample}}| \exp(j\phi_1) |^2 + |E_{\text{ref}}| \exp(j\phi_2) |^2 + 2E_{\text{sample}}E_{\text{ref}} \exp(j(\phi_1 - \phi_2)) \right]^{1/2}
\]

(6-1)

where \(\phi_1\) and \(\phi_2\) represent the phases of the sample and reference E-fields, respectively [47, 49, 51]. The first two terms of equation (6-1) represents the intensities from the sample and reference arms. In the absence of interference, the final intensity will just
be the sum of these two intensities. These terms will form the DC components in the inverse Fourier transform expression and does not provide useful information about the optical structure of the sample. The third term provides information about the relative phases between the sample and reference arms, allowing the optical structure of the sample to be determined.

Consider a system with a dispersive material in the sample arm and no dispersion management optics in the reference arm. The resulting OCT signal in $k$-space for a single reflecting surface can be written as

$$I(k) = \left( E_{\text{ref}} \exp(jk_{\text{ref}}d_1) + E_{\text{sample}} \exp(jk_{\text{sample}}d_2) \right)^2$$

$$= d.c. \text{ terms} + E_{\text{ref}} E_{\text{sample}} \exp[j(k_{\text{ref}}d_1 - k_{\text{sample}}d_2) + E_{\text{ref}} E_{\text{sample}} \exp[-j(k_{\text{ref}}d_1 - k_{\text{sample}}d_2)]$$

$$= d.c. \text{ terms} + E_{\text{ref}} E_{\text{sample}} \exp[j\phi(\omega) + \exp[-j\phi(\omega)]$$

where $\phi(\omega) = k_{\text{ref}}d_1 - k_{\text{sample}}d_2$, and $d_1$ and $d_2$ are the optical pathlengths of the reference and sample fields, respectively. For a dispersive material such the silica used in optical fibers, the wavenumber $k$ is a function of wavelength or angular frequency [34, 176]. In broadband sources, the wavenumber $k(\omega)$ can be expanded using Taylor’s expansion around the center angular frequency of a broadband source $\omega_0$ to give [177]

$$k(\omega)_{\text{broad}} = k_0 + \left. \frac{dk}{d\omega} \right|_{\omega = \omega_0} (\omega - \omega_0) + \left. \frac{1}{2} \frac{d^2k}{d\omega^2} \right|_{\omega = \omega_0} (\omega - \omega_0)^2 + \ldots$$

(6-3)
The first term represents the center wavenumber \( k_0 = \frac{2\pi}{\lambda_0} \). The second term
\[
\frac{dk}{d\omega} \bigg|_{\omega=\omega_0} = \frac{1}{\nu_g} - \frac{n(\omega_0)}{c} + \frac{\alpha_0 n'(\omega_0)}{c}
\]
represents the inverse of the group velocity with which all wavelengths appear to travel. The third term is the GVD or second order dispersion.

The OCT axial profile can be obtained by taking the inverse Fourier transform of the spectrum. For non-dispersive materials on both the sample and reference arms, the axial profile for a single reflecting sample surface corresponds to a shifted inverse Fourier transform of the light source spectrum. For a Gaussian spectrum with a center angular frequency of \( \omega_0 \) and a standard deviation of \( \sigma_\omega \) such that
\[
I(\omega) = I_0 \exp \left[ -\frac{(\omega-\omega_0)^2}{2\sigma_\omega^2} \right],
\]
the axial PSF will also have a Gaussian profile with standard deviation of \( \sigma_z \) and \( I(z) \propto \exp \left[ -\frac{(z)^2}{2\sigma_z^2} \right] \), where \( \sigma_z = c\sigma_t \) and \( \sigma_\omega \sigma_z = 1 \) [3].

The 3-dB bandwidths of the Gaussian spectrum and axial PSF are \( \Delta \omega = 2\sigma_\omega \sqrt{2\ln 2} \) and \( \Delta z = 2\sigma_z \sqrt{2\ln 2} \) respectively.

Re-writing equation (6-1) to consider angular frequency and time delay yields
\[
I(\tau) = \mathcal{F}^{-1}\{I(\omega)\} = \int I(\omega) \exp(j\omega\tau) d\omega = \int \left[ \text{DC terms} + I_0(\omega) \exp\left(jk_{ref}d_1 - jk_{sample}d_2\right) \right] \exp(j\omega\tau) d\omega = a\mathcal{F}^{-1}(I_{source}) + \text{(interference terms)} \tag{6-4}
\]
where the interference terms can be expanded as [154]:

\[
\int \exp \left[ j \left( a_{\tau} \frac{\omega}{v_x} + \frac{1}{c} (\omega - a_0) d_z - \frac{1}{2} (\omega - a_0)^2 d_t \frac{d^2 k}{d\omega^2} \right) \right] \exp(j\omega t) d\omega \\
= \exp \left( j \left( a_{\tau} \frac{\omega}{v_x} + \frac{1}{c} (\omega - a_0) d_z - \frac{1}{2} (\omega - a_0)^2 d_t \frac{d^2 k}{d\omega^2} \right) \right) \int \exp(j\omega t) d\omega \\
= \exp \left( j \left( a_{\tau} \frac{\omega}{v_x} + \frac{1}{c} (\omega - a_0) d_z - \frac{1}{2} (\omega - a_0)^2 d_t \frac{d^2 k}{d\omega^2} \right) \right) \int \exp(j\omega t) d\omega \\
= \exp \left( j \left( a_{\tau} \frac{\omega}{v_x} + \frac{1}{c} (\omega - a_0) d_z - \frac{1}{2} (\omega - a_0)^2 d_t \frac{d^2 k}{d\omega^2} \right) \right) \int \exp(j\omega t) d\omega
\]

(6-5)

Without loss of generality, a source with Gaussian spectrum is assumed, the optical path difference can be reduced to zero thereby eliminating the \(ji(\omega')\Delta t\) term, and setting \(\omega_0 = 0\) to simplify the integration. The infinite integral of the interference term is now

\[
\int \exp \left[ -\left( \frac{\omega^2}{2\sigma_\omega^2} + \frac{j \omega^2}{2} \frac{d^2 k}{d\omega^2} \right) d\omega + j\omega \tau \right] d\omega \\
= \int \exp \left[ -\left( \frac{1}{2\sigma_\omega^2} \right) \left( \omega^2 - \frac{j 2\sigma_\omega^2 \omega \tau}{1 + j 2\sigma_\omega^2 \omega \tau} \right) \right] d\omega \\
= \exp \left( -\frac{\sigma_\omega^2}{2(1 + j 2\sigma_\omega^2 \omega_0)^2} \right) \int \exp \left[ -\left( \frac{1 + j 2\sigma_\omega^2 \omega_0}{2\sigma_\omega^2} \right) y^2 \right] dy \\
= \exp \left( -\frac{(1 + j 2\sigma_\omega^2 \omega_0)^2}{2 \left( 1 + 4\sigma_\omega^4 \omega_0^2 \right)} \right)
\]

where \(x_0 = \frac{1}{2} \frac{d^2 k}{d\omega^2} d_z\) and \(y = \omega - \frac{j \sigma_\omega^2}{1 + j 2\sigma_\omega^2 \omega_0} \tau\). The axial PSF is obtained by taking

the magnitude of the Gaussian profile which reduces to
Thus, the effect of group velocity dispersion term broadens the axial PSF such that the new standard deviation of a Gaussian PSF becomes

\[
\sigma^* = \sigma + \left[1 + \frac{1}{2} \omega^2 \frac{d^2 k}{d\omega^2} d^2_2 \right]^4
\]

where \( \sigma_i \) is the original standard deviation of the PSF [3]. To minimize dispersion effects so that the theoretical minimum axial resolution can be achieved, the higher order dispersion terms should be zero.

In OCT systems, this implies that any dispersive terms, i.e. \( d \frac{d^2 k}{d\omega^2} \bigg|_{\omega=\omega_0} \), in one of the arms have to be cancelled out by a similar amount of dispersion in the other arm. In methods using a physical medium to compensate dispersion, the material and thickness have to be selected such that the dispersion is the same. With the RSOD approach [173], this is achieved by adjusting the value of \( \frac{d^2 k}{d\omega^2} \bigg|_{\omega=\omega_0} \) and distance \( d_2 \).

This method allows more freedom to compensate for dispersion. With numerical methods, the systemic dispersion can be calibrated by finding the impulse function using a single reflecting surface in one of the arms. Since the reflecting surface will theoretically produce the sharpest PSF, the additional dispersion which broadens the
PSF can be computed. In addition, the numerical dispersion method proposed by Wojtkowski et al. [154] attempts to maximize the sharpness metric which is a function of the axial resolution of the system.

6-3 Dispersion Compensation in Spectral Domain OCT

6-3-1 Proposed Method

To minimize the GVD or higher orders of dispersion, the spectrum for a single reflecting surface should only contain the first order term. Fourier transforming such spectra will result in a PSF that is shifted by an amount equal to the group delay

\[ I(z - z_i) \text{ where } z_i = c \times \left( \frac{d_1}{c} - \frac{d_2}{v_g} \right) \]  

In spectral domain OCT, this can be achieved by multiplying a phase term

\[
I(k) \exp \left\{ -j \left[ (\omega - \omega_h)^2 x_2 + (\omega - \omega_h)^3 x_3 + \ldots \right] \right\} \quad (6-9)
\]

\[
I(k) \times \exp \left\{ -j \left[ (\omega - \omega_h)^2 x_2 + (\omega - \omega_h)^3 x_3 + \ldots \right] \right\}
\]

to negate any higher order terms where

\[
x_2 = \frac{1}{2} d_2 \frac{d^2 k_{\text{sample}}}{d \omega^2} \bigg|_{\omega=\omega_h}
\]

\[
x_3 = \frac{1}{6} d_2 \frac{d^3 k_{\text{sample}}}{d \omega^3} \bigg|_{\omega=\omega_h}
\]

and

\[
x_n = \frac{1}{n!} d_2 \frac{d^n k_{\text{sample}}}{d \omega^n} \bigg|_{\omega=\omega_h} \quad \text{[154]}
\]

The original amount of dispersion has to be first determined before the additional dispersion can be compensated. Physically, this can be done by using an object with a single reflecting surface such as a mirror in the both arms. In the probe-based OCT system, the main objective is to cancel out the dispersion in the GRIN rod of the
sample arm if there is no equivalent GRIN rod in the reference arm. Hence the first step is to determine the amount of dispersion that the GRIN rod introduces to the system. The procedure to do this is summarized below:

a) Record the sample $I_{\text{sample}}(\lambda)$ and reference $I_{\text{ref}}(\lambda)$ spectrum separately.

b) Subtract the reference and sample spectrum from the spectrum to obtain the AC spectrum $I_{\text{ac}}(\lambda) = I_{\text{total}}(\lambda) - I_{\text{sample}} - I_{\text{ref}}$. In typical OCT signals, the sample spectrum is much weaker than the reference spectrum. However, to determine the amount of dispersion in the GRIN rod, a highly reflective mirror is recommended. This makes the sample spectrum comparable to the reference sample in terms of power. A DC free spectrum is necessary to obtain the analytic signal via the Hilbert transform.

c) A high pass filter may have to be applied if the AC spectrum contains a small DC term.

d) Hilbert transform is performed on the final spectrum to obtain the analytic signal. In the polar form, both the magnitude and phase of the spectrum can be obtained. In a dispersive medium, the phase will contain higher order terms.

e) The phase can be fitted to an $n^{th}$ degree polynomial to obtain their respective coefficients. In most cases, a $3^{rd}$ degree polynomial is sufficient to fit the phase function.

In many FD-OCT systems, the wavenumber domain is scaled to account for the double-pass of the optical path (i.e. $K=2k$). This has the advantage for inverse Fourier transforming the data such that it will yield only the actual optical path differences
(OPD) between each reflecting surface in the reference arm rather than the double-passed OPD. The extracted phase term is also scaled with respect to $K$ in the above algorithm.

The phase term is generally defined mathematically in angular frequency, i.e. $\phi(\omega)$. The phase equation expanded about $\omega_0$ is

$$\phi(\omega) = \phi_0 + \frac{dK}{d\omega}_{|\omega=\omega_0} (\omega - \omega_0)d + \frac{1}{2} \frac{d^2K}{d\omega^2}_{|\omega=\omega_0} (\omega - \omega_0)^2d + \ldots \quad (6-10)$$

where $K = 2k = \frac{2\omega}{c}$. Due to large absolute values of the angular frequencies in the optical region, the fitting of the polynomial may be ill-conditioned. To improve the numerical stability during the curve fitting process, the phase is scaled prior to the curve fitting process as follows:

$$\phi(K) = a_0 + a_1 \left( \frac{K - K_0}{10^4} \right) + a_2 \left( \frac{K - K_0}{10^4} \right)^2 + a_3 \left( \frac{K - K_0}{10^4} \right)^3 + \ldots \quad (6-11)$$

The fitted coefficients of the polynomial are

$$a_1 = c \times 10^4 \times 1 \times d \times \left. \frac{dK}{d\omega} \right|_{\omega=\omega_0} \quad \left. \frac{dK}{d\omega} \right|_{\omega=\omega_0}$$

$$a_2 = c^2 \times \left( 10^4 \right)^2 \times \frac{1}{4} \times d \times \left. \frac{d^2K}{d\omega^2} \right|_{\omega=\omega_0} \quad \left. \frac{d^2K}{d\omega^2} \right|_{\omega=\omega_0}$$

$$\vdots \quad \left. \frac{d^2K}{d\omega^2} \right|_{\omega=\omega_0} \quad (6-12)$$
Once the dispersion of the system is known, they can be used to cancel out the dispersion in subsequent OCT images. Essentially, the spectra of other images are first converted to an analytic signal which will be multiplied with the negation of the dispersion terms.

6-3-2 Results and Discussion

The OCT system with a Ti:Sapphire laser source (Femtolasers) of center wavelength 782 nm and a bandwidth of ~100 nm has a theoretical axial resolution of 2.7 \( \mu \)m. A 2×2 fiber coupler splits the input light into both the sample and reference arms. The interferometer system is configured such that the sample arm contains the GRIN rod (GRINTECH, 2 pitch, 203.73 mm) and the beam-steering optics, while the reference arm contains just a scan lens. A mirror is placed after the optical elements in each arm. The reflected spectrum from both paths are combined at the 2×2 fiber coupler and collected using the HR4000 spectrometer (Ocean Optics). The cross-correlation function obtained from the measurement is shown in Figure 6-1. Due to the huge amounts of dispersion in the GRIN rod, the axial PSF is observed to be extremely wide, spanning almost 2.5 mm.
Figure 6-1: The (a) cross-correlation function, (b) its corresponding spectrum and the (c) extracted phase plot of the OCT system.
The extracted phase of the spectrum was used to analyze the amount of dispersion. Ideally, the phase difference curve should just be a horizontal line with perfect dispersion compensation. Fitting a third order polynomial to Figure 6-1c yields $a_2 = -0.04381$ and $a_3 = 7.937 \times 10^{-6}$. Comparing the coefficients with the dispersion terms yield

$$\frac{1}{2} d_0 \frac{d^2 k}{d \omega^2} = -9.736 \text{ fs}^2$$

and

$$\frac{1}{6} d_0 \frac{d^3 k}{d \omega^3} = 1175 \text{ fs}^3.$$  

The corresponding values of GVD and TOD are given by

$$\text{GVD} = d_0 \frac{d^2 k}{d \omega^2} \bigg|_{\omega = \omega_b} = -19,472 \text{ fs}^2$$

$$\text{TOD} = d_0 \frac{d^3 k}{d \omega^3} \bigg|_{\omega = \omega_b} = 7,050 \text{ fs}^3$$

These numbers are compared against the gradient index properties in the form of Zemax glass catalog from GRINTECH. The GRIN rod has the following refractive and dispersion profiles:

$$n_2 = n_0 - 3.034 \times 10^{-3} r^2 - 4.783 \times 10^{-6} r^4$$

$$n^2(\lambda) = n^2(\lambda_{ref}) + \frac{K_1(\lambda^2 - \lambda_{ref}^2)}{\lambda^2}$$

(6-13)

where $K_1 = -0.051084309$ and $\lambda_{ref} = 0.656$. Computing the dispersion coefficients based on these parameters yield the following simulated values:

$$\text{GVD} = -18,830 \text{ fs}^2$$

$$\text{TOD} = 7,629 \text{ fs}^3$$

The discrepancies between the simulated and curve-fitted values of GVD and TOD are less than 8%. The preceding computation assumes that material dispersion dominates over the intermodal dispersion in the GRIN rod. The pulse broadening in graded index fibers due to intermodal dispersion can be defined as
\[ \tau_m = \frac{n_2 L}{2c} \left( \frac{n_0 - n_2}{n_2} \right)^2 \]  

(6-14)

where \( n_0 = 1.600 \) and \( n_2 = 1.59696 \). Thus, the pulse will be broadened by about 1.96 fs.

Given that the theoretical pulse width of the auto-correlation function or axial PSF is about 9 fs, the contribution of pulse broadening from intermodal dispersion is negligible at the relatively short GRIN rod length.

Figure 6-2: Axial PSF after numerical dispersion compensation.

Figure 6-3: OCT images of a mirror (a) before and (b) after phase correction.
Figure 6-2 shows the axial PSF after numerical dispersion compensation up to the third order. The FWHM of the PSF is found to be ~5 µm. The deviation from the theoretical minimum could be attributed to the non-Gaussian shaped spectrum and polarization mismatches. Figure 6-3 shows an OCT image of the single reflecting surface of a dielectric mirror. The post-process signal (Figure 6-3b) shows an improved SNR in addition to a better resolution.

Figure 6-4: OCT images of an onion with (a) no dispersion compensation, (b) numerical dispersion compensation only, (c) physical dispersion compensation only, and (d) both numerical and physical dispersion compensations.
To further evaluate the effectiveness of the numerical dispersion technique, physical dispersion compensation was also implemented in the reference arm. Three glass plates and a vertically-placed linear polarizer were inserted in the reference arm. Figure 6-4 depicts the OCT images of an onion under different modes of dispersion compensation. The images showed that numerical dispersion compensation is equally effective compared to physical compensation. When both methods are applied, the onion image appears to be the sharpest. While this is the most ideal situation, it may not be practical or economical to insert many optical elements in the reference arms just to compensate the dispersion mismatch.

This section has demonstrated that serious dispersion mismatches between the sample and reference arms can significantly distort the axial PSF. With proper dispersion compensation, the OCT image has better SNR and quality. Numerical dispersion through optical phase manipulation can correct systemic dispersion. The OCT images after applying numerical dispersion compensation exhibit a better SNR compared to that with simple physical dispersion compensation. The ability of numerical method can be applied to different OCT configurations, such as GRIN rod and catheter probes, making the technique very flexible.

6-4 Common-Path Axicon Fiber Probe

In the OCT rigid probe system presented in chapter 5, there is a need to match both dispersion and polarization between the reference and sample arms to optimize the

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axial PSF. While dispersion can be numerically compensated, it is more difficult to correct polarization mismatches. One disadvantage of the numerical dispersion method is that the compensation may not be numerically stable when the dispersion becomes very significant. In the earlier section, the fitting of the phase was subjected a scaling factor of $10^4$ to mitigate the issue with ill-conditioned matrices. In addition, the extraction of the phase through Hilbert transform requires the signal to be relatively noise-free. A low signal with low SNR will present inaccuracies in the determination of the phase and its subsequent compensation. When the probe was tested in the clinical setting, it is important to minimize any bends to the sample arm fibers. Since the SM600 fiber has a very thin core to maintain single mode operation at a wavelength of 800 nm, it is very susceptible to polarization changes when the fiber bends. Polarization changes in the sample arm will result in significant polarization mismatches as the reference arm is generally fixed in position. This manifests as a reduction in the SNR of the OCT image. To circumvent these issues, a common-path OCT system is proposed where the dispersion and polarization mismatches can be minimized. In a common-path system, both the sample and reference fields travel along the same path back to the interferometer. As a result, both fields experience the same degree of dispersion and polarization changes. Since the interference signals subtracts the phase between the sample and reference arms, these dispersion changes are cancelled out and the spectrometer records only the sample information. The common-path system is therefore highly tolerant of any fluctuations in the optical paths such as fiber bending and localized temperature variations which affects the refractive indices. The axial resolution thus approaches its theoretical limit under zero-dispersion condition.
In the original rigid probe design, the window face at the distal end is flat, i.e. at normal incidence to the scanning beam. The magnitude of the Fresnel reflections from the window face at the distal tip caused unwanted artifacts which manifested as horizontal solid lines across the OCT image. The location of these lines depends on the axial position of the mirror in the reference arm. In the worst scenario, these lines may overlap with the sample data thereby reducing the image quality. This issue was circumvented by using an angled probe design such that the unwanted Fresnel reflections from the window face were not collected by GRIN rod. With this change, the artifacts were removed. However, for a common-path system, the original flat window design is actually more desirable as the window face can provide a suitable common-path reference plane. Despite this advantage, this common-path design was not adopted. First, the presence of the scan lens at the proximal end resulted in a converging beam out of the GRIN rod. As the focus spot is outside the probe and the flat window, the Fresnel reflection from the window to be collected by the GRIN rod is relatively weak. This is not sufficient to provide the heterodyne gain to amplify the weak signals from the sample arm.

To address the problem of a weak reflection from the flat window and the need for an effective common-path reference field, a new scheme that uses a conical microlens was investigated. Conical lens has been proposed to be used in OCT [178, 179] where an extended focus range can be achieved. However, they rely on bulk optics which can be challenging to incorporate into small probes. For small probes, a large area photonic crystal fiber (PCF) coupled with a fused silica lens has been found to be able to focus the incident beam at the sample with a transverse resolution of 14.2 μm and a
working distance of 1.2 mm [180]. Following this work, a longer working distance of 5.2 mm were demonstrated using a common path design based on a TO-CAP with a ball lens [181]. The incorporation of a conical lens in a fiber can potentially overcome the limitation of the varying transverse resolution along the axial depth, and at the same time make it suitable to be placed in small probes or catheters. In this work, a microlens is chemically etched in the core of the fiber tip. The advantage of a monolithic conical microlens lies not only in its simple fabrication, but also its ability to generate the back reflected reference signal and focusing the incident light for imaging without the requirement of additional optical elements. Furthermore, a high coupling efficiency can be achieved since the conical microlens is already an integral part of the fiber core and there is no additional interface medium (air-glass) between the microlens and the fiber end. The conical-tipped fiber probe is fabricated via a straightforward single-step chemical etching technique [182] that is both time and cost efficient. Moreover, the choice of different chemical concentrations for the etching solution facilitates tunability of the cone angle which in turn modifies both the lateral resolution and imaging depth.

The beam generated from the conical-tipped fiber follows the form of pseudo-Bessel beam shape in free space [33]. Such an output from the conical-tipped fiber yields a narrowly focused beam with relatively low divergence [183-185]. The low divergence property of Bessel beams results in a relatively constant lateral resolution over a large depth [186]. This property can even be maintained when the incident beam is scattered upon entering the sample [187]. On the other hand, the conventional focused Gaussian beam has a larger divergence especially when high NA lens are used which results in
non-uniform lateral resolution over large depths. Alternatively, a low NA lens has to be used in the sample arms such that the divergence is minimized. One disadvantage of Bessel beams is that the illumination power is not concentrated at the imaging volume as the surrounding rings of the Bessel beam contains significant fractions of the incident power [187]. This may result in weaker back-scattering or back-reflection from the sample which affects the SNR of the OCT image.

6-4-1 Simulation and Fabrication

The in-fiber common path technique works by using the light field that is being reflected back from the glass/air interface at the end of the fiber. This reflection can be used as a reference field provided there is sufficient light being coupled back into the fiber core [188]. To verify this effect, a finite-element software (COMSOL) [33] was used to create a 2-D and a 3-D model of the conical-tip fiber. The 3-D model is used to calculate the global reflectivity coefficient of the fiber including polarization and geometry effects while the 2-D model, which is more robust and less computational intensive, is used to simulate the total tip-sample interaction. Both models consist of a single mode optical fiber and a conical microlens at one end, which has a base width of 8.2 µm (fiber core size), and a 128° cone apex angle and 340 nm radius of curvature curved tip as measured from the SEM image. The fiber eigenmode was used as input to the fiber and the reflection coefficient was determined by calculating the coupling efficiency of the back-reflected field to this fiber mode.

From the modeling results, most of the back-reflected light field from the conical tip did not couple back into the fiber core. This behavior is due to the fact that the angle between the base and the conical side is less than the critical angle for total internal
reflection (TIR) between the glass and air, which is \( \sim 43^\circ \) based on fiber core refractive index of \( n_{\text{core}} = 1.467 \) [183]. As with any convex lens that has no anti-reflection coating, light propagating towards the centre of the lens would be partially reflected back. The model estimates that approximately 0.35% of the light incident on the tip will be reflected and guided for the fiber used in the experiments at the operating wavelength of 1320 nm. The reference intensity of the conical-tip fiber can also be adjusted by coating the tip with a thin layer of gold. With an additional 5 nm thick gold layer, 3-D modeling indicates that the reflectivity from the conical tip can be increased from 0.35% to 0.55%.

At the shot noise limit, the sensitivity \( \frac{\rho \eta T}{\hbar} P_0 \gamma_s \) of the OCT system depends on the reflected power from the sample, \( \gamma_s \). The reflected power is a function of the transmittivity, illumination and collection efficiency which depends on the cone or apex angle of the conical tip. Qualitatively, the transmittivity, illumination and collection efficiency decreases with decrease in cone angle at the far field [184].

The lowest cone angle supported by the axicon occurs when total internal reflection is achieved where the angle between the base and the side of cone exceeds the critical angle. As such, \( \gamma_s \) decreases monotonically with decrease in cone angle. This implies that the optimization of the cone angle lies on achieving a suitable working distance and lateral resolution instead of sensitivity. The choice of a 128° cone angle attempts to match the lateral resolution over the working distance with the axial resolution of the system.
A buffered hydrofluoric acid (BHF) solution consisting of a volume ratio X:1 of ammonium fluoride solution (NH$_4$F) (40 wt %) : hydrofluoric (HF) acid (50 wt %) is used in the selective chemical etching technique. In the BHF solution, the concentration of NH$_4$F (denoted as X) controls the etch rate of the pure SiO$_2$ fiber cladding and the GeO$_2$-doped SiO$_2$ fiber core [11]. A difference in the etch rate, due to different concentrations (X), between the fiber cladding and fiber core will result in different cone angles. A conical-tip fiber using a 2:1 BHF solution yielding a cone angle of 128° was then fabricated. Using a mirror as a sample and a superluminescent diode (SLD) (DL-BX9-CS3089A, Denselight Semiconductors) with a center wavelength of 1320 nm and spectra width of 70 nm as a source, the measured axial resolution was approximately 12.1 µm. This is 10% larger than the theoretically predicted value of ~11 µm. The axial resolution was measured without any requirement for GVD and polarization compensations. The reflected power from the conical-tip fiber end, which was measured by replacing the spectrometer in the experimental setup with a photo-detector and removing the sample stage, was approximately 0.46%. The result is of the same order of magnitude as the modeled result of 0.35%.

6-4-2 Experimental Setup

The experimental setup of the common-path FD-OCT setup is shown in Figure 6-5. A circulator (OE-3-1310SFC-09-1, O/E Land Inc.) is used instead of a 2×2 coupler so that most of the incident light is channeled to the sample, thereby minimizing optical losses. A commercial spectrometer (BTC261E, B&W Tek, Inc), which incorporates a 16-bit 512 pixel TE-cooled InGaAs linear array detector with a 600 lines/mm grating
and a 25 µm slit width, was used for detection. The measured dynamic range of the system is approximately 80 dB. The optical power incident on the sample from the conical-tip fiber probe was measured to be approximately 5 mW using a photodetector (PDA10CS, Thorlabs Inc.). The motorized translation stage (KT-LS13-M, Zaber Technologies Inc.) provides the lateral scanning for the B-scan operation. A custom program was written with Labview (National Instruments) to operate and collect data from this experimental OCT system.

Figure 6-5: Schematic of the in-fiber OCT setup incorporating the conical-tip fiber probe.

6-4-3 Results and Discussion

Figure 6-6 shows the measured SNR and the axial resolution as a function of axial distance between the 128° conical-tip fiber end and mirror. The SNR was obtained by taking the ratio of the peak signal of the mirror surface, after inverse Fourier transform, and the noise floor. From these measurements it can be seen that an SNR of >30 dB is achievable at an axial distance of 0.5 mm from the fiber tip, demonstrating the extended imaging depth achievable with this system. The lower curve in Figure 6-6
also shows that the measured axial resolution of the system varies between 12–16 µm over the axial distance range. The lateral resolution of the 128° conical-tip fiber was measured using an USAF resolution target card (NT58-198, Edmund Optics). A cleaved fiber, using the same experimental setup shown in Figure 6-5, was used for comparison. Figure 6-7 presents the lateral plot from group 6, element E2 (width of each line = 6.96 µm) to E6 (width of each line = 4.4 µm) of a single OCT scan data performed with the 128° conical-tip fiber and the cleaved fiber. The results showed that the conical-tip fiber is able to clearly resolve the lines in element E6 whereas those from the cleaved fiber are barely resolvable.

Figure 6-6: Measured signal to noise ratio and axial resolution with the 128° conical-tip fiber for different axial position between the fiber tip and mirror.
Figure 6-7: Lateral plot from USAF target group 6, element E2 to E6 of a single OCT scan data performed with the 128° conical-tip fiber and the cleaved fiber.

Scanning of the group 7 element on the USAF target was also carried out to further assess the lateral resolution of this system. Group 7 consists of 6 line feature with a maximum line width of 3.9 μm. It was not possible to resolve any of the lines in this group with the conical-tip fiber. The lateral resolution that the 128° conical-tip fiber can achieve is taken as 4.4 μm since the smallest line thickness that the conical-tip fiber is able to resolve lies in element E6, group 6 of the USAF target. The lateral resolution of the system at the extreme end of the measurement range (0.6 mm from the fiber tip) was found to decrease to ~16 μm. An additional test was also carried out to determine the angular orientation sensitivity of the measurement using the conical-tip fiber and a mirror. The conical-tip fiber was first aligned perpendicular to the mirror and the peak intensity recorded. The measurement was then repeated with the same mirror tilted at different angles, while the fiber position remained unchanged. A comparison between the normalized signal intensity observed for the conical-tip fiber and the cleaved fiber plotted as a function of mirror tilt angle is shown in Figure 6-6.
The results show that the conical-tip fiber has a larger angular orientation tolerance, which gives a lower sensitivity to misalignment than the cleaved fiber. This larger angular orientation tolerance of the conical-tip fiber compared to the cleaved fiber also implies a larger acceptance angle. The maximum tilt angle that the conical-tip fiber can tolerate before the peak collected signal drops $1/e^2$ is approximately 15°.

To demonstrate the imaging capability of the conical-tip fiber through the common-path technique, a 2-D cross-sectional scan of a sample of fresh onion was performed. Figure 6-8 depicts a cross-section OCT tomogram of the onion sample obtained with the 128° conical-tip fiber. The image size is 256 × 1,000 pixels giving an area of 1.28 mm × 2 mm. The step-size of the translation stage used for the OCT scan was 2 µm. The image in Figure 6-8 is displayed in a logarithmic gray scale. From the cross-sectional OCT tomogram of the onion, the conical-tip fiber OCT system is found to achieve an imaging depth of approximately 600 µm. The cell wall layer making up the epidermis layer is clearly evident from the rest of the section. In addition the vascular bundle, which plays the vital role of transporting nutrients to the surrounding tissue, can also be clearly observed in the tomogram. Although the current OCT tomogram is obtained by fixing the conical-tip fiber probe and moving the translation stage, scanning at the fiber end itself could be achieved using small piezoelectric actuators. Since the conical-tip fiber is fabricated on a standard single mode fiber with an external diameter of 330 µm including the polymer buffer layer, this inbuilt microlens fiber probe can potentially be used to realize microprobes for ultra-small, needle-based diagnostic applications such as tumor diagnosis through refractive index measurement [189].
A proof of principle of a simply fabricated conical-tip fiber employing a common-path technique was demonstrated. The conical-tip fiber can be readily fabricated via a selective-chemical etching technique at low cost and with high reproducibility. This selective-chemical etching technique could be used to create an array of multiple conical micro lenses in a dedicated fiber bundle, which may prove useful in implementing a full-field OCT imaging system. This fiber bundle can also be used as an imaging system providing a useful field of view. Moreover, as the fiber bundle is more flexible than a rigid GRIN rod, it can be incorporated into flexible probes which allow orthogonal imaging of the nasopharynx. This would greatly improve the collection efficiency during the capturing of the OCT image. Thus, the common-path axicon OCT imaging system when coupled into a fiber bundle may be a viable solution for imaging the nasopharynx.
6-5 Summary

In this chapter, two methods of dispersion minimization were investigated. In the first method, a numerical algorithm to extract the phase was used to characterize the systemic dispersion in the GRIN rod probe system. This could be used to mitigate any dispersion in the system and optimize the PSF. The second method is based on a common-path axicon fiber probe. Dispersion and polarization mismatches were minimized resulting in a PSF that was close to the theoretically predicted value. Moreover, with the introduction of the axicon fiber tip, a long working distance imaging can also be achieved.
7-1 Conclusions

The work in this thesis is divided into two parts; the first part investigated the use of low coherence interferometry (LCI) techniques for particle tracking and detection of viral infection in plants, and the second part examined the feasibility of extending the LCI capabilities by incorporating them into existing endoscopy in the form of probes.

In LCI, interference effects are only observed when the optical path difference between the sample beam and a stable reference beam are within its short coherence length of the light source. Contrast in the form of coherence gating is thus provided at a very precise location at the sample. The high precision in LCI of the localization of a sample is the key in the achieving good tracking of optically trapped particles. Additional information such as the trapping dynamics can thus be derived. The detection of viral infection of the orchid was due to the ability of LCI to pick out changes in plant structures after the infection which manifested as scattering sites and their subsequent localization.

LCI, being incorporated into an optical tweezers system was used to observe and locate optically trapped microspheres. The broadband light source used was a super-luminescence diode with a center wavelength of 820nm and a bandwidth of 100 nm. The configuration is based on a spectral domain OCT platform and the axial resolution
achieved was 4.3 µm. This resolution was sufficient for imaging the front and back surfaces of microspheres with diameters of 10 µm and 50 µm used. A common-path design for the LCI portion was implemented to simplify the optical setup. Instead of the back focal plane (BFP) interferometry commonly used in conventional optical trapping systems, LCI was used to track the position of a trapped microsphere by examining the axial profile obtained from performing an inverse Fourier transform on the interference intensity spectrum arising from the sample (microsphere) and reference beam (cover slip). The long range tracking capability of the LCI system beyond 100 µm was demonstrated. This is significantly longer than that achieved by the standard BFP technique which has a tracking range of a few micrometers. Two trapped microspheres were also tracked directly using the LCI-optical trapping system. This demonstrated that the position of more than one microsphere can be monitored within an optical trap without the need for holographic imaging. The optical trapping portion, on the other hand, was used to characterize the axial resolution of the LCI portion. To accurately measure the axial resolution of the system, an optical trap was used to control the axial position of a microsphere and manipulating it relative to a cover slip. The distance where the surface of the microsphere can no longer be resolved from the cover slip was deemed the axial resolution of the LCI system. The measured axial resolution based on the Rayleigh criteria was 4.9 µm and is within 10% of the FWHM of the axial PSF. With axial tracking information collected using LCI over a period of time, the trapping dynamics (i.e. optical trap stiffness) can be determined. Optical trapping configurations with different numerical apertures (NAs) were tested using the combined system. The results using the LCI approach showed that the trap stiffness was higher in traps arising from systems with larger NAs. A dual counter-propagating beam trap was built to examine the effects of an additional trap on
the overall trap stiffness. The presence of a forward optical force from the top beam on
the trapped 10 µm microsphere to counter the bottom beam results in a stiffer trap
which was successfully quantified by the LCI technique. These results demonstrated
that LCI is a viable alternative to investigate the trapping dynamics in an optical
tweezers system with the added advantage of longer axial tracking range.

The axial profiles obtained through LCI at successive lateral position can be stacked
together to form a tomogram to analyze the sample. This is essentially the concept of
OCT. A spectral domain optical coherence tomography (SD-OCT) system was
designed and built to image orchid leaves. A Ti:Sapphire broadband light source with
center wavelength of 780 nm and bandwidth of 120 nm was used. A measured axial
resolution of 4 µm was achieved and the deviation from the theoretical resolution was
attributed to dispersion mismatches and the non-Gaussian shaped spectrum of the
Ti:Sapphire source. A SNR of 93 dB was achieved with a mirror placed in the sample
arm. The lateral resolution of 9 µm was achieved through the use of objective lens of
0.3 NA. The OCT system was able to differentiate Cymbidium Mosaic virus infected
orchids from normal orchids by observing their epidermis layers. In virus infected
orchids, the OCT images showed that the epidermides scattered more light compared
to normal plant leaves. This is also consistent with the results of virus-infected young
orchids without any visible symptoms. In stressed orchids where there is a lack of
water intake, the OCT images do not show higher scattering in the leaf epidermides. A
metric based on the average intensity values around the epidermis from the OCT
image was established to compare between virus-infected and healthy orchids. The
average intensity metric values of virus-infected leaves were found to be higher as
compared to those of healthy plants. When histological analysis of haematoxylin and
eosin stained virus-infect orchid leaf sections were conducted, no significant contrast
in the epidermis to indicate the presence of viruses was observed. These results
showed that OCT system can be suitable for fast, non-destructive diagnosis of orchid
virus infection.

The investigations of the LCI technique were conducted using bench top systems
which lacked portability. In addition, the working distance of these systems were
limited to that of the imaging microscope objective. An in-vivo OCT imaging probe
incorporated into a conventional white-light nasopharyngeal endoscopy was designed
and built to extend the capabilities of conventional system in difficult-to-access areas.
The light source used was the same as the one used for imaging orchid leaves
(Ti:Sapphire broadband light source with center wavelength of 780 nm and bandwidth
of 120 nm). Collaboration with clinicians from the Ear, Nose and Throat department in
Changi General Hospital was conducted for imaging the nasopharynx with OCT. One
of the major constraints in the design was to build an OCT modality around the
existing rigid nasopharyngoscope. A GRIN rod was inserted in the nasopharyngoscope to
relay the endoscopic image and facilitate the scanning of OCT beam. In the OCT path,
a 2D beam steering mechanism was incorporated into the probe to allow the sample to
be scanned at the distal end. The axial and lateral resolutions achieved for the OCT
images are 4 µm and 62.5 µm, respectively. In the endoscopic path, the field of view
was limited to the diameter of the GRIN rod (2 mm). The limitation of the field of
view was due to the lack of a wide-angle GRIN lens at the distal end which prevented
the focusing of the OCT beam at the sample. A high-speed spectrometer was designed
and implemented to enable fast OCT imaging. The line-scan CCD used in the
spectrometer had a line-rate of 29 kHz. With additional signal processing, a 2D frame rate of 18 frames per second was obtained. High spectral resolution was achieved by reducing coma aberration in the design of the spectrometer. Both the probe and high-speed spectrometer were incorporated for in-vivo imaging. Preliminary imaging on the finger showed that the system is fast and capable of resolving fine features such as the sweat ducts. However, preliminary OCT imaging of the nasopharynx was not successful. One of the main causes was the weak back-reflection from the nasopharynx which was beyond the sensitivity of the system. Arising from this investigation, some strategies for improving the probe design are identified and presented in the recommendation section.

One of the main drawbacks of a GRIN rod design was dispersion mismatches which distorted the shape of the axial PSF thereby worsening the OCT image quality. Two dispersion management methods to optimize the axial resolution of the OCT system were investigated. In the first method, the spectral phase of the axial point spread function (PSF) in a SD-OCT system with dispersion mismatches was extracted. The spectral phase was then subtracted from the phase of the spectrum to yield a transform limited axial PSF. This technique was used to improve the sharpness of OCT images. In addition, the spectral phase was used to determine the amount of dispersion in the GRIN rod with a group velocity dispersion of -19,472 fs$^2$ and a third order dispersion of 7,050 fs$^3$. The results were consistent with the simulation based on the dispersion properties of the GRIN material (GVD of -18,830 fs$^2$ and TOD of 7,629 fs$^3$) with a discrepancy of less than 8%. In the second method, an axicon fiber probe (cone angle of 128°) based on common-path principles was fabricated using standard
communications single mode fiber through chemical etching. The probe was used with a SLED source having a center wavelength of 1320 nm and a bandwidth of 70 nm. The center wavelength of 1320 nm was to match the single mode operation of the single mode fiber. The axial resolution of the system was 12.1 µm. The axicon fiber tip provided a weak back-reflection (0.46% of the incident light) that served as the reference plane in the common-path design. The cone angle essentially determines the transmittivity, spot size, and working distance. Due to the drop in transmittivity and subsequent illumination and collection efficiencies of the probe with cone angle and the dependence of system sensitivity (at the shot noise limit) on the reflected power from the sample, the cone angle was optimized to achieve a match between the lateral and axial resolution while maximizing the working distance. The common-path design helped to minimize dispersion and polarization mismatches which resulted in very little distortion in the axial PSF and a good SNR. The axicon fiber also provided a long working distance of about 600 µm where the SNR can be better than 30 dB for a mirror sample. A 2D image of an onion was obtained with the axicon fiber and the onion cell wells can clearly be observed. An imaging depth of 600 µm was also achieved. This result demonstrates the feasibility of using conical fiber tips as OCT probes.

7-2 Recommendations for Future Work

In this section, several recommendations for future work are proposed. These recommendations are divided into three areas; improvement to the probe design, enhancement of the LCI-trapping system and potential applications of techniques developed
7-2-1 Improvement to the probe design

Although the nasopharyngeal OCT probe was designed and tested *ex-vivo* on a human finger, a limited number of test was carried out to image the nasopharynx *in-vivo* due to a lack of samples and resources. One of the very few opportunities where the probe was tested did not yield an OCT image. This was attributed to extensive bleeding in the nasopharynx during the probing process. In addition, the presence of mucus in the nasopharynx of the volunteer reduced the incident optical power on the nasopharynx. Both blood and mucus absorbs or scatters near-infrared light. A model bench top tests where blood and mucus modeled using absorptive (e.g. India ink) and scattering (e.g. Intralipid) substances should be performed to determine the minimum power and fine tune system requirements to successfully image underlying tissues. To circumvent this issue, a more powerful light source may be needed. In addition, a small tube can be incorporated to the probe to aid the clearing of blood and mucus. A mechanical pump can be connected to the proximal end to drain excessive fluids via this tube. This would allow more successful attempts in studying the underlying tissue properties of the nasopharynx.

The limitations of the probe design manifested with the lack of a good SNR OCT image. This was exacerbated by the lack of a brightfield image to “guide” the scanning of the relevant zones of the nasopharynx. The requirement of the white light endoscopy with a wide field-of-view is in conflict with the need to focus the low coherence beam at the distal end when a single GRIN rod is used. A single scan lens prior to the GRIN rod cannot satisfy both requirements. To circumvent these issues,
variable focal lenses are proposed to be placed at the distal end. Electro-wetting technologies to adjust the curvature (and therefore focal lengths) can be utilized to achieve both requirements of the wide angle lens and scanning lens [47]. The proposed system will have two modes: imaging and OCT scanning modes. The system will alternate between both modes depending on the intended application. In this proposed system, two liquid lenses will be needed to achieve both requirements. A preliminary ray-tracing simulation of the proposed design was conducted using the Zemax software where the radii of curvature of the lens surfaces were optimized to provide both modes. In the imaging mode, the magnification is about -0.14 with the object at 10 mm from the distal end. The effective focal length is 1.733 mm with an equivalent F number of 6.9. The viewing angle at 10 mm object distance is ±30°. In the OCT scanning mode, the effective focal length adjusted to 21.5 mm with an F number of 21.4 yielding a lateral spot size of 9 µm. The lateral scan range is ±0.75 mm.

Although the OCT system achieved a frame rate of 18 frames per second, this capability was not fully demonstrated with real-time events. One of the possible demonstrations is to examine the opening and closing of stomata in plant leaves. The stomata can be triggered to open when the light intensity is high which can be externally modulated in a controlled environment. A sequence of images between the on-off states can be acquired to study the opening and closing of the stomata. A similar technique of characterizing the real-time capability using a sequence of OCT images with a timed event has been demonstrated by Wojtokowski et al for studying of human pupil dilation and contraction [190].
Enhancement of LCI-trapping system

The existing LCI-trapping system allows tracking of the trapped particles in the axial direction. However, a trapped particle can have more degrees of freedom. The LCI can incorporate a fast beam steering mechanism to sweep the low coherence beam laterally across the particle(s). The steering speed has to be fast enough so that the LCI system can capture its positional variation in the lateral directions. A suitable steering mechanism would be the resonant scanning mirrors with scan speeds up to 8 kHz. This would allow the construction of the three dimensional profile and its positional variation during the measurement period. The positional variations can then be used to calculate the trap stiffness in all three dimensions.

An alternative enhancement of the LCI-trapping system is to incorporate LCI in fiber dual trapping system. A dual-beam fiber trap using photonic crystal fibers has been demonstrated by Gherardi et al [191]. However, the monitoring of multiple trap particles is done with a CCD camera placed orthogonal to the particles. In their fiber trap, low coherence sources were used so that no deleterious interference effects disturb the optical binding effects of multiple particles. A possible extension of their work is to direct reflected light from the trapped particles to a circulator and then to a spectrometer to record the interferogram. This would allow the monitoring of trapped particles using LCI and the subsequent computation of their trap stiffness.
7-2-3 Potential applications of LCI techniques

7-2-3-1 Trapping dynamics of multiple particles

Optical binding in multiple trapped particles have shown to display intricate non-linear dynamics [85]. The dual beam trap presented in chapter 3 can be used to bind multiple particles. By using two beams with different wavelengths, interference effects at the trapping zone can be neglected. By adjusting the powers of the beams, the separation between the bound particles and their dynamics can be varied. The LCI system can be used to determine the trap stiffness of each particle and compare against the case where particles are trapped individually. A CCD camera system placed orthogonal to the dual beam trap can be incorporated in the system to observe and correlate the motion of the particles. The study of the trapping dynamics can aid the understanding of optical self-assembly of bounded arrays of particles.

7-2-3-2 Correlation between trapping dynamics of viruses and the OCT image at macro level

The long range tracking of LCI has been demonstrated in an optical trapping system. This work can be extended to characterize the trapping dynamics of different plant viruses (e.g. Cymbidium Mosaic Virus, Odontoglossum Ringspot Virus, Tomato Spotted Wilt Virus, etc.) in the form of optical chromatography where long range tracking will be useful. The trapping dynamics can infer certain characteristics of the viruses due to light-matter interaction. At the macro level, OCT has demonstrated the capability to detect infection due to CyMV. An intensity metric was used to classify the presence of CyMV but does not provide the concentration. Further work can be
conducted to correlate the virus concentration from the OCT images (e.g. extracting the scattering coefficient, spectroscopic analysis). There is scope for extending the OCT imaging to other types of viruses. This information can then be used to correlate back to the trapping dynamics of the different types of viruses.

7-2-3-3  **In-vivo imaging of plants**

Although the current system is fast compared to ELISA methods, it is still laboratory-based. More OCT imaging data of various plants can be collected to form a database for different types of virus infection. As the response to different types of virus infection on different plants may be vastly different, the database of OCT images will be able to determine which plant-virus combination will be most effective to be imaged by OCT. A portable OCT system can then be designed for quick and accurate diagnosis of virus-infection in plants that leverages on the database. In addition, different areas of the plant have shown to have different degrees of virus concentration [126]. An *in-vivo* OCT system can be used to probe different areas of the plant to study this variation using the GRIN rod-based probe. This scheme can potentially save time in sample collection and preservation as the OCT imaging system can give the results at the imaging site.
Appendix A: Alignment of Spectrometer

An article by Horiba Scientific describes the fundamentals of spectrometer and its alignment. In particular, it is recommended to use a HeNe laser to ensure that it passes through the optic axes of all optical components. The alignment procedure starts from the rear (i.e. focusing lens) without the CCD camera.  

1. Align the HeNe laser without the lens so that the beam is parallel relative to the optical table.
   a. Set the beam height so that it is roughly in optical axis of the lens.
   b. Place two irises at the same lateral position on the table.
   c. Tilt and/or shift the HeNe laser laterally to ensure that it passes through the two irises. Once these steps are completed, the beam is parallel in the lateral direction.
   d. Remove the front iris and place a distance behind its original position. The beam may be higher/lower at the new position.
   e. Adjust the vertical tilt and/or height of the HeNe laser until the beam passes through the iris at various positions. The height of the iris may have to be adjusted during this process. Once this is completed, the beam is parallel to the table.

2. Align the HeNe beam relative to the focusing lens.
   a. Place two irises in the beam path.

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Note: In adjusting the spectrometer, a possible method is also to adjust the focusing lens instead of the CCD’s position. This is to ensure that the diffracted beam after the lens hits the CCD at normal incidence. A pre-alignment of the CCD may be necessary to ensure this.
b. Place the lens in the beam path. The beam may be deflected by the lens so that it does not pass through the second iris.

c. Rotate the lens so that the back-reflected beam is incident on the front iris. This is to ensure that the lens surface is parallel to the wavefront of the beam.

d. Adjust the position (lateral and vertical shifts) of the lens so that the beam passes through the second iris. The vertical positions of the irises should not be adjusted as they will be used to align the forward path.

3. Place the grating in the path of the HeNe beam after the lens.

   a. Rotate the position of the grating such that its normal is about 30° with respect to the optical axis of the lens.

   b. Align the grating by shifting vertically and laterally so that the beam in the optical axis of the grating. This is to ensure that the designed center wavelength will pass through the optical axis of the lens.

4. Rotate the spectrometer setup to align to forward path. This step may require shifting the HeNe laser so step 1 may need to be repeated, but now the HeNe laser is adjusted to ensure that it passes through both irises.

   a. Shift and/or tilt the position of the HeNe so that it is 30° incident to the grating. The beam should pass through the optical axis of the grating.

   b. Check the position of the HeNe laser after the lens. The lateral position should be around the position that has been calculated using the grating equation

      \[ a \sin \theta_i + a \sin \theta_l = n\lambda \].

      For example, if \( a = 833 \) nm, \( \lambda = 633 \) nm and \( \theta_l = 30^\circ \), then \( \theta_l = 15^\circ \). If the grating is at a distance of 70 mm away from the lens, then
the lateral position of the first order diffracted HeNe beam should be at
$70\tan(30^\circ - 15^\circ) = 18.76$ mm from the optical center of the lens. For a lens of
diameter 50.4 mm, then the beam should be about 44.2 mm from the left edge.

5. Insert the fiber setup (fiber and collimating lens) into the forward path and ensure
that it matches the HeNe beam direction.
   a. Shift and rotate the fiber setup so that the HeNe beam passes through it. The
two irises can be reused to ensure that the beam height/vertical tilt is correct.

6. Replace the HeNe beam with the appropriate broadband source and place it to the
fiber setup.

7. Place the CCD in its position after the lens.
   a. Adjust the CCD position up/down, back/forward, rotate left/right to ensure that
      maximum power is incident on the CCD array.
   b. Move the CCD back/forth to check whether power is concentrated at some
      particular pixels to ensure that there is a proper rotational tilt. For example,
      if the power on the pixels increases from left to right when the CCD is moved
      forward, while the power, this implies that there is a rotational tilt that has the
      lower wavelengths nearer to the lens. Hence the CCD should be rotated
      clockwise.

8. Adjust the rotation of the grating to ensure that the CCD reading
increase/decreases evenly across all wavelengths when it is moved up/down.
9. Use a simple common-path interferometer setup to check the spectrum against a calibrated spectrometer. If the steps are done properly, the SNR of the spectrometer should be fairly good.

10. Use a calibrated light source with known spectral lines to calibrate the pixel position to its corresponding wavelengths. The measured spectrum from a commercial calibration source (Avantes) with the custom spectrometer setup implemented in this work is in good agreement with that obtained from a commercial spectrometer (Figure A-1).

![Figure A-1: Measured spectrum of the Avantes calibration source from the (a) Basler setup implemented in this work and the (b) Ocean Optics HR4000 spectrometer.](image)
Appendix B: Reduction of Coma Aberration in Spectrometer

Quantifying the Coma Aberrations

To quantify the improvement with a two-achromat compound lens system, the OSLO ray tracing software was used to simulate the amount of coma aberration. A useful quantity to check for coma aberration is the Seidel coma coefficient which states the degree of deviation from a perfect spherical wavefront after a lens. The Seidel coma coefficient for two back-to-back 100 mm focal length achromats was 0.000209 with a back focal length of 46.26 mm for $\lambda = 900$ nm and a field angle of $\pm 8.58^\circ$. With a single achromat of 50 mm focal length, the coma aberration was much higher at 0.003795.

The separation distance between the two achromats has an effect on coma aberration. Table B-1 shows the results for the 75 mm achromats pair simulated with OSLO using $\lambda = 900$ nm at an angle of $\pm 8.58^\circ$. The coma is observed to be the least when the lenses are in contact with one another. The simulation was performed without changing the stop position from the first achromat surface.
Table B-1: Simulated Seidel coma aberration for different separation between two achromats.

<table>
<thead>
<tr>
<th>Lens Separation (mm)</th>
<th>Seidel coma aberration value</th>
<th>Back focal length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$5.169 \times 10^{-5}$</td>
<td>67.573168</td>
</tr>
<tr>
<td>5</td>
<td>$11.1 \times 10^{-5}$</td>
<td>66.232634</td>
</tr>
<tr>
<td>10</td>
<td>$16.7 \times 10^{-5}$</td>
<td>64.845084</td>
</tr>
<tr>
<td>15</td>
<td>$22.1 \times 10^{-5}$</td>
<td>63.407999</td>
</tr>
<tr>
<td>20</td>
<td>$27.2 \times 10^{-5}$</td>
<td>61.918678</td>
</tr>
</tbody>
</table>

With proper alignment of the spectrometer, the maximum depth that can be resolved is fairly close to the theoretical maximum. This was not achieved previously with only one achromat.

**Dependence of the Seidel Coma Aberration on the Position of Focusing Lens and Grating**

Based on the simulated values from OSLO, the Seidel coma aberration is $6.804 \times 10^{-7}$ when the distance is 72.85 mm between the imaging lens and the grating. However, when the distance is at 60 mm, the Seidel coma aberration is found to be -0.000451 (Figure B-1). The simulation results showed that the minimum coma aberration occurs when the distance between the lens and the grating is about 73 mm.
Figure B-1: Simulated Sidel coma aberration plotted as a function of distance between the focusing lens and grating.
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CONFERENCE PAPERS


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