ULTRA HIGH RESOLUTION AND CONTRAST SENSITIVE OPTICAL COHERENCE TOMOGRAPHY

by

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# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>OCT</td>
<td>Optical Coherence Tomography</td>
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<tr>
<td>FDOCT</td>
<td>Fourier Domain OCT</td>
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<tr>
<td>SSOCT</td>
<td>Swept Light Source OCT</td>
</tr>
<tr>
<td>LCI</td>
<td>Low Coherence Interferometer</td>
</tr>
<tr>
<td>UHR-OCT</td>
<td>Ultra-high resolution OCT</td>
</tr>
<tr>
<td>SLED</td>
<td>Super Luminescent Diodes</td>
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<tr>
<td>SCG</td>
<td>Super Continuum Generation</td>
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<tr>
<td>PCF</td>
<td>Photonic Crystal Fiber</td>
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<tr>
<td>SPM</td>
<td>Self Phase Modulation</td>
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<tr>
<td>FWM</td>
<td>Four Wave Mixing</td>
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<tr>
<td>SRS</td>
<td>Stimulated Raman Scattering</td>
</tr>
<tr>
<td>CSOCT</td>
<td>Contrast Sensitive OCT</td>
</tr>
<tr>
<td>SPR</td>
<td>Surface Plasmon Resonance</td>
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<tr>
<td>PT</td>
<td>Photothermal</td>
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<td>NR</td>
<td>Nanorods</td>
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Ultrahigh Resolution And Contrast Sensitive Optical Coherence Tomography

Abstract

by

HUI WANG

In this thesis, I first demonstrate three novel broad band light sources at above 1 µm for ultra-high resolution optical coherence tomography (OCT) based on multiple SLED combination and supercontinuum generation. The light sources are compact, reliable, smooth and ready for practical applications. The images achieved with those light sources showed superior quality in comparison to the images achieved under normal resolution OCT with axial resolution around 12 to 15 µm. In the second part of the thesis, the feasibility of using gold nanorod (NR) as a probe for contrast sensitive OCT is described. Two methods, photothermal effect (PT) and spectroscopic analysis, have been investigated. The method based on PT cannot provide sufficient contrast for imaging due to the thermal lensing effect. Although external modulation is preferred for contrast sensitive OCT, more sophisticated probes are required in future studies. In spectroscopic analysis, contrast due to absorption and scattering of NR has been observed. However, absorption is dominant for the small size of NR used here. Larger NR will be advantageous for use as a scattering probe and for avoiding the ambiguity of contrast sensitive OCT. In the last part of the thesis, three biological applications are demonstrated. The results show that intact mouse embryo and fiber orientation of intact mouse heart can be readily imaged with ultrahigh resolution OCT. These findings pave the way for future imaging, with a high speed system, of those subjects in living states. As an additionally investigated application, the sliver glucose sensor was imaged in the mouse skin. For reliably tracking sensors with OCT, the implant depth should be around the interface of the epidermis and dermis. For tracking of the inflammation process over the long term, multiple optical imaging modalities may be required.
CHAPTER 1. BACKGROUND

This chapter includes a first introduction to the basic principles of OCT. Three different implementations for OCT will be discussed briefly. The parameters for evaluating OCT will then be introduced, including the background for developing ultra-high resolution OCT (UHR-OCT). The chapter concludes with a comparison of OCT with other imaging modalities by way of an observation of the various advantages and disadvantages of each modality. Additionally, the concept of contrast sensitive OCT will be introduced.

1.1 MICHELSON INTERFEROMETER

OCT originated out of the Michelson interferometer, where a light source with a long coherence length was split into two arms by a beam splitter. The light reflected from each arm is recombined by the splitter then directed to a photo detector. Interference fringes can be observed by moving the mirror in one of the arms. Fig.1.1 displays the typical set up.

Fig. 1.1. Typical Michelson interferometer and interference fringes
The electrical field reflected from the two arms can be written as

\[
E_R = E_{R0} \exp[-j(\omega t - K \cdot 2l_R)] \\
E_S = E_{S0} \exp[-j(\omega t - K \cdot 2l_S)]
\]

(1.1)

Where \( E_{R0} \) and \( E_{S0} \) are the amplitude of reflected electrical fields \( E_R \) and \( E_S \); \( K \) is the wave number. \( L_R \) and \( L_S \) are the distances from the two mirrors to the detector. The intensity measured at the photo detector is

\[
I \propto |E_{R0}|^2 + |E_{S0}|^2 + \text{real}(E_S \cdot E_{R0}^*)
\]

\[
\text{real}(E_S E_{R0}^*) = 2E_{R0} E_{S0} \cos(2KL)
\]

(1.2)

By taking the real part of the signal at the detector, the interference signal can be expressed as a Cos function of the optical path mismatch between the two arms.

1.2 **Low Coherence Interferometer (LCI)**

It is straightforward to consider coupling light composed of many different wavelengths into the interferometer simultaneously. With each wavelength, the interference fringes can still be observed by using a filter before the detector. Fig. 1.2 shows the interference fringes at three different wavelengths 1.25 \( \mu \)m, 1.3 \( \mu \)m and 1.35 \( \mu \)m. At position zero, where the optical path is perfectly matched, all the fringes reach the maximum value. However, the fringes will be out of phase when the position is away from zero.
Now, considering a broad band light source (Fig. 1.3), we can define the bandwidth of this light source as the full width of half maximum (FWHM). By summing all the fringes at different wavelengths, the final fringes can be observed only around the position zero and appear like a wave packet. This wave packet is called the pointed spread function (PSF) of LCI and the FWHM of PSF is named as the axial resolution of LCI.

Mathematically, the spectrum and PSF can be related by Fourier transform.
By assuming the spectrum has a Gaussian shape, the axial resolution between the PSF and band width can be derived as

\[
AxialResolution = 0.44 \frac{\lambda_0^2}{\Delta \lambda}
\]  

(1.4)

where \(\lambda_0\) is central wavelength and \(\Delta \lambda\) is the FWHM of the light source.

1.3 **Optical Coherence Tomography**

Optical coherence tomography (OCT) is originated from LCI. LCI was proposed by several authors in 1987 to test miniature optical components [1, 2]. Continued development of LCI lasted into the next decade [3-6]. A typical application of this technology involves finding faults in optical components based on one-dimensional scanning. Later, people realized LCI could be used for biological tissue, such as measuring depth-resolved tissue structures. In 1986, Fercher and Roth proposed the use of LCI to measure both short and long intraocular distances with high precision [7]. In 1991, LCI was adapted by adding a focusing lens and a transverse scanning mirror for cross-sectional images. This technique, then, was named Optical Coherence Tomography (OCT) [8] and began to be used extensively in numerous biomedical areas.

The readily apparent difference between the LCI and OCT is that OCT takes a B-Scan by integrating many A-Scans into image at high speed. Recently, one of the main efforts in OCT research has been to increase the speed of data acquisition achieving real time images. Now it is possible to reconstruct 3D images in mere
seconds by using multiple B-Scans. Fig.1.4 shows the typical scanning scheme of OCT.

1.3.1 Implementations of OCT

Since the first generation of time domain OCT (TDOCT), Fourier domain OCT (FDOCT) and swept source OCT (SSOCT) represent the new generation of OCT with ultrahigh-speed and ultrahigh-axial resolution. New advances in OCT often usher in a new generation of related technologies, such as high speed CCD and femtosecond lasers. In this section, we will briefly discuss three implementations of OCT and associated advantages and limitations. The possible resolutions for these limitations will also be described briefly.

1.3.2 TDOCT

Fig. 1.5 shows a typical, fiber based TDOCT. The light from a broad band light source is split by a 50:50 fiber coupler into the reference arm and the sample arm of the apparatus. The reflected light from the reference arm and the sample arm is recombined by the fiber coupler and detected by a photo detector. For the broad band
light used here, only the interference located in the coherence gate can be detected. By varying the optical path of the reference arm with respect to time, the coherent gate can be moved sequentially through the sample. The reflected intensity from each layer in the sample can be resolved by demodulating the interference signal to form an A-scan. Due to the fact that an A-scan is achieved in a time series, this implementation is then called TDOCT.

![Optical layout of typical TDOCT](image)

The OCT signal at the detector can be expressed as

\[
I_i \propto I_{DC} + I_{AC} + \left[ e^{j2\omega_g \Delta} \int_{K_q-\Delta K}^{K_q+\Delta K} I(\Delta K) e^{j\Delta \omega \Delta K} d\Delta K + CC \right]
\]

(1.5)

Where CC is the conjugated term and \(I_{DC}\) and \(I_{AC}\) represent background light from the reference arm and the sample arm. By varying the optical path, a carrier wave is generated by the blue term in Equ. (1.5) and is associated only with the interference term. A TDOCT signal is an amplitude modulated wave that can be demodulated by quadrature demodulation. The rate of image acquisition is dependant on the speed of each A-scan. Image acquisition, therefore, is determined by the speed of optical path delay. Currently, the highest speed achieved by TDOCT is around 8K A-scans per
second by using rapid scanning optical delay line. Further improving speed will be
dependant on the new generation of high speed optical delay line [9, 10].

1.3.3 Fourier Domain OCT (FDOCT)

Instead of detecting OCT signals at all wavelengths simultaneously using a photo
detector, we also can detect OCT signals at each wavelength with a spectrometer. The
signal at each pixel on the CCD is the interference signal at a specific wavelength K
and can be written as

\[ I_K \propto I(K)_{DC} + I(K)_{AC} + I(\Delta K)[e^{i2K_K\Delta z/v_g} e^{i\Delta \phi/\lambda_K} + e^{-i2K_K\Delta z/v_g} e^{-i\Delta \phi/\lambda_K}] \] (1.6)

By doing Fourier transform to the interference term, we can get

\[ I_i \propto e^{i2K_K\Delta z} I[(\tau + \Delta z / v_g)] + e^{-i2K_K\Delta z} I[(\tau - \Delta z / v_g)] \] (1.7)

In FDOCT, each A-scan can be achieved by one exposure of the CCD. During the
exposure, all the photons from each layer of the sample will be integrated to generate
an OCT signal. The longer exposure time represents a high signal noise ratio.
However, in TDOCT, only the interval during which the optical paths are matched is
used for OCT signal. This interval is very small for high speed TDOCT. Thus,
FDOCT has sensitivity advantages over TDOCT [11, 12]. With the commercial
availability of high speed CCD, FDOCT can now be run at very high speeds, up to 20K-80K A-scans per second [13, 14].

The limitations of FDOCT can be attributed to three primary aspects. The DC background from the reference arm and the AC background from the sample arm cannot be totally eliminated, even by subtraction. These backgrounds can reduce the sensitivity of the system. Another limitation is the mirror item in the Equ. (1.7). FDOCT cannot distinguish the signal from the two layers as having the same distance from, but opposite direction to the reference mirror. Several solutions have been proposed and are based on introducing an extra phase shift either on the A-scan or on the B-scan [15-20], although these methods still have their own drawbacks. The image depth is limited by the fall off and resolution of the spectrometer, which makes the FDOCT complex and sophisticated.

1.3.4 Swept Source OCT (SSOCT)

The principle of SSOCT is more like FDOCT. However, the setup of SSOCT is nearly identical to TDOCT, with the exception that the reference arm in SSOCT is immobile [21]. Swept source is similar to the tunable laser, but covers broader band and is tuned at a much higher speed. Instead of using a sophisticated spectrometer to detect light at each wavelength, SSOCT can use photo detectors (PD), which makes the system compact and more reliable. By using high-speed and balanced PD, it is possible to realize heterodyne detection by modulating the phase of interference terms [22]. The DC, AC backgrounds and mirror images can be eliminated easily. Fig. 1.7. shows the
typical setup of SSOCT.

One drawback of SSOCT is the imaging depth, which is determined by the line width of each wavelength [23-26]. For deep imaging depth, SSOCT requires a narrow line width at each wavelength. Although current filters permit SSOCT to achieve around a 3-4 mm imaging depth – still insufficient for tissue, which has rough surfaces. Recently developed Fourier domain mode locking (FDML) swept source represents ultrahigh-speed imaging, up to 300K A-scans per second with effective imaging depth to ~ 6 mm [25, 27]. The cost and size of the FDML laser is not ideal for a portable system. However, we should acknowledge that the ultra high speed will provide us enough freedom to perform either real time speckle reduction or high lateral resolution enface imaging. In addition, ultrahigh-speed imaging can help us capture dynamic biological process in vivo.

Further development of SSOCT should focus on the design of an inexpensive ultrahigh-speed swept light source for ultrahigh-speed and ultra-high resolution system.

Fig. 1.7.Optcial layout of swept light source OCT
1.4  PERFORMANCES OF OCT

1.4.1  Axial resolution

As we discussed in section 1.2, the axial resolution of OCT can be expressed by equation (1.8), when assuming the light sources have a Gaussian shape.

\[ A_s = 0.44 \frac{\lambda_0^2}{\Delta\lambda} \quad (1.8) \]

There, \( \lambda_0 \) is the center wavelength of the light source and \( \Delta\lambda \) is the full width of half maximum (FWHM) of the band width of the light sources. It is clear that axial resolution of OCT is determined by the bandwidth and the central wavelength of the light sources. The corresponding plot of bandwidth vs. axial resolution is shown in Fig. 1.8. Three wavelengths, 0.8 µm, 1 µm and 1.3 µm, are now popularly used in current OCT systems and are plotted in the Fig. 1.8. Red and green areas represented the commercial available SLED light sources. Blue areas represent the ultra-high axial resolution. For achieving ultrahigh axial resolution less than 5 µm in tissue, the band width is required to be above 50 nm at 0.83 µm, 80 nm at 1.06 µm and 120 nm at 1.3 µm. It is not difficult to find a SLED with a bandwidth above 50 nm at 830 nm. However, there is no single SLED at 1.3 µm with a bandwidth above 120 nm. Many efforts have been made to develop broad band light sources at 1.3 µm for ultra-high resolution OCT. We will further discuss this later in this chapter.
1.4.2 **Lateral Resolution**

Lateral resolution of OCT is determined by the spot size of the light beam after passing through the focusing lens. Typically, the light beam is assumed to be a Gaussian beam. For diffraction-limited, the focal spot will be

\[
D = \left( \frac{4 \lambda}{\pi} \right) \left( \frac{F}{D} \right)
\]  

(1.9)

Where \(F\) is focal length and \(D\) is the collimated beam diameter before focusing. Depth of focus (DOF) will determined the consistency of the lateral resolution at different imaging depths. Due to the angle divergence, a Guassian beam’s lateral resolution will be degraded at the area away from the focal point.

\[
DOF = \left( \frac{8 \lambda}{\pi} \right) \left( \frac{F}{D} \right)^2
\]  

(1.10)

Typically, this performance can be estimated by DOF. Considering a beam at 1.3 \(\mu\)m with size of 4 mm, for a 30 mm focal length, the spot size is 12 \(\mu\)m and the DOF is
around 190 μm. Assuming the focusing point is located around 1 mm below the surface of the sample, the lateral resolution at the surface will be around 36 μm.

1.4.3 Center Wavelength

The center wavelength of the light sources is not only related to the axial resolution but also determines the penetration depth of OCT. Penetration depth of OCT depends on the absorption and scattering of the subjects. Historically, two center wavelengths, 830 nm and 1300 nm, have been preferred for OCT.

![Water absorption spectrum](http://omlc.ogi.edu/spectra/water/index.html)

**Fig. 1.9.** Water absorption spectrum. The original data consult from http://omlc.ogi.edu/spectra/water/index.html

Absorption attenuates the light in the subjects and is wavelength dependent. Because more than 60% of biological tissue consists of water, the absorption of tissue is mostly attributed to the water. Examining the water absorption spectrum shown in Fig. 1.9, we can find a window with lower absorption, termed “therapeutic windows” for biomedical optics, existing around 600 nm to 800 nm. For retinal imaging, a photon must pass through the corneal and anterior chamber and lens. In order to avoid water absorption, light sources centered at around 800 nm are often used in
ophthalmology. However, this is not absolutely true when scattering and dispersion are also considered. In addition, the spectrum reflected back from studied objects can also be modulated by the absorption, which may affect the PSF of OCT.

Scattering also attenuates the forward light, but it generates OCT signal by backscattering some of photons. For tissue, scattering decreases with an increase in wavelength and can be defined as Rayleigh scattering when the size of the scattering particles are comparable with the wavelength. At 800 nm, although absorption is low, the scattering is high so light cannot penetrate deeply. At longer wavelengths, although the absorption is increased, the decreased scattering permits imaging deeper than at 800 nm. Previously, 1300 nm was chosen for imaging opaque subject for better penetration. Another reason for choosing 1300 nm is due to commercial availability of the fiber components in this band. However, this should not prevent us from exploring other wavelengths, such as 1500 nm [28].

Dispersion is induced by the mismatch of material dispersion between the reference arm and the sample arm and can reduce the axial resolution of the system. Dispersion can be classified variously as system dispersion or sample dispersion. System dispersion can be perfectly compensated for when the two arms are exactly the same or carefully matched. Sample dispersion is difficult to compensate for fully, because dispersion can be matched only at a specific depth. Still, the image’s information comes from the full depth. For normal resolution OCT, the influence of sample dispersion can be neglected if a relatively narrow-band light source is used. However, for UHR-OCT, the sample dispersion can seriously degrade the axial
resolution. For this reason, light sources centered at around 1-1.1µm should replace the 830 nm light source for ophthalmology. The dispersion around this area is close to zero. In addition, the increased absorption of water can be compensated for by increasing incident power. According to ANSI standards, the maximum permissible light exposure in the human eye is 1.7 mW at 800 nm and 5 mW at 1060 nm for continuous wave light. Another advantage of using a longer wavelength is an increase in penetration depth. The retinal imaging depth is limited by the highly absorbing and scattering retinal pigment epithelium (RPE). The signal from the choroidal layers is insufficient for an 800 nm light source. However, choroid vessels can be visualized with light sources centered on 1-1.1 µm [29-31].

1.4.4 Spectral shape

As described above, the PSF of OCT system is the inverse Fourier transform of the power density spectrum of the light source.

\[
PSF = IFFT[SP(k)]
\]  \hspace{1cm} (1.11)

Here, the SP (k) is a power density spectrum defined with wave vector k. For a Gaussian spectrum, the PSF is also Gaussian-like shape. However, for a rectangular spectral shape, the PSF is a Sinc function, where small lobes aside of the main lobe can be observed and are called sidelobes. Sidelobes will be present in the images as ghost lines around the strong reflection layer. In order to avoid this, Gaussian or Lorientz spectral shape is desired for OCT.
1.4.5 Signal Noise Ratio

In an OCT system, signal noise ratio determines sensitivity and image quality. Typically S/N can be defined as

\[
\frac{S}{N} = 10 \log \left( \frac{\rho^2 (R_r R_s) P_0^2}{4 \rho NEP B + 2eI_{dc} B + \sigma^2} \right)
\]  

(1.12)

Where \( R_r \) and \( R_s \) are the reflection from the reference and sample arms; \( P_0 \) is the power from the light source and \( \rho \) is quantum efficiency of the detectors; \( B \) is the detection bandwidth, \( NEP \) is Noise equivalent coefficient of the detector, which is either a photodetector or CCD, and \( e \) is the electron charge; \( I_{dc} \) is the average photocurrent excited by photons; \( \sigma_I \) is intensity noise, which is determined by laser and can be modeled [32] or measured. For typical TDOCT the bandwidth \( B \) is related to the carrier frequency, band of light source and the scheme of optical delay line [12]. For FDOCT and SSOCT, at the same acquisition time, intensity noise and shot noise will be decreased about \( M \) times, where \( M \) is the pixel number covered by the light source.

As we described in this section, many performances of OCT are determined by light sources. For a high quality OCT system, an ideal OCT light source should have following properties:

a) Enough bandwidth to support high axial resolution in tissue;

b) Enough output power for more than 100 dB;

c) Smooth and Gaussian-like spectrum for avoiding ghost lines in the images;

d) Above 1 \( \mu \)m central wavelength for better penetration depth.
In this section, we will introduce the current status of UHR-OCT. In particular the development of the light source that is the core of the OCT technology and some basic concepts of nonlinear effects, which is essential to the thesis. Superilluminencesnt LED (SLED) is typically used for OCT due to its compact size, reliability, low noise and smooth spectrum. However, the bandwidth of SLED is still limited, especially above the 1µm wavelength range. After decades, several different broad band light sources have been developed based on newly developed SLED, laser and fiber. However, both have limitations.

### 1.5.1 SLED

SLED is a diode similar to the laser diode (LD) with amplified simulated emission. However, in SLED, lasing action cannot be built up due to insufficient feedback for oscillations. Current SLEDs operate at various wavelengths, such as 0.8 µm, 1 µm, 1.3 µm and 1.55 µm. All of these wavelengths have been used for OCT. At the 830 nm range, SLED with up to 70 nm bandwidths and 3 mW fiber pigtailed output power (corresponding to around 3.3 µm in the tissue) are commercially available. For ranges above 1 µm, the typical bandwidths are around 50 nm to 60 nm, which limits the axial resolution when larger than 10 µm in the tissue.

### 1.5.2 Broadband Laser

With the development of ultrafast laser, it is possible to achieve broad band spectrum
directly from the oscillator. The most successful ultrafast laser is Ti:sapphire lasers, which emit light at around 700 nm to 900 nm. The bandwidth and pulse duration of a laser can be roughly calculated by

\[
\Delta \lambda (nm) = 1.47 \times 10^{-3} \frac{\lambda^2 (nm)}{\tau (fs)}
\]

Figure 1.10 plots the relation between time and bandwidth. For achieving high axial resolution, the femtosecond laser should emit less than 20 fs pulses at 800 nm. Currently, the Ti:sapphire laser, with pulses down to 7 fs, has been commercialized. With a compact design and low cost, Ti:sapphire lasers at 800 nm with axial resolution up to 1.2 µm in the tissue have been utilized for corneal and retinal imaging [33]. However, at 1 µm and 1.3 µm, the available lasers can emit pulses only above 50 fs. Especially for the 1.3 µm laser, bulkiness and maintenance issues continue to be problematic, particularly outside of a lab.

![Fig.1.10 Pulse width vs. band width of femtosecond lasers](image)
1.5.3 Supercontinuum Generation

Nonlinear optical effects have been observed and studied since the availability of ultrafast lasers. The most valuable applications of non-linear effects are to generate new frequencies by pumping with monochrome lasers. Mathematically, the nonlinear effects can be described on the molecular scale by the following total polarization:

\[ P = m + \varepsilon_0 \left( \chi^{(1)} E + \chi^{(2)} E^2 + \chi^{(3)} E^3 + \cdots \right) \]  \hspace{1cm} (1.14)

Where \( P \) is the total polarization of the molecule; \( m \) is the permanent dipole moment; \( E \) represents the electric field; \( \chi^{(1)} \) is the linear polarization; \( \chi^{(2)} \) and \( \chi^{(3)} \) are the first and second hyperpolarizability coefficients. The most noticeable terms are the second and third hyperpolarizabilities and the related phenomena are called second harmonic generation (SHG) and third order nonlinear effects, including self phase modulation (SPM), four-wave mixing (FWM), stimulated Raman scattering (SRS) and others.

Broad band light sources can be generated through efficient non-linear effects. In order to enhance non-linear effects, two conditions must be satisfied, extremely high power and long walking distance for keeping such high power. Ultrafast lasers such as femtosecond lasers can produce very high peak power. For a Nd:glass 100 fs laser at 1.06 \( \mu \text{m} \) with repetition rate 80 MHz, the peak power is 12.5 KW with an average output power of 100mW. Optical fiber can be used to confine this power in a very small area and to conduct pulses for a long distance. By using pulsed lasers and optical fibers, broad band spectra called continuum generation (CG) have been demonstrated and utilized for ultrahigh resolution OCT [34]. The CG can be divided into two pumping schemes: pumping in normal dispersion and pumping in anomalous...
dispersion.

1.5.3.1 Dispersion of fibers

The phase shift introduced by the material with length $L$ is

$$\Phi(\omega) = n \frac{2\pi}{\lambda} L = \Phi(\omega_L) + \Phi'\omega\Delta\omega + \frac{1}{2} \Phi''\omega^2\Delta\omega^2 + \frac{1}{6} \Phi''\omega^3\Delta\omega^3 + \ldots$$

and

$$\Phi''(\omega) = \frac{d^2\Phi(\omega)}{d\omega^2} = \frac{\lambda \Delta^2 n(\lambda)}{2\pi c} \frac{d^2n(\lambda)}{d\lambda^2}$$

Where $\Phi''(\omega)$ is defined as group dispersion delay (GDD). Group velocity dispersion (GVD) is defined as

$$GVD = \frac{GDD}{L} = \frac{dv_g}{d\lambda} \begin{cases} >0, \text{ Normal Dispersion} \\ <0, \text{ Anomalous Dispersion} \end{cases}$$

Conventionally in industry, the dispersion is described as

$$D(\text{ps} / \text{km.nm}) = \frac{2\pi c}{\lambda^2} GVD(\text{ps}^2 / \text{m})$$

So the normal dispersion is negative and anomalous dispersion is positive.

1.5.3.2 Pumping in normal dispersion

Pumping in normal dispersion means that the pumping wavelength exists in the normal dispersion area of the fiber. Typically, the conventional single mode fiber (SMF) has zero-dispersion wavelength (ZDW) around 1.3 µm or above. The pumping wavelength can be chosen according to the ZDW. The CG is mostly attributed to the SPM when the pumping pulses are on the order of femtoseconds.

The first demonstration of CG for OCT is based on the conventional dispersion shifted fiber with a core diameter of 9 µm and a Cr:fosterite fs laser at 1280 nm [35]. An axial resolution of 6 µm was achieved in the tissue. However, as we stated in the previous section, even now the Cr:fosterite laser is very bulky, expensive and hard to maintain outside of a lab, which makes it difficult to use in a clinical setting.
Ti:sapphire lasers also can be used for pumping SMF. The bandwidth of the CG will range from 40 nm to 80 nm, dependant on the pumping power. The drawback of using a Ti:sapphire laser is that the spectrum is centered around 800 nm, which is not preferred for opaque tissue imaging. Further, broadening the spectrum is attained by using ultrahigh numerical aperture (UHNA) fibers, which have a small core size of 2.3 µm [36]. By pumping with a Ti:Sapphire laser, the bandwidth can reach about 100 nm. However, due to the dope of GeO₂, this kind of fiber cannot be used for any appreciable length of time if the pumping power is very high [37]. In addition, the spectral modulation can be observed, possibly due to multimode interferences. UHNA fibers also can be pumped with a femtosecond laser at around 1µm. By using a Nd:glass fs laser, up to 5 µm axial resolution has been achieved [38].

Examining the CG generated by pumping the fiber in the normal dispersion area, we find the generated spectrum is smooth, Gaussian-like and free of additional noise, excepting laser noise. These benefits are advantageous for OCT applications. However, the bandwidth is relative narrow due to the large normal dispersion, which will stretch the pulses quickly. Unfortunately, there are no convenient lasers operating at around 1.3 µm for this application.

1.5.3.3 Pumping in anomalous dispersion area

CG can also be generated by pumping the fiber in the anomalous dispersion area. In this situation, many non-linear effects will be involved basically due to the formation of solitons [39]. For conventionally single-mode fiber, 1.55 µm has to be used for this pumping scheme. The spectrum of CG by pumping in anomalous
dispersion area ranges from 1.1 µm to 1.7 µm. Therefore, this CG is often called supercontinuum generation (SCG) due to such a broad band width. Up to 2 µm axial resolution has been achieved, but the power around 1.3 µm is very weak for real applications [40].

The invention of photonic crystal fiber (PCF) permits the possibility of SCG by using pulsed lasers at other wavelengths. PCF is different from SMF in that PCF shows the internal structure of the fiber. Figure 1.11 shows the cross section of a PCF scanning by SEM. The core of PCF is surrounded by air holes, which work as cladding in SMF. The most important property of PCF is that the dispersion profile can be engineered by modifying the diameter of the air holes and the pitch distance between the air holes. ZDWs of PCFs can be shifted between 700 nm to 1500 nm, which permits Ti:sapphire or other lasers for pumping in anomalous dispersion areas. The first SCG created by pumping a PCF with a Ti:sapphire laser was demonstrated in 2000 [41]. The PCF used there had ZDW around 720 nm and was pumped by a 100 fs Ti:sapphire laser centered at 800 nm. The SCG spectrum covered from 400 nm to 1500 nm. This kind of PCF was then used for generating broad band spectrum for UHR-OCT. A broad band spectrum centered at ~1.3 µm was achieved by filtering SCG and feeding it into an OCT system. Up to 2.5 µm axial resolution has been demonstrated [42]. Subsequently, several other lasers or PCFs have been used for generating broad band spectrum for UHR-OCT based on the similar mechanism [43-45].
Although the spectrum of SCG is very broad, SCGs are rarely used for clinical applications except as demonstration in research labs. This pumping scheme is either limited by the performance of the generated spectrum or by the lasers employed. In regards to lasers in the range of ~100 fs, first, since SCG is originated from higher order solitions fission, the spectrum is unstable and is very sensitive to the fluctuation of laser intensity \([46, 47]\). Therefore, the noise, including both shot noise and intensity noise, are amplified together, which will reduce the S/N of OCT. Second, the output spectrum is not smooth and requires that it be filtered when a specific wavelength range is desired. For lasers with ~20 fs pulses, the spectrum is smoother and the noise is lower. However, ~20 fs lasers are more expensive and difficult to manage for routine operation. For a wavelength range outside of the capabilities of Ti:sapphire lasers, it is still challenging to achieve such short pulses.

Due to the commercial availability of femtosecond lasers and broadband SLED at 0.8 µm, it is not difficult to achieve ultrahigh axial resolution at this wavelength range. However, for opaque tissue, a wavelength range between 1 µm to 1.4 µm is desired.
First, using a longer wavelength, a larger penetration depth can be achieved as a result of a reduction in scattering. The penetration depth at 1.3 µm is almost twice that at 0.8 µm. Second, a water absorption peak at 1.44 µm should be avoided because of a resultant deterioration in the system’s performance. Third, for InGaAs detector, the spectral respondence is maximized between 1 µm and 1.7 µm. As we discussed in this section, currently available light sources in this wavelength range still cannot satisfy the requirements of UHR-OCT. High performance broadband light sources should be further developed. In addition to the performance we described in section 1.4, the light source should be compact, reliable, fiber-based and easily usable, especially in a clinical setting.

In the following two chapters, we will propose three novel light sources based on the combination of multiple-SLEDs and SCG. Our goal is to develop compact and reliable broad band light sources for clinical application of UHR-OCT. The advantages and disadvantages of each light source will be discussed at the end of each chapter. Future improvements will also been noted.

1.6 COMPARISON OF OCT WITH OTHER IMAGING MODALITIES

In cancer diagnostics, excisional biopsy and histopathology are the gold standards. However, in many cases, they suffer from a high rate of false negative as a result of sampling errors. OCT enables noninvasive, high resolution, in vivo, two- or three-dimensional cross-sectional imaging of microstructure in biological tissue. This will help the early diagnosis of neoplastic changes without the need to excisionally
remove and process a sample, as is the case in conventional biopsy. By combining with optical fiber, OCT can be designed as a small fiber probe to image different organs in the body. However, since OCT is based on optical technology, it will be limited by some basic physical principles.

![Diagram showing comparison between different imaging modalities](image)

**Figure 1.12.** Comparison between different imaging modalities. $: cheap; $$: middle; $$$: most expensive.

Figure 1.12 compares the resolution, cost, imaging speed and depth of current popular imaging modalities. OCT fills the gap in resolution between several μm to tenths of μm with imaging depth around 1 to 3 mm. The cost of OCT is far less than other modalities. As with other optical imaging methods, OCT is still limited by imaging depth due to photon scattering and absorption, although it has been improved by almost one order. The imaging information of OCT comes from the coherent scattered photons; this makes OCT non-sensitive to fluorescence emission and permits OCT to image the original tissue conveniently without stain. However, OCT cannot image the molecular events that are resultant from molecular-level change—such as fluorescence. In order to circumvent this obstacle, recently contrast sensitive OCT has been proposed to image the signature of scattering or absorption, which is associated with probes. We
will discuss this topic later, specifically regarding the use of gold nanorods as probes.

1.7 AIMS OF THIS THESIS

1.7.1 Aim 1. Novel light sources for UHR-OCT

As we stated above, the currently developed broad light sources can not be readily used in clinical setting for reliably achieving high quantity ultrahigh resolution OCT especially above 1 μm. We are going to develop three novel light sources based on either SLEDs or supercontinuum generation at above 1 μm. For UHR-OCT, the expected axial resolution should be less than 5μm in the tissue. The newly developed light sources should be reliable, compact and low noise and have the ability for achieving high quantity images.

1.7.2 Aim 2. Using gold nanorods as a contrast for OCT

The success of the contrast sensitive OCT (CSOCT) depends on the contrast probes. In order to unambiguously distinguish the contrast, the scattering or absorption of the probes should have significant marks or can be external modulated. Scattering probes are highly desired for it can be potentially used for UHR-OCT and enlightening some invisible structure under normal OCT. Gold nanorods bears most of the properties for being ideal probes for CSOCT. We aim to develop the appropriate methods for unambiguously extract contrast information originated from gold NRs. Both external modulation method and spectroscopic analysis will be studied.

1.7.3 Aim 3. Biological applications with OCT

With available high speed UHR-OCT, we will study the feasibility of imaging intact mouse
embryos and fiber orientation of mouse embryo hearts. This is the essential step of imaging those samples in living state in future. Currently available technologies only can achieve 3D images through histological sectioning with intensive labor. With developed sliver glucose sensors, there is a requirement of tracking sensors \textit{in vivo} for a long time for inflammation responses. We are going to discuss the possibility of using OCT imaging implanted sensors in the mouse skin for long term tracking.
CHAPTER 2. COMBINATION OF MULTIPLE-SLED AT 1.3 µM FOR
UHR-OCT

2.1 INTRODUCTION

SLEDs are the most popular light sources for OCT because they are inexpensive, reliable and fiber based. These benefits allow SLEDs to be easily integrated into an OCT system and to be used in a number of various situations. Currently, the wavelengths of commercially available SLEDs can cover from 0.6 µm to 1.5 µm. For OCT, SLEDs centered at 0.8 µm, 1 µm and 1.3 µm are generally chosen for different applications. For ophthalmology, 0.8 µm and 1 µm are preferred, due to either the lowest water absorption or dispersion. For opaque tissue, the SLEDs at 1.3 µm are preferred for better penetration depth. Compared with other light sources such as supercontinuum generation based light, SLED is preferential [1-3]. However, SLED with a bandwidth above 1 µm cannot support a resolution of less than 5 µm in tissue.

The bandwidth of single SLED at 0.8 µm can reach between 60-70 nm, which corresponds to approximately 4-5 µm axial resolution in air. However, the bandwidth of single SLED at 1.3 µm is limited to approximately 40-75 nm, resulting in axial OCT image resolution of 12-15 µm in air (9-12 µm in tissue). OCT images with higher axial resolution clearly show improved image quality due to smaller speckle size and the ability to display finer structures with higher contrast, which can possibly
improve diagnosis in the clinic [1, 4].

The most straightforward method of generating broad band light sources is to combine multiple-SLEDs. Schmitt et al proposed improving OCT image quality by combining light emitting diodes (LED) with offset center wavelengths around 1300 nm, and demonstrated OCT imaging using two LEDs [5]. This demonstration resulted in OCT imaging with approximately 10 μm axial resolution in air, but optical power output was low. In this chapter, we demonstrate the use of an OCT light source centered at ~1.3 μm for high resolution OCT imaging that consists of four SLEDs with offset center wavelengths optimally combined using fiber couplers and current tuning. With ~145 nm of bandwidth and over 10 mW of output power, the combined SLED is fiber based, reliable, compact and can be easily integrated into an OCT system. OCT images of a stage 30 embryonic chick heart, using the combined light source and a single SLED, are compared using identical instrumentation and sample location.

2.2 METHOD AND RESULTS

The newly combined broadband SLED source was generated by combing four SLEDs, which were independently driven. To generate a broadband spectrum with the smallest ripples possible, each of the SLEDs was chosen according to its central wavelength and output power. The layout of the combination is shown in Fig. 2.1. S1325 (Inphenix, USA) has a central wavelength of 1325 nm, a full width half maximum (FWHM) bandwidth of about 70 nm, and an output power of 15 mW.
S1325 has similar specifications as the SLEDs popularly used singly in OCT systems. SLED S1355 (Denselight, Singapore) has a central wavelength of 1350 nm, a FWHM bandwidth of 50 nm, and an output power of 15 mW. The third and fourth SLEDs (S1265 and S1380) were custom built by WT&T (Canada) and have center wavelength at 1265 nm and 1380 nm. The center wavelength of S1265 and S1380 can be tuned over a 10 nm range. The spectrum of each SLED is shown in Fig. 2.2 (a).

The four SLEDs were combined using three broadband fiber couplers (FCs). The S1325 and S1355 were combined employing a FC (FC1) with a splitting ratio of 80:20, which is chosen for smooth combined spectrum shape. The S1355 was used for filling the gap between S1265 and S1380. S1270 and S1380 were combined using a 50:50 FC (FC2). The output of FC1 and FC2 were then combined by using another 50:50 FC (FC3) to generate the final output spectrum. The combined spectrum is plotted in Fig. 2.3 (a). The final output spectrum had a FWHM bandwidth of 145 nm, an output power of over 10 mW, and ripples less than 1 dB.

![Fig.2.1. Schematic of the OCT system with a broadband combined SLED light source for high resolution OCT imaging. PD: photodetector; Cir: circulator; TD: optical delay line; FC: fiber coupler; Ga: galvanometer scanner;](image-url)
A time domain OCT system (see Fig. 2.1) was built for coherence function measurement and for demonstrating the performance of the light source for OCT imaging. The combined broadband source was coupled into a 50:50 fiber coupler (FC4), which split the light into a reference arm and a sample arm. In the sample arm, the light was collimated and then focused onto the sample. In the reference arm, a galvanometer was used to generate a time delay. The light returning from the sample and reference arms interfered at the balanced detectors. The OCT signal was amplified, band-pass filtered and logarithmically demodulated before being sampled and displayed. The delay line scanned 3 mm at 15 Hz repetition rate. The center frequency of the filter was set to 300 KHz with 60 KHz of bandwidth.

System performance was characterized by measuring the coherence function by using a mirror as the sample. The coherence function of each SLED was measured and plotted in Fig. 2.2 (b). The shortest coherence length (13 µm) was measured with the S1325, which represents the typical coherence length of SLEDs commonly used for OCT. Fig. 2.3 (a) shows the coherence function of the combined spectrum. A coherence length of 6.5 µm was measured in air, corresponding to an axial point spread function of ~5 µm in tissue. Compared with typical single SLED light sources, the combined SLEDs improve the axial resolution by half and are comparable to the demonstrated resolution of broadband light sources generated by SCG [6, 7]. However, the combined SLED light source is more compact, inexpensive, reliable and portable. Although the spectral ripples are small, small side-lobes can be observed on the coherence function. These arise mainly due to the rectangular shape of the
combined spectrum.

Fig. 2.2. Individual SLEDs. (a) The spectrum of the SLEDs used for combination. The spectrum has been scaled according to output power. (b) Measured point spread functions and coherence length of each SLED.

Fig. 2.3 (c) presents the logarithmically demodulated signal. The plot has been scaled to reflect a 56 dB attenuation in the sample arm. The power from the reference arm was optimized to maximize the sensitivity, which was -102 dB for 3 mW on the sample. The lateral resolution was approximately 12 µm which is determined by the lens group used in the sample arm scanner.
Fig. 2.3. Combined SLEDs (a) Combined spectrum of SLEDs shown in Fig 1(a); (b) Measured point spread function of combined spectrum. (c) Measured demodulated point spread function in logarithmic scale.

Fig. 2.4 presents OCT images of an excised embryonic chick heart at stage 30 of development. The arrows in Fig. 2.4 (a) point to trabeculae in the heart. The image in Fig. 4 (a) was acquired using the combined broadband light source with an axial
resolution of 4.8 µm in the tissue. As a comparison, the OCT image in Fig. 2.4 (b) was obtained using a single SLED (S1325) with an axial resolution of 9.6 µm in tissue. Acquired images were ~ 2 mm × 4 mm (axial × transverse) with 2440 × 1000 pixels. For the single SLED image, the power on the sample was 1.2 mW. In order to keep the same sensitivity for the two acquisitions, the power on the sample with combined SLEDs was attenuated to the same level. Except for the power, the sample and optics instrumentation were kept identical for both acquisitions. Figures 2.4 (9Xa) and 2.4 (9Xb) are 9 times zoomed images of the boxed areas in 4(a) and 4(b) respectively.

The high resolution image in Fig. 2.4 (a) and Fig.2.4 (9Xa) demonstrates improved image quality when compared to the lower resolution image in Fig. 2.4 (b) and Fig.2.4 (9Xb). Specifically, the speckle size in (a) is just about half of that in (b) due to the shorter coherence length, and the fine structure of the trabeculations are better defined with higher contrast and sharp edge. These differences are important for quantitative image analysis, reducing speckle noise and improving segmentation of structures of interest.
Fig. 2.4. OCT images of an excised embryonic chick heart at stage 30 of development. (a) OCT image acquired with the combined SLED light source (axial resolution of ~5 \( \mu \)m in the tissue). (2440(h)×1000(v) pixels, 2mm(h)×4 mm(v)) (b) OCT image acquired with a single SLED light source (axial resolution of 9.5 \( \mu \)m in the tissue). (2440(h)×1000(v) pixels, 2mm (h) ×4mm (v) (9Xa) 9 times zoomed image of the boxed area in (a). (9Xb) 9 times zoomed image of the identical boxed area in (b). LV: Left Ventricle; RV: Right Ventricle; Tr: Trabeculae.
The combined broadband light source demonstrated here was realized with four SLEDs from different vendors. They are all compact, reliable and portable. The main drawback of this light source is the observable side-lobes shown in the coherence function. The side-lobes can be attributed to the rectangular spectral shape achieved when the multiple SLEDs were combined. We optimized the ripple to less than 1 dB by carefully selecting the component SLEDs and by adjusting the power and center wavelengths. As demonstrated in Fig. 4, the side-lobes are small enough that they are not apparent when imaging opaque tissue and image contrast was not compromised. If necessary, the side-lobes could be further reduced by digital spectral shaping [8]. This light source can be easily extended to FDOCT. By controlling the gain of each pixel, we may reshape the spectrum to suppress the
side-lobes.

A limitation of this light source, however, is its inefficiency. Of necessity, each fiber-optic coupler used to combine the SLEDs imposes, on average, a 50% loss. The approximately 10 mW output of this source represents approximately 25% of the total optical power output of the four individual SLEDs combined. While 10 mW is lower than the optical output available from some single SLEDs (e.g. S1325), it is sufficient for real time OCT imaging. Additionally, such output is significantly higher than previously available broadband LED or SLED combinations.

The bandwidth of the light source could be further extended by integrating more or broader SLEDs at the two sides of the spectrum. However, extending the spectrum longer than 1400 nm is not preferred due to a strong water absorption peak at 1440 nm, which will strongly attenuate that spectral range in tissue. The commercially available combined SLEDs (SuperlumDiodes Ltd.) has a shorter coherence length—around 4.5 μm in air, corresponding to a bandwidth of 230 nm. However, because the center wavelength is approximately 1375 nm, a significant component of the spectrum is beyond 1400 nm and can be expected to be seriously attenuated in biological tissue. In addition, the optical power is limited to 5 mW. Extending the spectrum less than 1250 nm would be advantageous, but would depend on the availability of SLEDs at this wavelength range.

In conclusion, we have demonstrated a broadband light source for high resolution OCT by combining four SLEDs. The smooth spectrum has a bandwidth of ~145 nm and over 10 mW of output power. The measured coherence length is
approximate 6.5 µm in air (4.8 µm in tissue), which enables imaging at approximately half the axial resolution of OCT imaging with a single SLED. The resolution performance of this light source is similar to previously demonstrated broadband light sources, which pump fibers with femtosecond lasers—such as the Cr:foesterite laser—but is less expensive, more compact and more portable. For direct comparison, two images with different axial resolution were acquired under identical conditions. High-resolution imaging with the new light source revealed improved visibility of fine structures. When implemented in real-time OCT, this light source could be well suited for biomedical applications of OCT imaging, especially for clinic applications.
CHAPTER 3. SUPERCONTINUUM GENERATION BASED LIGHT SOURCES FOR UHR OCT

As discussed in Chapter 1, previously SCG light sources were generated by pumping a photonic crystal fiber (PCF) in the anomalous dispersion area. They have been limited either by insufficient power at the desired wavelength [1] or by spectral modulations [2]. The intensity noise from the pump laser can be amplified during the SC generation [3], which will affect the image quality. Continuum generated in a single-mode fiber by pumping in the normal dispersion area do not amplify intensity noise [4], but the continuum is limited to a band around the wavelength of the pump laser. While femtosecond Cr:forsterite lasers emitting at 1300 nm are commercially available, they are expensive and less compact and convenient for clinical applications [5].

In this chapter, we will discuss two novel broad band light sources for UHR-OCT based on the SCG and PCF. The light sources we demonstrated here both have advantages of two pumping schemes, broad band and low intensity noise. In order to understand the mechanism behind the SCG, we will first describe the numerical simulation of SCG with non-linear fiber equations. Then we will discuss two light sources based on SCG with different PCFs. One PCF with two closely spaced ZDWs is used to generate a dual-band broad band light source at 0.8 μm and 1.3 μm simultaneously. Another PCF with no ZDW can generate a broad band light source centered at 1.15 μm. Both PCFs were pumped by a compact and turn key Nd:glass
femtosecond laser centered at 1.06 μm. The possibility of further improvements will be discussed at the end of the chapter.

3.1 Numerical Simulation of Pulses Propagation in the Single Mode Fiber

3.1.1 Nonlinear Schrödinger Equation (NLSE)

From the Maxwell equation and the assumption of one dimensional propagation of light in the fiber, the nonlinear Schrödinger equation (NLSE) can be used to describe the temporal propagation of pulses in the fiber [6].

\[
\frac{\partial A(z,\tau)}{\partial z} = \sum_{k=0}^{n} \left( -i \frac{(\beta_0^k)}{k} \frac{\partial^k A(z,\tau)}{\partial \tau^k} \right) - \Gamma(\omega_0)A(z,\tau) - (i + \frac{1}{\omega_0} \frac{\partial}{\partial \tau}) Q_{\text{ker},A}(z,\tau) \left| A(z,\tau) \right|^2 - (i + \frac{1}{\omega_0} \frac{\partial}{\partial \tau}) Q_{\text{Raman},A}(z,\tau) FT^{-1} \left\{ S_{\text{Raman}} FT_\Omega \left[ \left| A(z,\tau) \right|^2 \right] \right\} \tag{3.1}
\]

It can also be written in the frequency domain by inverse Fourier transform [7] as

\[
\frac{\partial A(z,\Omega)}{\partial z} \cong A(z,\Omega) \left[ -i \Delta(\Omega) \right] - \{\alpha(\Omega)\} \left( \text{Dispersion} \right) - i(1 + \frac{\Omega}{\omega_0}) Q_{\text{ker},FT_\Omega} \left[ A(z,\tau) \left| A(z,\tau) \right|^2 \right] \left( \text{Kerr effect} \right) - i(1 + \frac{\Omega}{\omega_0}) Q_{\text{Raman},FT_\Omega} \left[ A(z,\tau) \left| S_{\text{Raman}} FT_\Omega \left[ \left| A_{\text{total}}(z,\tau) \right|^2 \right] \right| \right] \times \frac{1}{A(z,\Omega)} \left( \text{Raman effect} \right) \tag{3.2}
\]
3.1.2 Parameters Definition

We define the corresponding parameters as follows:

- **Total field** $A(z, \Omega)$ and $A(z, \tau)$

The electrical field of each frequency in the fiber is

$$E(z, \omega, r) = \psi(\omega, r) E_0(\omega, z) \exp[i(\omega \tau - \beta(\omega) z)] \quad (3.3)$$

Where $\psi(\omega, r)$ is effective mode radius. By integrating the entire field of frequencies, we can get the total field expression

$$E_{\text{total}}(z, \tau, r) = \frac{1}{2\pi} \int_{\omega_0}^{\infty} \psi(\omega, r) E_0(\omega, z) \exp[i(\omega \tau - \beta(\omega) z)] d\omega \quad (3.4)$$

where $\omega_0$ is the center frequency and $\Omega$ is the offset of frequency to center frequency.

Replacing all $\omega$ with $\omega_0 + \Omega$ and extending the wave number according to the central frequency, we get

$$E_{\text{total}}(z, \tau, r) = \frac{1}{2\pi} \psi(r) \exp[i(\omega_0 \tau - \beta(\omega_0) z)] \int_{\omega_0}^{\infty} E_0(\omega_0 + \Omega, z) \exp[-i(\Delta \Omega \tau)] \exp[i\Omega(\tau - \beta(\omega_0) z)] d\Omega$$

$$= \frac{1}{2\pi} \psi(r) \exp[i(\omega_0 \tau - \beta(\omega_0) z)] \int_{\omega_0}^{\infty} E_0(\omega_0 + \Omega, z) \exp[-i(\Delta \Omega \tau)] \exp[i\Omega \tau] d\Omega$$

$$= \psi(r) \exp[i(\omega_0 \tau - \beta(\omega_0) \tau)] E_0(\tau, z) \quad (3.5)$$

Here $\Delta \Omega$ is the sum of higher order dispersions. Because only power $P_{\text{scale}}$ can be measured, it is reasonable to express the electrical field intensity with power.

$$E_0 = \left[ \frac{2P}{\gamma_{\text{cu}}} A_{\text{eff}} \right]^{\frac{1}{2}} = \left[ \frac{2P_{\text{scale}}}{\gamma_{\text{cu}}} A_{\text{eff}} \right]^{\frac{1}{2}} A(z, \tau) = \kappa A(z, \tau) \quad (3.6)$$

So

$$E_{\text{total}}(z, \tau, r) = \kappa \psi(r) \text{Re}[A_{\text{total}}(z, \tau) \exp[i(\omega_0 \tau - \beta(\omega_0) z)]]$$

$$A(z, \tau) = \frac{1}{2\pi} \int A(z, \Omega) \exp[i\Omega \tau] d\Omega \quad (3.7)$$
Here, $A_{\text{eff}}$ is effective radius of the fiber.

- **Fiber loss**

Fiber loss is often defined as dB/Km. However, for SCG, at most only several meters of fiber are employed, so this parameter is often neglected. For simulation with hundreds meters of fiber this parameter should be considered.

- **Kerr Nonlinear Coefficient $Q_{\text{kerr}}$**

The Kerr effect includes SPM and FWM. This effect is described by the measured nonlinear refractive index $n_E^2$, which comes from the susceptibility $\chi^3$ as defined by units measured as $(m/V)^2$ and is defined as $\frac{3\chi^{(3)}}{8n_0}$. The change of refractive index can then be defined as

$$\Delta n = n_E^2 |E|^2 = \frac{2In_2}{\varepsilon_0cn_0} m^2/W = \frac{3\chi^{(3)}}{4\varepsilon_0cn_0} (m^2/W) \frac{P}{A_{\text{eff}}}$$

So the Kerr nonlinear coefficient is

$$Q_{\text{kerr}} = \frac{\omega_0}{c} \frac{3\chi c n_0}{4n_0^2} \frac{P_{\text{scale}}}{A_{\text{eff}}} = \frac{2\pi}{\lambda} n_2 \frac{P_{\text{scale}}}{A_{\text{eff}}}(m^{-1}) = \gamma P_{\text{scale}}$$

Assuming $n_2 = 2.6 \times 10^{-20} m^2/w$

$$\text{The } Q_{\text{kerr}} = 0.165 \frac{P_{\text{Scale}}}{\lambda A_{\text{eff}}} \text{ and } \gamma = \frac{2\pi n_2}{\lambda A_{\text{eff}}}(mW)^{-1}$$

- **Raman Gain Coefficient**

When a molecule interacts with incident photons, most of photons will be elastically scattered; this is termed Rayleigh scattering. However, a small part of the radiated photons will be transferred into the vibration modes of the molecule and is downshifted to a longer wavelength. This is called Raman effect. When the pumping power is high, stimulated Raman scattering may happen. One of the essential parameters for this process is Raman coefficient, which defines the gain of Raman
scattering and is frequency dependent.

Raman coefficient in Eq. (3.2) can be equated with Eq. (3.10) by considering silica as fiber material

$$Q_{\text{Raman}} = \frac{1}{2} g_{\text{max}} \frac{P_{\text{scale}}}{A_{\text{eff}}} = \frac{1}{2} \frac{1.2 \times 10^{-11}}{\lambda(\mu m)} \frac{P_{\text{Scale}}}{A_{\text{eff}}}$$  \hspace{1cm} (3.10)

where $g_{\text{max}}$ is the maximum value of Raman gain of silica. The $S_{\text{Raman}}$ in the equation is Raman gain spectrum and is a function of frequency shift, which can be measured. In our simulation, we digitalized the measured data from the reference [8].

3.1.3 Numerical solution

NSLE can be solved either in the time domain or in the frequency domain. In the time domain, the typical algorithm is called the split-step Fourier (SSF) method [6]. In this thesis, we solved NSLE in the frequency domain because it has distinct advantages over the time domain. Typically, in pulsed lasers, higher order dispersion and Raman gain spectrum are all measured and easily defined in the frequency domain. Thus, it is more natural to solve them in the frequency domain. Francois’ paper [7] offers excellent insight into this issue.

Runge-Kutta Fehlberg Method was used here to solve NLSE. During simulation, the number of sampling points varied from 2048 to 4096, so in fact 2048 to 4096 equations should be solved simultaneously, which requires more time than the SSF method. The dispersion order was included to the 17th order to accurately fit the dispersion profile of fibers. All the code was run in the Matlab.
3.2 Dual Band Broad Band Light Source for UHR-OCT

As discussed in previous sections, a light source with improved performance can be achieved by pumping a fiber in the normal dispersion area. However, they are limited either by band width or by femtosecond lasers. In order to achieve broad band spectrum, the ideal pumping wavelength should rest at a wavelength close to zero dispersion, but this could potentially induce non-stable SCG due to the fact that part of the wavelength will go into an anomalous dispersion area [2]. Ideally, if the dispersion profile can be near zero dispersion in a relative large wavelength range, the generated spectrum will be broad and non-stable SCG can be simultaneously avoided.

Previously, a dual band SC light source was demonstrated in a PCF with two ZDWs at around 0.8 μm. However, these two bands are either visible or too narrow at 1.1 μm due to fiber loss at longer wavelength and are thus not attractive for OCT applications [9]. In this section, we present a continuum spectrum by pumping a novel PCF, which has a convex dispersion profile and two closely spaced ZDWs at around 1 μm. For convenience, we named this PCF as PCF1. A dual band spectrum at 0.8 μm and 1.3 μm can be generated simultaneously. Both of the two bands can be used for UHR-OCT. We will first demonstrate the experimental output spectrum by pumping the PCF1 with a Nd:glass laser. Many small modulations can be observed in the two bands. Numerical simulation will be used to disclose the mechanism of continuum generation and to compare with our experiments. Then we will propose two methods of smoothing the continuum spectrum, demonstrating an improved dual-band continuum light source with superior properties for OCT imaging, especially at 1300
First, we experimentally demonstrate optimization of each band separately by using polarization control of the pump laser light. Polarization dependence of supercontinuum generation (SC) has been studied in PCF with one ZDW [10, 11]. Even PCF designed for low birefringence exhibits appreciable birefringence. Using this method, we present ultrahigh resolution, \textit{in vivo} images of biological tissue at 830 nm and at 1300 nm. Second, based on the simulation, we further propose the use of very short pump pulses (<50fs) as well as polarization control to smooth the spectrum. In this case, both bands are simultaneously broad and smooth enough for ultrahigh resolution OCT without appreciable side-lobes. OCT imaging of human colon tissue is demonstrated \textit{in vitro}. We directly compare ultrahigh-resolution OCT imaging achieved with this novel light source and conventional-resolution OCT imaging using a super luminescent diode (SLD) light source by imaging the same sample with the same OCT system and switching the light sources. This experiment clearly showed improved image quality under higher resolution.

\subsection{Continuum generation with PCF with two close spaced ZDWs}

We used an NL-1050-ZERO-2 fiber (Crystal Fiber A/S) for continuum generation. For use in this work, we calculated the dispersion profile of the PCF with mode solver (Mode Solutions, Lumerical) using air-hole pitch and diameter measured by scanning electron microscopy (SEM) (Fig. 3.1 inset). The calculated dispersion profile is shown in Fig. 3.1, with error bars generated by considering the ±2\% standard deviation of the air-hole diameter measured from the SEM. The PCF has a
convex dispersion profile that results in two closely-spaced ZDWs at 990 nm and 1123 nm. The maximum dispersion is very low, approximately 2 ps/nm/km.

The pump laser used in our experiment was a mode locked Nd:glass femtosecond laser (IC-150, High Q) operating at a repetition rate of 74 MHz at 1059 nm. The laser is a compact turn-key system and has been successfully used for ultrahigh resolution OCT imaging at around 1.05 μm [4]. The pulses have a FWHM length of 130 fs and an average power of 150 mW. About 50% of the pump light was coupled into the PCF by an aspheric lens (C230 Thorlabs). A half-wave plate was located in front of the aspheric lens to allow for control of the polarization state of the light coupled into the PCF. The output end of the PCF was then spliced to HI1060 single-mode fiber coupled into a fiber-based or free-space interferometer for measuring the OCT axial PSF.

---

**Fig.3.1.** Dispersion profile of the photonic crystal fiber (NL-1050-ZERO-2) used in this work. Two zero dispersion wavelengths are shown at 990 nm and 1123 nm.
1123 nm. The inset is a scanning electronic micrograph of NL-1050-ZERO-2 used to calculate the dispersion profile.

We experimentally measured continuum generation in five lengths of PCF, 0.15 m, 0.3 m, 0.7 m, 1.2 m, and 1.6 m and found that the spectrum of the continuum was relatively smooth when the fiber length was less than 0.7 m, but the two bands were relatively narrow. The continuum bandwidth was fully extended after 1.2 m, so 1.2 m of PCF was used in our subsequent OCT experiments (with the exception of the compressed-pulse experiment described below). Fig. 3.2 (a) shows the continuum spectra generated in five lengths of PCF without polarization control. Fig. 3.2 (b) shows the equivalent numerically simulated spectra (see below). The numerical results are in good qualitative agreement with the experimental results, especially in the 1300 nm band. The obvious features of the spectra are the two bands centered at 830 nm and 1300 nm. Almost all of the pump power at 1059 nm is efficiently transferred into these two bands. Spectral modulations were observed on the two bands with 1.2 m PCF, which will induce side-lobes in OCT axial PSFs. In the 830 nm band, the average power is about 27 mW and FWHM of the band is 83 nm, measured with short-pass optical filter and optical spectrum analyzer. The measured power of the 1300 nm band using a long-pass filter is about 42 mW and the FWHM bandwidth of the band is around 120 nm. These two bands represent the most common wavelengths used for OCT imaging and both bands provide sufficient power and bandwidth for ultrahigh resolution, real-time OCT imaging.
Fig. 3.2. Continuum generated experimentally (a) and numerically (b) with different lengths of NL-1050-ZERO-2 PCF. The continuum spectrum is relatively smooth when PCF is less than 0.7 m, but the side bands are only fully extended after 1.2 m. Significant modulations can be observed on the two side bands after 1.2 m PCF.

3.2.2 Smoothing continuum spectra

The major limitation of the continuum spectra demonstrated here and previously is the presence of significant modulations on the spectrum, which reduce the contrast and quality of OCT images by introducing high and broad side-lobes on the axial PSFs. We have investigated two methods of reducing these undesirable modulations for discussion here. First, we will introduce polarization control of the
pump light launched into the PCF and experimentally demonstrate the resulting reduction of spectral modulations. Second, we will discuss the possible origin of the modulations based on numerical simulation and numerically and experimentally demonstrate generation of a smooth continuum spectrum by pumping the PCF with very short pulses (<50fs).

3.2.2.1 *Smoothing continuum spectrum with polarization control*

We have observed that the continuum spectrum can be significantly modified by controlling the polarization state of the pump laser. The continuum generation can be attributed to self-phase modulation (SPM), degenerate four-wave mixing (DFWM) and stimulated Raman scattering (SRS) as described in section 3.2. It is understood that SPM and DFWM in fiber are sensitive to the polarization state of pump light if the fiber has birefringence [6]. The gain of SRS is also polarization dependent [12]. As shown in the SEM image in Fig. 3.1, the core of the PCF is surrounded by six air holes and is more hexagonal than circular. Irregularities in the diameter, pitch, position and shape of the air holes, could induce appreciable birefringence [13]. Considering only the standard deviation of the diameter of the air holes, we estimate the birefringence of the PCF we studied to be roughly $5 \times 10^{-5}$ at 1059 nm [13]. Therefore, by controlling the polarization state of the pump laser, we expect to be able to manipulate the modulations on the continuum spectrum.
Fig. 3.3. Smoothing of the spectrum of continuum at 1300 nm (a) and 830 nm (b) with polarization control. The dashed line is the spectrum of the pump laser.

A half-wave plate was placed in front of the focusing lens to generate different linear polarization states of the pulses launched into the PCF. Fig. 3.3 (a) displays the continuum spectrum with the 1300 nm band optimized and Fig. 3.3 (b) shows continuum spectrum with the 830 nm band optimized. Compared with Fig. 3.2, the optimized spectral bands are obviously smoother. However, we found that it is difficult to smooth the continuum in the two bands simultaneously. The axial PSFs corresponding to the optimized bands are presented in Fig. 3.4. The coherence length of the smoothed 830 nm band is about 3.8 μm in air (corresponding to an axial PSF in tissue of about 2.8 μm), which corresponds well with the measured bandwidth of 83 nm FWHM. The 1300 nm band has a coherence length of around 6.3 μm (corresponding to an axial point-spread function in tissue of about 4.5 μm), which
corresponds well with the 120 nm FWHM bandwidth. In order to demonstrate the suppression of side-lobes with polarization control, Fig. 3.5 compares the axial PSF envelopes in the 830 nm band (a), and the 1300 nm band (b) at the two input polarization states resulting in best case and worst case spectral modulation. We observe an average suppression of the side lobes in the 1300 nm band and the 830 nm band by up to 7.7 dB and 5.5 dB, respectively, by choosing the optimum polarization state for each. In our experiments, we observed that the magnitude of the modulations was sensitive to the angle of linear polarization, with the optimum angle existing in a narrow range of about ten degrees. We neither defined nor monitored the birefringent axis of the PCF, so the absolute optimum angle was not determined. Although polarization control does not completely avoid the spectral modulations, this method is a practical way to optimize the spectrum when pumping the PCF with the direct output of a laser that is currently commercially available.

Fig.3.4. Point spread function (PSF) of the smoothed continuum at 830 nm and 1300 nm by polarization control.
Fiber-based time-domain OCT interferometers were built to separately acquire images at 830 nm and 1300 nm. Two different broadband couplers and detectors were used for the two bands. Due to the losses in the various fiber connectors, the average power on the sample was 10 mW at 1300 nm and 3 mW at 830 nm. Spectral filtering was not used to separate the two bands because the bands are sufficiently distant from each other. Thus, the wavelength response of detectors and fiber couplers was sufficient to prevent cross-talk. Using spectral filters before the detectors, we confirmed that the presence of the 830 nm band at the 1300 nm detector did not introduce additional photon noise, and vice versa. Because we spliced HI1060 single-mode fiber to the output of the PCF, it was not necessary for us to separate the bands in order to couple the light into the interferometers. Dispersion mismatch in the OCT interferometer was avoided by using the same lens group in the two arms of the interferometer. The lateral resolution of the sample scanner was around 10 μm with a NIR achromatic lens with 40 mm focal length. The A-scan rate was 12 Hz for the 1300 nm image and 8 Hz for the 830 nm image. A balanced InGaAs detector was used at 1300 nm. A single Si detector was used at 830 nm. The measured sensitivity of the systems was 108 dB at 1300 nm and 100 dB at 830 nm.
Fig. 3.5. Suppression of the PSF side lobes due to modulation on the 1300 nm band and the 830 nm band using linear polarization control. Shown are the best case (dashed line) and the worst case (solid line).

Fig. 3.6 shows images of a human finger nail bed \textit{in vivo} at 1300 nm and a mouse ear \textit{in vivo} at 830 nm. These images demonstrate that fine structures can be resolved using both wavelength bands, although the full benefit of the resolution may not be realized due to incomplete suppression of the side-lobes induced by spectral modulation.

Fig. 3.6. (a) OCT image of a human finger nail bed \textit{in vivo} at 1300 nm. The top surfaces are a glass slide and transparent gel. (740(h)×1000(v) pixels, 6mm(h)×1.5mm(v)) (b) OCT image of a mouse ear \textit{in vivo} at 830 nm. The thickness of mouse ear is between 300\(\mu\text{m}\) and 400\(\mu\text{m}\). (800(h)×750(v) pixels, 4mm(h)×0.6mm(v))

3.2.2.2 \textit{Pulse length and fiber length optimization}
In order to further investigate the optimization of the continuum spectrum, we simulated the pulse propagation in the PCF with the total-field nonlinear Schrödinger equation [7]. Self-phase modulation (SPM), four-wave mixing (FWM) and stimulated Raman scattering (SRS) were all included in the simulation. In the model, we assumed that the effective mode area is independent of wavelength and neglected polarization sensitivity of the PCF. Except where otherwise noted, the simulations were designed to match our experimental parameters (130 fs pulses at 1059 nm pumping 1.2 m of PCF and 45 fs pulses pumping 0.2 m of PCF). Based on the results illustrated in Fig. 3.7, we conclude that the SC develops in the PCF as follows: When the femtosecond pulse is coupled into the PCF, the spectrum is quickly broadened by SPM as a result of the flat and low group dispersion during the first tens of centimeters in the PCF. We observe that after 50 cm the spectrum is not farther broadened. However, the energy distribution in the two bands is not uniform. SPM seeds new frequencies for SRS, which can help to transfer and redistribute the energy in the two bands at 830 nm and 1300 nm and induce some modulations in the spectrum. Maintaining high peak power after tens of centimeters facilitates the occurrence of SRS and is possible because the pulses are first compressed in the abnormal dispersion area and reach higher peak power than the original pulse. After 30 cm propagation, the peak power in the two narrow bands at 900 nm and 1200 nm is still nearly as high as 1.8 kW. The convex dispersion profile at the two ZDWs also permits a longer walk-off distance because of the flat group velocity area. With further propagation, the contribution of SRS becomes obvious and induces some
spectral modulation on the inner side of the two side bands, where the walk-off
distance is longer than at the wavelengths at the outside of the two bands. The
contribution of SRS also has been observed previously, when 109 fs pulses were used
to pump a one-ZDW single-mode fiber near the ZDW [14]. In general, we observe
that the contribution of SRS is not negligible when longer pulses and longer lengths of
PCF are used. In this case, the spectral modulations become serious after about 0.7 m.

Fig. 3.8 shows the simulated continuum spectrum without (Fig. 3.8a) and with (Fig.
3.8b) SRS included in the model. Two peaks at 936 nm and 978 nm, separated by
about 13.2 THz; the characteristic Raman shift for silica fiber, can be observed in Fig.
7.8b. However, the red shift peaks induced by SRS are easily merged by the
interaction with other effects such as SPM. It is apparent that SRS significantly
contributes to the modulation on the spectrum.

![Fig. 3.7. Simulated spectrum development as pulse propagates in the PCF. Dashed lines show the two ZDWs.](image)

Other effects could also contribute to the modulation on the two bands. By careful
inspection of the spectrum in Fig. 3.7 (a), small ripples can be observed. We found
that these ripples become more obvious when the dispersion profile is up-shifted. The up-shift will increase the maximum GVD and further separate the two ZDWs. This phenomenon can be attributed to cross phase modulation (XPM) and the interference of light that is temporally separated, but spectrally overlapped [15]. However, further up-shift of the dispersion profile would introduce soliton self-frequency shift and amplification of dispersive waves [16], which were not observed in our case. The positions of the two ZDWs and the maximum dispersion of this type of PCF are very sensitive to the microstructure of the fiber, as shown in Fig. 3.1, such as air hole diameter. This sensitivity may have caused variation of the dispersion profile along the fiber and error in our estimation of the dispersion profile, which could contribute to the modulations apparent on the outer side of the two bands, but not present in our simulation in Fig. 3.2. These ripples are also sensitive to pulse width and fiber length. Therefore, the optimum pump scheme and fiber length may vary with particular PCF specimen.

Fig.3.8. Numerically simulated continuum generated in 1.2 m of NL-1050-ZERO-2 PCF. (a) Only includes Kerr effect (SPM and DFWM); (b) Kerr and Raman effects are
Contribution of SRS to spectral modulation is apparent in (b). Two peaks at 936 nm and 978 nm in (a) is separated about 13.2THz.

The contribution of the SRS and interference can be reduced when fiber length is short as shown in Fig. 3.2—where the spectrum is smooth at the length of 0.3 m PCF. However, the pulses must be both short and of sufficient intensity to fully extend the spectrum. We observe in the simulation that using shorter pump pulses and a shorter length of PCF allows the modulations to be almost completely eliminated. With 0.2 m of PCF and a 45 fs pulse at 1059 nm with 40 mW of average power in the PCF, our numerical simulation generates two smooth bands with near-Gaussian shapes, as shown in Fig. 3.9, except a dip as shown in the spectrum. This could be an excellent OCT light source, providing ultrahigh resolution with little or no contrast reduction due to extended side-lobes.

Due to the lack of a commercially available ultra-short-pulse fs laser (<50fs) at around 1 μm, we experimentally generated such pulses using the previously-described Nd:glass laser. The 130 fs pulses were first coupled into a 30 cm single-mode fiber (SM980). The spectrum was broadened to around 43 nm (FWHM) with a 100 mW output average power by SPM, as shown in Fig. 3.10 (a). Two SF11 prisms were separated by about 1 m, to compress the pulses from 130 fs to around 45 fs, as measured using an autocorrelator by assuming sech pulses, as shown in Fig. 3.10 (b). The compressed pulses were then coupled into a 20 cm PCF after passing through a half-wave plate. The average output power from the PCF was around 40 mW, which corresponds to 25% coupling efficiency after pulse compression and two fiber couplings.
Fig. 3.9 (b) shows the spectrum generated by pumping with the compressed pulses and optimizing using polarization control. Compared with the spectrum generated with 130 fs pump pulses, the spectrum generated with compressed pump pulses is significantly smoother. Both bands show much lower modulations. The smooth and near-Gaussian shapes, especially at 1300 nm, are excellent for OCT imaging because the resulting axial PSFs should exhibit negligible shoulders and side-lobes (see below). The FWHM bandwidth is 90 nm with 12 mW average power at 830 nm and 130 nm with 26 mW average power at 1300 nm. The large dips shown in the simulated bands are not present in the experimentally generated spectrum, apparently merged by XPM. The numerical simulation does not account for XPM because it does not include polarization. Fig. 3.11 shows the PSFs for the two bands, displayed both linear (a) and (b) and demodulated in log scale (c) and (d), measured with a free-space interferometer. Both PSFs show clean and short side-lobe tails that do not extend far above the noise floor because of the smooth spectrum shape.
Fig. 3.9 (a) simulated spectrum; and (b) spectrum experimentally generated with polarization control by pumping 0.2 m PCF with 45 fs pulse and 40 mw average power in the PCF.

Fig. 3.12 (a) and (b) demonstrate OCT images of human colon tissue in vitro achieved with this dual-band light source at 830 nm and 1300 nm. As a comparison, an SLD at 1310 nm with axial resolution of 14 μm was used to generate the OCT image shown in Fig. 12 (c) with the identical instrumentation and tissue sample as we used with the 1300 nm band of dual-band light source. The improved OCT image quality under higher resolution is readily apparent. Specifically, the edges are sharper and speckles are smaller. With the 830 nm band of the dual-band light source, the image quality is further improved. However, penetration depth is about half that of the 1300 nm images due to higher scattering.

The drawback of using pulse compression could be the complexity of the setup and relatively lower power due to the losses associated with pulse compression and fiber coupling. These drawbacks may be eliminated as appropriate sub-50 fs pulsed lasers become commercially available in the 1.05 μm wavelength range. Then, the PCF could be directly pumped with ultra-short pulses. A 47 fs [17] diode pumped laser and a 33 fs [18] fiber laser have been recently realized although the average output power is not yet sufficient for this application. However it is likely that an appropriate pump laser for this proposed OCT light source will be commercially available in the future.
Fig. 3.10. Spectrum generated in 0.3 m SC980 (a) and autocorrelation trace of short pulse generated by prism compression (b).

Fig. 3.11. Point spread functions measured with a free space interferometer with spectrum optimized by polarization control.
Fig.3.12. (a) OCT image of human colon in vitro at 830 nm with axial resolution of 2.8 μm in tissue. (1500(h)×3500(v) pixels, 5mm(h)×2.4mm(v)) (b) OCT image of human colon in vitro at 1300 nm with axial resolution of 4.5 μm in the tissue. (1500(h) ×3500(v) pixels, 5mm (h) ×2.4mm (v) (C) OCT image of the same tissue and using the same interferometer as used in (b), but using a common SLD light source at 1310 nm with axial resolution of 11 μm in the tissue.
3.2.3 Discussion and Conclusion

In conclusion, we have presented a dual-broadband continuum light source for OCT that utilizes PCF with two closely-spaced ZDWs. The two bands are generated at 830 nm and 1300 nm, the primary OCT imaging wavelengths, with broad bandwidths and sufficient power for ultrahigh resolution, real time OCT imaging. Implemented by pumping the PCF with a commercially available femtosecond laser at 1050 nm, the spectrum exhibited modulations that resulted in undesirable side-lobes on the axial PSF. We have demonstrated generation of a smooth spectrum by use of polarization control and by using shorter pump pulses (<50 fs). We have observed birefringence in the PCF, probably due to irregularity of the PCF structure. By using polarization control, we demonstrate up to 7.7 dB suppression of the side-lobes. We have demonstrated in vivo ultra-high resolution OCT imaging at 830 nm and at 1300 nm using this light source.

According to our numerical investigation, we discussed the origin of the spectral modulation, showing an obvious contribution by SRS. The spectral modulation may also be influenced by XPM and by interference due to variability of the dispersion profile along the fiber. We demonstrated that the spectrum can be significantly smoothed by using shorter pump pulses (<50 fs) and the appropriate length of PCF, which depends on the particular pulse parameters. By fiber-prism pulse compression, we experimentally generated a smooth dual-band light source at 830 nm and 1300 nm by pumping 0.2 m PCF with 45 fs pulses. Broadband superluminescent diodes at 830 nm are commercially available. However, a smooth, broad band, near-Gaussian
shaped, highly efficient and low noise OCT light source at 1300 nm is not currently commercially available and has been demonstrated only using Cr:fosterite femtosecond lasers [5, 19]. The light source we have demonstrated here represent a promising alternative to Cr:fosterite lasers for OCT imaging at 1300 nm. This work demonstrates the feasibility of a compact, high-power dual-band light source for ultrahigh resolution OCT imaging in the near future, as ultrafast high-power femtosecond fiber lasers are being rapidly developed.

3.3 Broad Band Light Source at 1.15 μm for UHR-OCT

We have presented a dual band broad band light sources based on pumping a PCF with two close spaced ZDWs for UHR-OCT. Although the axial resolution has been achieved to around 6 μm in air at 1.3 μm band, it will be attractive to improve axial resolution farther to 2 or 3 μm axial resolution.

The band width of the light source is proportional to the axial resolution. For 2 μm or 3 μm resolution, the band width will be more than 200 nm in NIR range. As we have discussed, in order to achieve better penetration depth in opaque tissue and to cover the effective responsivity of InGaAs detectors, it is reasonable to choose a wavelength range of the light source between 1 μm to 1.4 μm [20]. It has been shown through numerical simulation that for light sources with center wavelengths above 1.2 μm, it is difficult to achieve axial resolution better than 2 μm to 3 μm at depth in tissue due to absorption and imperfect dispersion compensation [21]. Therefore, a broad-bandwidth light source for UHR-OCT centered between 1.1 μm and 1.2 μm
could be advantageous for mitigating resolution degradation, covering the effective wavelength range of InGaAs detectors and maximizing imaging penetration depth.

Similar resolution has been demonstrated by pumping a PCF in the anomalous dispersion area with a Ti:Sapphire laser. Broad band light sources centered at around 1.3 µm and 1.1 µm have been used to achieve 2.5 µm and 1.8 µm axial resolution (in air) with UHR-OCT [22, 23]. Further, by using a compact fiber laser at 1 µm to pump a PCF just below the ZDW, a SC covering from 800 nm to 1300 nm was also demonstrated for UHR-OCT [24]. However, when femtosecond laser pulses are launched near the ZDW of PCF, SC is generated by the excitation of unstable solitons [25]. Therefore, these light sources show large spectral modulations and tend to be noisy [3, 26]. 15 dB dynamic range reduction due to noise amplification has been reported as compared with SC based on self-phase modulation (SPM) [27]. In addition, a spectral filter was required for achieving the desired spectral range, which reduced the output power and increased the complexity of the system [1, 22]. Therefore, SC generation in the normal dispersion area by SPM is preferred for UHR-OCT, because this can achieve a smooth spectrum, low noise and high output power [27]. However, SC based on SPM in conventional single-mode fiber generally results in a relatively narrow bandwidth and the output wavelength is limited to sizes approximating the pump wavelength. By pumping a UHNA fiber with a Nd:glass laser centered at 1.059 µm, a bandwidth of 139 nm has been demonstrated. The axial resolution in the air is around 5 µm [4]. Further extension requires higher peak power coupled into the fiber, which will increase the cost and size of the laser.
In this section, we demonstrate a new SC light source centered at 1.15 µm by pumping another PCF with the same femtosecond laser at 1.059 µm. The SC is attributable to SPM, so the spectrum is smooth and Gaussian-like with very low side-lobes and low noise, because there is no noise amplification by solitions excitation. Also, because of the unique dispersion profile of this PCF, the SC is extremely broad, resulting in a clean, narrow point spread function (PSF) of only 2.8 µm (in air). Moreover, the center wavelength of SC is shifted from the pump wavelength of 1.059 µm to 1.15 µm, which is desirable, as described above. This PCF transfers most of the power of the pump laser to a very broad band in the desired wavelength range, but still maintains the benefits of SC generation by SPM and has sufficient power for real-time UHR-OCT.

3.3.1 Experimental Setup

Fig.3.13. (a) Dispersion profile and cross section SEM image (inset) of photonic crystal fiber (PCF); (b) experimental setup: BS, beam splitter; HWP, half-wave plate; RM, reference
The fiber used for SC generation is a commercially available PCF (NL-1050-NEG-1, Crystal Fiber A/S). For convenience, we named it PCF2. This fiber has a core size about 2.3 μm and a convex dispersion profile without ZDWs. It looks like the down shift of the dispersion profile of PCF1. It is possible to carefully control the microstructure of the PCF, such as air hole diameter or pitch distance. The convex dispersion profile results in a relatively flat dispersion area from 0.9 μm to 1.1 μm, which is very close to zero dispersion (Fig. 3.13 (a)). The dispersion profile is digitalized from the specification of the manufacturer and the tolerance is slightly modified so that the simulation best fit the experimental result shown below. The cross section of PCF is also shown in the inset of Fig. 3.13 (a), where air hole diameter and pitch distance is eventually determined by the dispersion profile of the PCF. The schematic of the experimental setup is shown in Fig. 3.13 (b). 130 fs pulses were launched from a turn-key, compact Nd:glass laser (High Q) into a 0.8 m length of PCF, which was spliced to a 0.5 m length of conventional single-mode fiber (HI1060). HI1060 is used to simplify coupling to the interferometer. The output of the pump laser centered at 1.059 μm was passed through a half-wave plate (HWP) and was focused on the PCF core by an aspheric lens (C230, Thorlabs). About 70% of the pump energy was coupled into the PCF, corresponding to an average power of 100 mW at 75 MHZ repetition rate. Although the PCF was designed to be non-polarization maintaining, we observed appreciable birefringence, presumably caused by the irregularity of the air holes and pitches of the PCF. The HWP adjusted
the polarization of the light launched into the PCF to optimize the SC generation for OCT.

3.3.2 Numerical Simulation and Experimental Result

The numerical simulation of the SCG was conducted based upon the way in which the SCG was developed during pulse propagation by using the algorithm described in section 3.1. The numerical simulation of spectral development along the PCF was shown in Fig. 3.14. The spectrum broadening starts from spectral oscillation, which is due to SPM. The low-dispersion point of the PCF2 is at around 0.95 μm, and it is clear that the pump energy is distributed to bands on either side of 0.95 μm during spectral development. However, the majority of the energy is distributed to the longer-wavelength side. This is attributable to the asymmetric dispersion profile. Due to the flat and low dispersion area, around 1 μm, the spectrum first extended very quickly to the short and long wavelength side. However, the slope of dispersion at the long wavelength is smaller than that at the short wavelength. This permits the spectrum to be more easily extended to the longer wavelength side than to the shorter wavelength side. Therefore, most of the spectral energy concentrates at the long wavelength side and the center wavelength shifts from 1.059 μm to about 1.15 μm. After 0.5 m propagation, the spectrum has been fully extended. Further propagation did not appreciable modify the spectrum. In Fig. 3.15, we compare the simulation with and without SRS, only a small spectrum change can be attributed to SRS. Thus, the SC generation can be primarily attributed to SPM, because SC is generated only in the normal dispersion regime.
The SC spectra measured experimentally and generated by numerical simulation are compared in Fig. 3.15. During experimentation, the spectrum was been broadened to span from 0.8 µm to 1.3 µm, with a full-width at half-maximum (FWHM) bandwidth of 240 nm centered around 1.15 µm (black solid line, Fig. 3.15 (a)), when optimized with the polarization control. The spectrum of the pump pulse is also shown in Fig. 3.15 (a) (dotted blue line), Fig. 3.15 (b) presents the numerical simulations of the spectra. The simulation agrees well with the experimental results. Including stimulated Raman scattering in the simulation reproduces the small modulations observed in the experiments (green solid line).

Fig.3.14. Numerical simulation of spectral development with pulses propagating in PCF2. After 50 cm propagation, there is no obvious change of the spectrum.

![Graph showing SC spectra comparison](image)
3.3.3 OCT imaging system and results

To demonstrate ultra-high resolution OCT imaging using this light source, the SC light output from the fiber was coupled with a free space time-domain OCT as shown in Fig. 3.13 (b). The average power output of the fiber was about 40 mW, due to 4 dB splicing loss between the PCF and the HI1060. An achromatic lens with 45 mm focal length was used to focus the light onto the sample, resulting in approximately 7 μm lateral resolution. The scanning optical delay in the reference arm is generated by using a retroreflector mounted on a galvanometer. Dispersion compensation was achieved with two pairs of prisms made of SF11 and Lakn22. The axial PSF of the system was measured by recording the interferogram with a mirror as the sample and is plotted in Fig. 3.16 (a). The measured axial resolution was about 2.8 μm in air, (corresponding to approximately 2.1 μm in the tissue). The inverse Fourier transform of the PSF is shown in Fig. 3.15 (a) (red dashed line) to match very closely with the spectrum of the SC input to the interferometer, indicating that the interferometer did not significantly filter the SC light. The shorter wavelengths were cut off at around 0.95 μm due to the responsivity of the InGaAs photodetector, which eliminates the requirement of a long pass spectral filter. The slight asymmetry of the PSF was due to residual, unmatched higher order dispersion. As shown in the logarithmic plot in Fig. 3.16 (b), the PSF is very clean down to -40 dB. Two small
side lobes are observed at 6 μm aside and -35 dB down from the main peak, caused by small, low frequency modulations in the spectrum. This PSF performance represents a significant improvement over previously reported UHR-OCT light sources near this wavelength range using 1 μm femtosecond laser [24]. Fig. 3.16 (b) was scaled to reflect 60 dB sample arm attenuation. By considering the 135dBc/Hz relative intensity noise of the laser at a carrier frequency of 300 KHz [4], 100 KHz signal detection bandwidth, 8 mW average light power on the sample and 0.1 mW power to the detector from the reference arm, the expected sensitivity was calculated to be around 103 dB with single detector. This is plotted as the dashed line in Fig. 3.16 (b), and corresponds closely to the measured noise floor (solid line), confirming that there was no appreciable noise amplification during the SC generation based on SPM [4]. This is expected for SC based on SPM, but not for SC generated by pumping around ZDWs with conventional PCF [22, 23]. The theoretically expected shot noise floor of 113 dB (dotted line) is also plotted in Fig. 3.16 (b) for comparison. This performance could be approached by using balanced detection.
An in vivo ultrahigh-axial resolution OCT image of human skin and nail bed is shown in Fig. 3.17 (a). Stratum corneum, epidermis, nail pad and blood vessels can be readily identified. The penetration depth is comparable to OCT imaging with 1300 nm light. An ultrahigh-resolution in vitro OCT image of de-epithelialized porcine cornea is demonstrated in Fig. 3.17 (b), where Bowman’s layer, characterized by denser collagen lamellae, Descemét’s membrane and intrastromal morphology (for example, collagen lamellae) can be clearly identified. No obvious side lobes are observed in the images even at the specular reflection surface.
3.3.4 Discussion and Conclusion

The SC spectrum demonstrated here shows obvious center wavelength shifting from pumping wavelengths of 1.059 μm toward 1.15 μm, which is not observed in normal SC based on SPM [4, 5, 19, 28]. It is worth discussing whether or not this center wavelength shifting is advantageous. For ultra high axial resolution, such as...
around 3 \(\mu m\), the axial resolution cannot be kept in the tissue due to absorption, scattering and dispersion. In reference, the effect of water dispersion and absorption in UHR-OCT was numerically studied by assuming that a Gaussian broad band light source doubly passes 1 mm water. According to the upper center panel of Fig. 3, at 3 \(\mu m\) axial resolution, if dispersion has been fully compensated for, the axial resolution will be almost unaffected by absorption until 1.2 \(\mu m\). Considering no dispersion compensation, we can observe a low and flat area from 0.98 \(\mu m\) to 1.15 \(\mu m\), which represents the optimal center wavelength range for UHR-OCT without dispersion compensation. This is due to the fact that the second order zero dispersion of water is around 1 \(\mu m\). However, we can observe that resolution is still degraded to about 4.5 \(\mu m\) due to even higher order dispersion. With full 2\textsuperscript{nd} and 3\textsuperscript{rd} order dispersion compensation, the flat area still exits, but it seems that a center wavelength below 1 \(\mu m\) is better. However, for opaque tissue, scattering must be considered. In reference, three representative opaque tissues were studied. Better penetration depth can be achieved when the wavelength is above approximately 1 \(\mu m\), excepting the area around 1.44 \(\mu m\), due to strong absorption. We can conclude that the optimal center wavelength range for UHR-OCT should be set at between 1 \(\mu m\) and 1.15 \(\mu m\). However, choosing 1.15 \(\mu m\) can provide other benefits.

First, with longer wavelength, we can image deeper. Second, the effectively responsive wavelength of an InGaAs detector starts from 0.9 \(\mu m\). By choosing a center wavelength around 1.15 \(\mu m\), we can cover the range from 0.9 \(\mu m\) to 1.4 \(\mu m\), which not only overlaps with the effective range of InGaAs detector, but also avoids
the absorption peaks at 1.4 µm. Third, a light source centered at 1.15 µm is probably appropriate for both ophthalmology and opaque tissue. Granted, this is not a comprehensive study, but the results of references [20, 21] give evidence that a center wavelength at 1.15 µm maybe a good choice for UHR-OCT.

In conclusion, using a compact and commercially available 1.059 µm femtosecond laser and a novel PCF, we have developed a new light source for UHR-OCT. Compared with previous SC based light sources at above 1 µm wavelength range with conventional PCF, it has a smoother spectrum, resulting in a very clean PSF with negligible sidelobes and no noise amplification. The axial resolution, about 2.8 µm (in air), is almost two times greater than the demonstrated results of SC generated with conventional single mode fiber. We have demonstrated OCT imaging with ~2.1 µm axial resolution (in tissue). The SC generation is mainly attributed to SPM because the PCF has no negative dispersion area and the dispersion profile is close to zero dispersion at the pump wavelength. The PCF is commercially available and no tapering or further modification of the fiber is required. The spectral shift from the pump wavelength to 1.15 µm provides an effective overlap with the responsivity of InGaAs detectors as well as good penetration depth in opaque tissue. The performance of this light source suggests that PCF with this type of convex, normal dispersion profile is more suitable for SC generation than conventional PCF or single mode fiber for UHR-OCT.
In this chapter, we presented two SCG based broad band light sources by pumping two different PCF with convex dispersions profile. We emphasize two advantages of this pumping scheme.

- The laser we choose was centered on 1 μm. Although, a Nd:glass laser is chosen here, there are many choices in this wavelength range. Particularly, recently developed fiber femtosecond lasers will be very attractive for this application, because fiber lasers are compact, reliable, inexpensive and can be easily integrated into fiber based OCT system by eliminating free-space coupling [18, 29, 30]. However, currently the performance of fiber lasers is still unable to satisfy this pumping scheme due to poor pulse quality. The fiber laser is based on amplifying the seeded fs pulses. Due to inherently higher order dispersion of the fiber, pulses are seriously chirped and have many side lobes. It is well known that the SPM is sensitive to chirp, so the generated spectrum is distorted and cannot be used for OCT. One possible solution is to compensate for higher order dispersion with chirp mirrors [18]. Another choice of femtosecond lasers is the use of a solid-state laser. The lasers we used here can be called compact and free-from alignment. However, it still came with a chiller and the size is still not small enough for a portable system. Several more compact lasers at around 1 μm with air-cooling have been commercialized, such 1 μm laser from One Five®. Some of them with built-in
fiber coupling systems, which will make this pumping scheme promising for a portable UHR-OCT system.

- As we demonstrated, this pumping scheme is mainly generated by SPM, so the noise is limited only to the lasers. But the spectral ranges are maximized due to larger dispersion range around zero dispersion.

- As SCG is caused by SPM, the phase of SCG will be smooth in all the spectral ranges. This will provide the possibility of compressing the spectrum to ultrashort pulses. For a dual band spectrum, it is possible to compress the spectrum to two-color, ultrafast pulses. This will be useful for two-photon microscopy and CARS microscopy [31]. For 1.15 μm spectrum, it is hard to compress the entire band into one pulse. However, by partially choosing the corresponding spectrum, we can generate tunable pulses from 1 μm to 1.2 μm.

We also need to note, due to the extreme broad band used here, instead of building fiber based OCT, we built a free-space OCT. For real applications, fiber-based OCT is definitely more desirable. Broad band fiber components such as fiber couplers are required and customer designed lens are also needed to avoid spectral filtering due to fiber coupling. In addition to this, the system we built here was a slow speed system, which means each frame took about a tenth of a second. This is due to the unavailability of high-speed broad band optical delay line or an InGaAs photo detector array (PDA). This problem can be solved in the near future with the development of PDAs. In the next chapter, in order to take advantage of UHR-OCT
based on currently available technology, we setup a UHR-OCT at 800 nm for real application.
CHAPTER 4. CONTRAST SENSITIVE OCT WITH GOLD NANORODS

4.1 INTRODUCTION

Contrast agents have been popularly used in different imaging modalities when inherent contrast is not sufficient or specific molecular events are desired. [1, 2] In fluorescence microscopy, including confocal and multiphoton microscopy [3], organic dye is used to stain specific molecule. By exciting the dye with short wavelength light, one can image events at the molecular level by filtering the emitted fluorescence out of the dye. In ultrasound, gas bubbles are used as a contrast to enhance the back-reflected signal at the resonance frequency [4]. For other modalities, such as PET and SPET, radioisotopes are required for imaging [1, 2]. Contrast sensitive imaging offers several advantages over histology. They can speed up the evaluation of drug candidates by reducing the workload of tissue preparation. Additionally, the biomarkers of a disease can be identified for early diagnosis.

For OCT, contrast sensitive imaging is often used for imaging tissue samples without involving contrast agents. The OCT signal is originated from the back scattered photons due to the variation of the refractive index in the tissue. The images of OCT include information regarding optical scattering and absorption of the tissue. However, fluorescence cannot be detected by OCT, because it is not coherent. The contrast for OCT must have detectable signature either in scattering or in the absorption spectrum. Many efforts have been made to construct CSOCT [5, 6]. The success of CSOCT relies on the discovery of appropriate probes for contrast and
novel imaging methods. The proposed CSOCT can be categorized by either the properties of probes or the methods.

4.2 PRINCIPLES OF CSOCT

For a subject, from the coherent confocal volume at a given depth \( z \), the light back-reflected by the scatters can be expressed as

\[
I(\lambda, z) = S(\lambda)H_r(\lambda, z)H_m(\lambda, z)
\]

\( S \) : Incident spectrum; \( I \) : back reflected spectrum

\( H_r \) : Spectrum backscattered from coherent confocal volume at layer \( z \)

\( H_m \) : attenuation function

\( H_m(\lambda, z) \) is the attenuation of the sample above the layer \( z \) and \( H_r(\lambda, z) \) is the backscattering strength in the coherent confocal volume. They can be further extended as

\[
H_m(\lambda, z) = \exp\left\{-2\int_0^z [\mu_a(\lambda, z') + \mu_s(\lambda, z')]dz'\right\}
\]

\( H_r(\lambda, z) = \kappa \mu_s(\lambda, z) \) \hspace{1cm} (5.2)

Where \( \mu_a \) and \( \mu_s \) are the absorption and scattering coefficient and \( \kappa \) is backscattering coefficient which can be defined as

\[
\kappa = \frac{\mu_{bs}(\lambda, z)}{\mu_s(\lambda, z)}, \quad \mu_{bs}(\lambda, z) \text{ is backscattering coefficient;}
\]

(5.3)

a) Absorbing probe (AP)

The use of absorbing probes (APs) is based on the regional attenuation due to absorption. By considering probes located in a small volume at a depth of \( z \), the OCT signal can be written as

\[
I(\lambda, z) = \exp\left\{-2\int_0^{z-\Delta z} [\mu_a(\lambda, z') + \mu_s(\lambda, z')]dz' - 2\int_{z-\Delta z}^{z+\Delta z} \mu_{bs}(\lambda, z'),dz'\right\}\kappa(z)\mu_s(\lambda, z)
\]

(5.4)
Here, the $\mu_a$ and $\mu_s$ are the absorption and scattering coefficients of the subject and $\mu_{ca}$ is the absorption coefficient of APs; $\Delta z$ is the optical path of the volume occupied by the absorbing probes. It is clear that the OCT signal containing the contribution from the APs is related to the optical path length of the APs and the strength of backscattering surrounding the APs, mixed with intrinsic scattering and absorption of subjects

b) Scattering probes (SPs)

Probes having significant scattering ability can be sensed directly by OCT. The signal originated from SPs at layer $z$ can be expressed as

$$I(\lambda, z) = \exp\left\{-2\int_0^z [\mu_s(\lambda, z') + \mu_a(\lambda, z')]dz'\right\}\kappa\mu_{sp}(\lambda, z')$$  \hspace{1cm} (5.5)

where $\mu_{sp}$ is the scattering coefficient of the probes. The photons scattered from the SPs cannot be directly identified from OCT images because they have been mixed with intrinsic scattering and absorption. Although engineered micorspheres have been demonstrated to be usable as SPs by directly monitoring the enhanced signal from the scatters, it is difficult to distinguish them from the intrinsic scattering of subjects [7].

For OCT, SP is advantageous over the AP. First, the contrast of the AP relates to the optical path length and relies on the scattering of the surrounding medium. In order to achieve reasonable contrast, the concentration of APs must be either high enough or at a large volume within an environment with detectable backscattering. Therefore, it is hard to achieve high resolution with AP. Second, SP with strong scattering, such as gold nanoshell [8], can be used to enhance the OCT signal to brighten the structures that were previously invisible.
4.3 METHODS FOR CSOCT

However, compared with fluorescence-based imaging modality, CSOCT cannot directly map the probes because the probe signal is either originated by or mixed with intrinsic background. The primary job for CSOCT is to extract contrast information from normal OCT images unambiguously. Several methods have been developed for extracting contrast for OCT images. They can be classified into three categories.

4.2.1 External modulation

External modulation is realized by externally modulating the optical properties of the probes, such as scattering and absorption cross section area. The modulation will not obviously affect other contents in the tissue, except the probes. This method includes pump-probe methods and magnetic modulation with iron particles [9-12]. External modulation can avoid background interference resultant from the surrounding medium and probes can be unambiguously identified. However, currently proposed methods suffered lower signal strength, slower speeds or required intensive pumping power. Magnetic modulation could be one of the best options, but practical applications have not been demonstrated until recently, which may be due to lower signal in opaque tissue.

4.2.2 Spectroscopic analysis

With the probes having a marked signature in the extinction spectrum, the spectrum reflected from each layer can be analyzed to extract this signature for
contrast imaging [13, 14]. Spectroscopic OCT was first demonstrated with normal OCT images whose pixels are colorfully mapped with the spectral center of the mass. By introducing probes, such as organic dye or nanoparticles with a marked signature on extinction spectrum, the contrast images may be extracted by spectroscopic analysis. However, this method suffers from several limitations. First, the speckle noise is inherent to the OCT images and is generated by the waves with random phases [15]. The spectrum extracted from OCT images will not be faithfully recovered due to the speckle noise. Triangulation spectroscopic analysis [13] has been proposed to overcome the scattering for the medium surrounding the probes. However, the spectrum modulation induced by speckles is wavelength-dependent and cannot be eliminated by this method. In order to enhance the sensitivity of spectroscopic analysis, real time speckle suppression is required for development. Second, the chromatic aberration induced by the optical components may affect the sensitivity when broad band light sources are used. This effect maybe corrected by using achromatic lens or calibration.

4.2.3 Nonlinear coherent emission

Some inherent coherent emission also can be used for CSOCT. Second harmonic generation (SHG) and coherent anti Raman scattering (CARS) have both been utilized for OCT imaging [16-18]. However, corresponding nonlinear microscopy is inherently depth resolved and easily constructed. Before these nonlinear OCTs can be accepted, the full comparison between microscopy and OCT must be accomplished.
In this chapter, we will discuss the possibility of using gold nanorods (NRs) for CSOCT. Ideally, we are looking to use NRs as SPs to extract contrast information unambiguously. Previously, NRs have been proposed as lower backscattering albedo, but prior knowledge of backscattering of tissue is required. This is not feasible for real application [19]. NRs have also been demonstrated as SPs, but the method used was not practical for real images [20]. As discussed, the best method for CSOCT is external modulation. We will discuss the possibility of using this method for NRs. Since NRs have a remarkable signature on its extinction spectrum, spectroscopic analysis is also feasible.

### 4.4 Gold Nanoparticles as a Probe for Contrast Sensitive OCT

For a particle in the nano-size scale, its electronic properties change because electrons are confined in a very small volume. This is due to the reduced density of states and spatial space for electron motion [21]. A typical effect is the size dependence of optical properties. One example is quantum dot (QD), which is made of semiconductor materials. With quantum size effects, the fluorescence of QD can be tuned from visible to NIR. This property makes QD an attractive contrast for multicolor fluorescence imaging.

However, for OCT, we expect a dramatic extinction spectral marker that can be used for contrast imaging. Gold nanoparticles have long been used as a pigment for ruby-colored stained glass, due to its strong absorption at ~530 nm.

The physical origin of this absorption can be imagined simply as coherent
oscillation of the conduction band electrons induced by an electromagnetic field. This is called surface plasmons resonances (SPR). At the wavelength around SPR, gold nanoparticles will show strong absorption and scattering. SPR is shape and size related. We can tune the SPR from visible to NIR by changing the shape or size. This property makes gold nanoparticles a viable candidate for CSOCT. Until now, several different gold nanoparticles have been proposed for OCT imaging.

Gold nanoshell have silica core encapsulated in a gold layer. By controlling the thickness of the gold layer, the gold nanoshell shows large scattering at NIR. It has been demonstrated that by injecting gold nanoshell into a mouse, the particles can accumulate into a tumor area because of the leaking of the blood vessels around the tumor [22]. The OCT images showed enhanced imaging depth and contrast due to the nanoshell. Gold nonocages are cubic particles with a hollow core, which have strong absorption peaks at NIR [23, 24]. They have also been used as AP for OCT by using spectroscopic analysis.

4.4.1 Properties of Gold Nanorods

NRs are rod-like particles that have two SPR modes—transversal mode and longitudinal mode. The SPR in the longitudinal mode can be tuned by the aspect ratio of the length to the width, or finely tuned by controlling the shape [25, 26], (e.g. dumbbell or round cap). NRs were first synthesized using an electrochemical method. However, only small amounts could be produced each time. Later, a photochemical method was proposed to synthesize NRs with longitudinal SPR up to 800 nm [27].
This method suffered from a low yield of NRs. Seed growth method [26] is a currently popular method for synthesizing NRs. The NRs used in our experiments were synthesized with the seed growth method as described below:

a) **Synthesis of Au seeds**

   In a typical protocol, 5 ml 0.5 mM aqueous H\text{AuCl}_4 solution was added and mixed with 0.2 mM 5 mL aqueous CTAB. The color of the mixed solution quickly changed from light yellow to orange. Finally, 0.6 mL of an aqueous 10 mM NaBH4 solution, cooled in an ice bath, was added, followed by intense stirring for two minutes and the solution was then kept at a temperature above 30 degree until further use.

b) **Synthesis of Au nanorods**

   The synthesis of nanorods greatly depends on different parameters, such as seed size, temperature and concentration of agents. It is hard to reproduce uniform NRs from batch to batch. The growth solution containing 0.2 M 5 ml CTAB and 0.3 mL Ag\text{NO}_3 was mixed and immersed in an ultrasonic cleaning bath for ten minutes. Then, 1 mM 5 mL H\text{AuCl}_4 and 78.8 mM 70 μl ascorbic acid were added. Finally, 12 μl Au seed solution was added to the growth solution. The mixed solution was shaken vigorously for 30 seconds and then left undisturbed for 30 minutes at room temperature. The nanorods were not stable, especially those with a large aspect ratio. Usually, this band will shift to blue after several hours. According to the required SPR band, the 4:1 Na2S can be added at some time by monitoring the spectrum [28]. Subsequently, the SPR band will be arrested and may still be shifted to the blue side.
about 20 nm after several weeks. The NRs were centrifuged for one minute at a speed of 12500 g and then dispersed into 1 mL DI water. The NRs should be centrifuged again at 4000 g for several minutes if a large peak was observed at visible, which means some junk particles, such as nanospheres and nanocubes, exist.

**c) Equipment and characterization**

Transmission electron microscope images were obtained using a JEOL 100 CXII at 100 kV. The TEM grids are prepared as follows: a drop of the solution obtained after centrifugation was deposited on the grid and allowed to dry in air. Absorption spectra were recorded from aqueous solution by using a CAR UV–vis-NIR Spectrophotometer (Varian).

Figure 4.1 shows one typical batch of NRs synthesized with seed growth method. Figure 4.1 (a) shows the TEM images of NRs with a scale of 50 nm. Through close examination of the images, we can find the NRs have a dumbbell shape, not exactly a rod shape. The yield of NRs is above 90%. However, large nanospheres (NS) and nanocubes (NC) are still observed in the images. Those big junk particles can be filtered by centrifuging at low speed. Figure 4.1 (b) shows the extinction spectrum as measured with a spectrophotometer. Two obvious SPR bands represent transversal and longitudinal modes. A small peak at 550 nm has been attributed to the NS and NC. Several properties of NR should be emphasized by comparing them with organic dye and other nano particles.

a) The longitudinal SPR band shows much stronger extinction and has a FWHM band width of about 150 nm centered at 800 nm. This band can be further tuned to an
even longer wavelength range by adjusting the aspect ratio of the NRs. The NRs shown here have an aspect ratio of about 4.5:1 with deviation around 15% to 20%. The average length is 43.09 nm and the average width is about 9.52 nm. Organic dye in NIR, such as ICG, has a FWHM band between approximately 70 nm and 80 nm at a concentration of less than 1 mM. ICG has been demonstrated as AP for CSOCT [13]. However, due to non-uniformity of current NRs, the band width is almost twice that of ICG. This may hinder NRs for spectroscopic analysis because band width of the light source is required to be at least comparable to that of the extinction band. However, this band may be narrowed by synthesizing more uniform NRs. Other methods can also be adapted and we will discuss this possibility later. Nanoshells also have an extinction band at 800 nm, but the band width is more than 200 nm, which makes it hard for spectroscopic analysis.

b) The SPR band of individual NRs is Guassian shape and can be maintained well in different environments [30], except for a bit of shift due to small refractive index changes around the NRs. This property is different from ICG, which has an extinction that will be changed at different solute and concentration levels. For OCT, a well-defined extinction spectrum is important in order to be reliably used for extracting contrast information and quantifying concentration.

c) NRs may have large absorption and scattering cross sections. The relation between absorption and scattering cross section and coefficient is defined as

\[ \sigma_{e} (cm^{-2}) = Q_{e} A (cm^{2}) \quad \text{and} \quad \mu_{e} (cm^{-1}) = \rho (cm^{-3}) \sigma_{e} (cm^{2}) \]  

(5.6)

Here, \( \sigma \) is the cross section and \( Q \) is the efficiency without unit; the density of
particles (number of particles/cm$^3$) is defined as $\rho$ and $\mu$ is the coefficient. For light through the optical path length $L$, the transmission is defined as

$$T = \exp(-\mu_e L)$$

(5.7)

The extinction coefficient can be converted to a molar extinction coefficient, which is often used to define organic dye.

$$\varepsilon (M^{-1} cm^{-1}) = \sigma_e (cm^2) \times 6.02 \times 10^{20} (M^{-1} L^{-1}) / 2.303$$

(5.8)

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<th>Type</th>
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<td>Nanocage</td>
<td>$r=23nm$</td>
<td>800</td>
<td>149.8</td>
<td>1.994</td>
<td>7.12</td>
<td>0.289</td>
<td>0.143</td>
</tr>
<tr>
<td>Nanorod1</td>
<td>$R=3.9$ $r=11.43nm$</td>
<td>797</td>
<td>907.09</td>
<td>1.41</td>
<td>70.86</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>Nanorod2</td>
<td>$R=4.6$ $r=11.43nm$</td>
<td>863</td>
<td>1003.87</td>
<td>1.95</td>
<td>102.05</td>
<td>0.195</td>
<td>0.10</td>
</tr>
<tr>
<td>Nanorod3</td>
<td>$R=3.9$ $r=21.86nm$</td>
<td>842</td>
<td>449.34</td>
<td>5.14</td>
<td>242.58</td>
<td>2.79</td>
<td>0.54</td>
</tr>
<tr>
<td>ICG</td>
<td></td>
<td>790</td>
<td>1.08x10^-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1. Comparison of different absorption and scattering coefficients[31], between NR, nanoshell, nanocage, and ICG

Compared with ICG, gold nanoparticles show the absorption cross section at least five orders larger. The more important property is the scattering ability of gold nanoparticles, which indicate nanoparticles can be SP. Commonly, people compared the extinction ability by considering different particles packed into the same volume and use $\mu_E$ as a standard. However, for cell targeting, it is not clear whether the size or shape will affect the number of particles bound on the cell surface. For a cell with a size of 10 $\mu$m, we can assume that there are $10^6$ receptors on the cell surface and 10%
can be accessed by particles. Although the area of cell surface can accommodate about 2.4e5 NRs with a size of 20 by 80 and 10e5 NRs with a size of 10 by 40, it is possible that the cell can be bound by 10^5 without regard to the size or shape difference. In this situation, relatively bigger NRs are favorable because they have a larger scattering cross section. In our study, the NRs have sizes similar to NR1 and NR2, which have a very strong absorption and low scattering.

Fig. 4.1 (a) The TEM images of synthesized NRs at different areas and scale; (b) The extinction spectrum of typical NRs with SPR bands labeled.
4.4.2 Feasibility of imaging Gold NRs with Photothermal Effects

We realized that the external modulation method maybe the best one for CSOCT. Here, we want to explore the possibility of externally modulating the extinction of NRs for imaging. NRs have a very large absorption cross section as compared to ICG at SPR. The photons absorbed by the NRs will be transferred into heat and conducted to the surrounding medium. This effect is called the photothermal effect (PT) and has long been used for therapy and cellular imaging. By targeting NRs to tumor cells in vitro, it has been shown that tumor cells can be selectively killed by illuminating them with a light beam [32-34]. This phenomenon was thought to be useful for PT therapy. Nanoshells have been used in small animal models for treating cancer.

![Fig.4.2. The illustration of photothermal effects of gold nanorods and laser illumination.](image)

During heating of the NRs, there are many effects accompanied with the resultant thermal expansion [35]. Figure 4.2 illustrates the effects that may come at different stages. The thermal expansion of NRs can generate an acoustic wave, which has been used in photon acoustic imaging. Theoretically, infrared emission can also be used for
imaging. However, it is too low to detect. The change in the refractive index around the NRs can modify the wave front and path length of photons. This effect has been used to image single gold nanoparticles down to 1.4 nm [36]. The typical principles was described as

$$I = \left| E_{\text{ref}} + E_{\text{scatt}} \right|^2 = \left| E_t \right|^2 \left( r^2 + |s|^2 - 2r|s|\sin \theta \right)$$ (5.9)

Here, $E_{\text{ref}}$ references the light generated from non-scattered light, which is the same as the light from the reference arm of the OCT; $E_{\text{scatt}}$ denotes the scattered light induced by the refractive index change. Although scattered light is very weak, it may be amplified through reference light. By modulating the pumping beam that is used to generate thermal effect, the scattering change can be monitored by another probe beam. PT interferometer (PTI) has been successfully used to image gold nanospheres in a cell [37]. We expect it can also been used in OCT based on the same principle. However, for enough contrast, OCT needs high backscattering.

Fig.4.3 The scheme of photothermal spectroscopy with NR. The 530 nm continue wave (CW) used as a pump beam and 830 nm SLED used as probe beam
Fig. 4.4 The thermal lensing effects measured with photothermal spectroscopy. Three responses can be observed by changing focal point in the solute.

In order to prove the possible scattering induced by the PT, we first set up a PT spectroscopy as shown in Fig. 4.3. As a pump beam, a green light at 530 nm was combined with a probe at 830 nm generated by a SLED. The dual color beam was then combined and focused on the NRs in a 1 mm cuvette. The transmitted light beam was then detected either by a photodetector or by a spectrometer. Fig. 4.4 shows an example of a signal detected with photodetector captured by transferring the lens focal point in the NR solution. By turning the pump beam on and off at different locations of the focal point in the solution, the transmission has different responses. The transmission can be enhanced, reduced or kept the same. This phenomenon is contrary to previous explanations of PTI [37] where the induced scattering should reduce only the transmission. We now can explain this with the photothermal lensing effect, which also has long been used to detect very low absorption molecular events in solute. Figure 4.5 shows the typical principle of the photothermal lensing effect [38]. In relatively transparent media, the heat generated from the molecules will be conducted
to the environment. This will change the refractive index of the surrounding medium. Due to relative transparency of the solute and the gradient of the heating expansion, the weak lens will be formed as shown in Fig 4.5. Those lenses will converge or diverge the probe beam, then affect the intensity detected by the photodetector. Converging and diverging can be balanced if the number of weak lenses formed before and after the focal point of the probe beam is identical.

OCT images were achieved by sandwiching the NR solute between two glass slides. The two images shown in Fig. 4.6 were designed to show the possible contrast change determined by whether the pump was on or off. Obvious contrast change can be observed at the glass slide’s surfaces below the NRs. However, we did not observe obvious contrast change from the NRs themselves, which is desired for the use of NRs as a SP. This is because thermal lenses may deflect the beam and make the focal point of the probe beam close to the glass slide’s surfaces. However, the backscattering induced by the thermal lens is too small for OCT. Due to a lack of contrast directly from the NRs, it is hard to use this method to externally modulate NRs for CSOCT, especially in opaque tissue. But from another point of view, we
proved OCT can be used for monitoring photothermal lensing effects. This maybe useful for improving the quantification of the concentration of an analyte, because the signal of thermal lens spectrometry is high in relation to the geometry of the optical scheme. In addition, with multiple wavelength detection, it also implies that the sensitivity can be further improved, which will facilitate the development of “chemical analysis on a chip” [38, 39].

Fig. 4.6. The OCT images with photothermal lensing effect. (a) Image with pump off; (b) Image with pump off; (c) Typical OCT signal with pump on and off; (d) The difference of OCT signals.

4.4.3 Imaging NRs with Spectroscopic Analysis

4.4.3.1 Narrowing SPR band by photothermal melting

As discussed above, the bandwidth of SPR of NRs is normally between 120 nm and 150 nm at around 800 nm. Theoretically, for a single ideal NR, the band width is about 50 nm at 800 nm and will increase with the red shift of SPR [29, 40]. However, in order to enhance the contrast of CSOCT during spectroscopic analysis, a narrow bandwidth (i.e. a bandwidth below 100 nm) is desired. The most straightforward
spectroscopic analysis is to achieve two images at different wavelength ranges. The peak of the SPR band should be overlapped by one of them. By directly comparing the two images, the difference may be attributed to the existence of NRs. Narrow or sharp SPR bands will permit the offset of the two wavelength range to be small. Thus, the speckle noise and interference from wavelength dependent background scattering can be minimized. With the seed growth method, the growth of NRs can be affected by many factors, such as temperature, seed quality and concentration of the agent. The exact mechanism of NR growth has not been clearly understood. As such, the deviation in the size of NRs can be as large as 20%. The deviation of the NRs we made was estimated at around 15%.

The best method for narrowing the SPR band is to produce uniform NRs. However, this is still limited by current technologies. Another method is to melt NRs with photothermal effects using a pulsed laser. The laser pulses can heat the electrons of the gold nanorods. The interaction between the electrons and the lattice conduct this heat to the lattice, which then melts and isomerizes the nanoparticles to become more thermodynamically stable until they reach a spherical particle shape. Numerical simulation has shown that NRs can gradually shrink to shorter and thicker rods below their melting point [41]. This implies that the SPR band will gradually begin a blue shift. By carefully choosing the laser wavelength, we can selectively melt part of the NRs quickly while keeping other NRs stable. This will melt the NRs beginning with those possessing a larger aspect ratio before those with a smaller one, thus narrowing the SPR band. Previously, Links et al. have demonstrated that the SPR band can be
narrowed down to 100 nm from longer wavelengths by choosing the wavelength of the burning laser a bit larger than the peak wavelength of the SPR [42-44]. However, obvious reduction of the SPR peak can be observed during the melting. Ideally, the NRs are narrowed, but are made to keep the same extinction peak, which means the SPR band is sharpened.

A small amount of NRs were placed in a 1 mm glass cuvette. A laser beam from an oscillator or OPA was steered by a galvanometer from left to right and focused as a burning beam to a spot size of 200 μm. A motor drive translation stage moved the cuvette up and down so the burning beam could scan the entire area with NRs. Fig. 4.7 shows the narrowing of the SPR band when burning at the red side of the peak of the longitudinal SPR band. The laser used here was a Clark CPA-2001 with a pulse energy of 200 nJ and a pulse width of 150 fs at a repetition rate of 1 K. The wavelength of the burning beam was 850 nm, which is about 70 nm from the peak of the SPR band. Fig. 4.7 (a) shows the narrowing of the SPR band with respect to time. The slop at red side is increased about twice before and after melting. This implies that contrast can be enhanced about 3 dB after narrowing. In addition, we observed that the peak of the SPR band is increased a little during the initial stages and is then reduced for further melting. This is different from the findings reported before [42-44] and may be attributed to the fact that the burning wavelength was far from the peak of the SPR band. Further melting did not further narrow the NRs. For OCT, a longer wavelength is desired for better penetration depth. NRs may be melted from the blue side. The same laser was used here. However, the burning wavelength was tuned to
720 nm with pulse energy of about 80 nJ. Smaller narrowing was observed during several initial scans. The pulse energy was then increased to 120 nJ. After only one scan, the peak was decreased very quickly, which indicated that the rods had been melted to produce spheres. This was also confirmed by the increase of the SPR band at the transversal mode.

![Graph](image)

Fig. 4.7 (a) The narrowing of SPR band by photothermal melting at red side of SPR peak; (B) Photothermal melting of NRs at blue side of SPR peak. The arrow in the (a) shows the slope of SPR band before and after narrowing.

In conclusion, by carefully choosing the burning wavelength, it is possible to further unify the NRs without reduction of the SPR peak. The FHWM bandwidth can be narrowed to around 100 nm. Further improvement is possible using high energy picosecond or nanosecond lasers, which have a more narrow line width than femtosecond lasers.

4.4.3.2 Imaging NRs by spectroscopics analysis

NRs have a well-defined extinction peak, which can be used as a signature for CSOCT. The extinction is from scattering and absorption, so NRs can be used as scattering probe or as an absorbing probe or both. With the size of the NRs used here,
less than 10% of extinction is from scattering. In order to determine the possible origin of contrast from the NRs, the most straightforward method—utilizing two light sources—was used here. One was generated from a SLED centered at 840 nm and another one was generated by pumping a normal single mode fiber with a tunable Ti:Sapphire laser. The two light sources were combined by a fiber coupler to generate a dual band light source. The tunability of the Ti:Sapphire laser gave us the option of choosing the optimal wavelength range for CSOCT. The typical spectrum of the light source and NRs are shown in the Fig. 4.8. Two NRs were used in the images. One shows the NRs as sharpen with photothermal melting and the other shows the NRs without further processing, with SPR band at around 840 nm. It is clear that dual band light source can cover either of the SPR bands of the two NRs.

![Normalized Intensity vs Wavelength](image)

**Fig. 4.8** The extinction spectrum of 740 and 840 nanorods and dual band light sources used in spectroscopic analysis.

The images were achieved with spectral domain OCT, which can cover a wavelength range from 700 nm to 900 nm. Therefore, the OCT images with dual separated band light sources can be achieved in one attempt. This is very important for future practical applications, especially in vivo imaging. The single attempt can
eliminate the noise induced by separated scans, especially a motion artifact induced by speckle noise. This makes the direct comparison of the images at different spectral bands in real time possible.

Due to the broad band spectrometer and the separated light sources used here, optical system must be calibrated at these two bands. We calibrated the signal strength with glass slides at different positions using dual band light sources. Figure 4.8 shows the calibration and ratio of the light sources. Actually, the optical system just showed small difference at the depth around 600 μm. The factor were then corrected in imaging processing.

![Fig. 4.9. The calibration of the optical system with two light sources at different wavelength ranges and the ratio of their signal strength](image)

About 500 μg/ml 740 nm NRs were placed into a capillary with an external diameter of 1 mm. The image was achieved in a single attempt. The two images at different bands were separated in the frequency domain. The difference between the two images was achieved by subtraction using a logarithmic scale and consideration of the correction factors. Fig. 4.10 shows the images at two bands and their respective subtractions. The image at 740 nm has a stronger signal than that the one at 840 nm,
as expected, since NRs have strong scattering at 740 nm. However, the image at 840 nm shows better penetration depth due to lower extinction. By examining Fig. 4.10 (c), we find that the contrast has positive value at the upper area of the capillary but negative at the lower part.

Fig.4.10. The OCT images of 740 nm NRs achieved with dual band light sources. (a) Image with 740 nm light source; (b) Image with 840 nm light source; (c) The subtraction between (a) and (b); (d) The differential image of (c)

The images shown in Fig. 4.11 were achieved with 830 nm NRs. Two capillaries were set side by side; one was filled with NRs and another one was filled with 650 nm microspheres as a control. From Fig. 4.11 (c), we find that they show an opposite trend. For the microspheres, light was scattered more strongly at 740 nm than at 840 nm, so the OCT signal was strong at the upper part of the capillary but weak at the lower part. However, for 840 nm NRs, due to strong extinction around
840 nm, the OCT showed a stronger signal with the 840 nm light source than with the 740 nm light source at the upper part of the capillary. The differential image of NRs shows a trend opposite that of the microspheres. For clarity, Fig. 4.12 plots the two A-scan signals at two different positions, which represent the typical signal of NRs and microspheres. The slope of the OCT signal indicates the extinction of the samples. The OCT signal from NR decay slows at 740 nm but quickens at 830 nm. This confirms the strong extinction of NRs at 840 nm. For microspheres, this trend was reversed as is similar in normal tissue.

Fig.4.11. The OCT images achieved with dual band light sources with 840 nm NRs and 650 nm microsphere. (a) Image with 740 nm light source; (b) Image with 840 nm light source; (c) The subtraction between (b) and (a). (d) The differentiation of (c).
4.5 DISCUSSION

As we have shown above, NRs show contrast by differentiation of the images achieved with light sources in different wavelength ranges. As compared to gold nanoshells, NRs have a relatively narrower band width of SPR and can be further narrowed down to 100 nm. This value will be comparable with NIR organic dyes, such as ICG. We demonstrated here that photothermal melting is one of the ways to create more uniform NRs. Compared to nanocages, NRs have greater feasibility for finely tuning the SPR to different wavelength and have stronger absorption and scattering at similar sizes. Compared to ICG, the absorption of NRs is six orders larger, which make NRs an efficient agent for photothermal therapy.

However, unlike engineering microspheres or ICG, NRs cannot be treated as pure AP or SP. The contrast of NRs shown above actually originates from both of them. This will introduce ambiguity in explaining the CSOCT images and this ambiguity is what we want to avoid. As shown in Fig. 4.10, most of the contrast is
positive. However, for normal tissue images, this differentiation may also be positive as shown in the microsphere images. It will be hard to identify contrast when NRs are in a multiple scattering environment. The same situation also happens to 840 nm NRs, as shown in Fig. 4.12.

Inherent wavelength-dependent scattering can be minimized by narrowing the extinction peaks and doing spectral triangulation analysis [13]. This will require broadband light sources, as indicated previously, and real-time speckle noise reduction. Another way to improve outcomes is to maximally enhance the OCT signal, so the contrast is well above the threshold of the inherent scattering difference. In this situation, SP will be desired not only for using as probes but also for helping to light some structures that are invisible under normal OCT imaging. Although here we are looking for SP, the NRs used here are predominantly absorptive. A high concentration must be used to generate sufficient signals, this will limit the image depth. However, by tuning the size or shape of NRs, it is possible to enhance the ratio of scattering to absorption to a similar value as the nanoshells. NRs have an anisotropic shape and the scattering cannot be modeled with Mie theory. In addition, the signal comes from back scattering, which may be related to the shape of NRs—such as rod or dumbbell shapes. Previous work considered only the scattering or extinction. Less attention was paid to back scattering. By considering optical geometry and using the DDA model, numerical study should be conducted in order to find the optimal size and shape of NRs for CSOCT.

The limitations of NRs as probes can be described in three aspects. First, NR is
coated with CTAB, which is toxic and may be non-selectively uptaken by cells [45]. Other biocompatible coats should be developed to replace CTAB and to keep SPR extinction as well. Second, CSOCT relies on efficiently delivering the probes to the target area. However, for NRs, this has been demonstrated only in in vitro to cells. Due to the special shape of NRs, it is unknown if the NRs can be efficiently accumulated into tumorous areas with different aspect ratios. Third, OCT above 1 μm is preferred; NRs can be tuned to above 1 μm with larger aspect ratio, but the bandwidth of the SPR will be more than 100 nm, even with a single perfect rod. For a batch of NRs, this bandwidth can easily reach to 200 nm, which is too broad to do spectroscopic analysis.

It is important to quantify the concentration of the probes for some applications. However, this information is not straightforward for CSOCT. For pure absorption probes, the signal strength is determined not only the concentration but also the scattering media around the AP and extinction coefficient of the media above the probes. For the APs buried in a homogenous media, the concentration can be easily mapped by differentiating the OCT signal as showed in the Fig.4.10 (d) and Fig.4.11 (d). However, the signal is weak and difficult to identify since the images are achieved in one shot. The speckle noise overwhelms the slope of the OCT signal. For contrast OCT, one of the important techniques is to develop real time speckle noise reduction.

Although we did not get successful results by using external modulation to achieve contrast, it is still the best way of doing CSOCT. For this purpose, the first priority is to pursue appropriate probes. NRs maybe still a good candidate if one were
to synthesize composites or surface modifications. For example, NRs could be
combined with iron oxide particles, which could permit a change in the orientation of
the NRs. Due to high polarization sensitivity of the NRs, the scattering of NRs can be
modulated.
CHAPTER 5. BIOLOGICAL APPLICATIONS OF OCT

In this chapter, we will evaluate three potential applications of OCT. Ultra high resolution OCT (UHR-OCT) is always expected for OCT applications, especially with the light sources having wavelengths above 1 μm. In the previous two chapters, we have introduced three novel broad band light sources at this wavelength range. However, current technologies still limit these light sources for high-speed image acquisition. Here, we demonstrated two applications by employing UHR-OCT at 800 nm, since high speed and low cost CCD is commercially available at this wavelength. The conclusion can be easily extended to UHR-OCT above 1 μm with enhanced imaging depth.

5.1 IMAGING EARLY MURINE EMBRYO WITH UHR-OCT

Embryonic development is a dynamic process with dramatic morphological change occurring during a relatively short time. Understanding the developmental process is important to many applications, such as vaculogensis [1] or the development of stem cells [2]. Many different technologies have been studied in an attempt to achieve 3-D images of embryonic tissues at differing stages. Traditionally, histology was used to reconstruct complex 3-D structures. However, this method is laborious and time consuming. MRI microscopy can be used for imaging larger, fixed tissue with resolution of 20 -30 μm, but the time requirement is still onerous. Other technologies, such as high resolution ultrasound or optical projection tomography,
have also been employed [3-6]. However, to this point in time, there is still a lack of solid tools for imaging embryos in vivo, under higher resolution or tracking dynamic processes in the embryo.

Depending on the wavelength and subject, OCT can image opaque tissue up to 1-3 mm invasively. 3-D images can be reconstructed by integrating multiple 2-D OCT images. Therefore, OCT can be a powerful tool for imaging an embryo. Previously in our group, chicken embryonic hearts have been imaged with gated or ultrahigh speed OCT with axial resolution about 10 μm in tissue [7, 8]. OCT demonstrated excellent ability of imaging the dynamic processes in embryonic hearts.

In this section, we studied the feasibility of using OCT to image whole murine early embryos will be discussed. The mouse is one of most popular research animal models for biologists due to its well-characterized genotype and established knock out models. For early-developed embryos, in order to image the fine structures, an ultrahigh resolution FDOCT at 800 nm was built. The reason we used UHR-OCT at 800 nm was that the high speed CCD, with up to 4K pixels, was available. This permitted us to built high speed UHR-OCT for 3-D images. The broad band light source was generated by pumping a normal single mode fiber, HI780, with a 100 fs Ti:sapphire laser. The spectrum was broadened to around 60 nm due to SPM. This band represents about 4 μm axial resolution in tissue. The lateral resolution on the sample was around 7 μm determined by Zmax simulation. About 10 mW power was used on the sample. A high-speed line scan CCD camera with 4K pixels and 14 KHz line rate was employed for a high-speed and high-resolution spectrometer. The
achieved spectrum was inverse Fourier transformed and combined to generate a B-scan. Multiple B-scans were later reconstructed into 3-D images in Amira®. The image areas were set at 2x2 mm with C-scan lateral resolution of about 7 µm.

At day 9 of development, the mother was euthanized and the embryos were surgically removed from the uterus. The embryo was then fixed in formalin for in vitro imaging. The embryo was pinned to silicone between two platinum plates in a container filled with saline solution. Fig. 5.1 (e) shows the sagittal cross-section of the embryo, which was achieved directly by an OCT B-scan. After 3-D reconstruction, the transverse cross-section at different positions was demonstrated in Fig. 5.1 (a-d). The corresponding positions were marked in Fig. 5.1 (a). The relatively lower resolution in the transverse images was due to lower lateral resolution along the C-scan. It is clear that the OCT images can be used directly to identify the internal structures of the embryo without sectioning the tissue. Corresponding organs were labeled by speculating from Atlas. 3-D reconstruction was also shown in Fig. 5.2. We put 3-D OCT images and 3-D images from emap Atlas together (http://genex.hgu.mrc.ac.uk/) for a direct comparison. As seen from the images, OCT is a powerful tool for the study of the development of whole embryos. Images were obtained in vitro. It is very possible to achieve total embryo imaging in vivo by adequately controlling the environment of the embryos and by employing high speed OCT. The broad band light sources above 1 µm we have developed will be preferred for deep penetration. However, a photodiode array with more than 1K pixels is
required for UHR-OCT. Currently, a spectrometer at above 1 µm is being developed in our lab.
Fig. 5.1. The sagittal and transversal cross sections of mouse embryo at E9.5. Labels are listed as below.  

Fig. 5.2. Left figure: 3D images of E9.5 mouse embryo reconstructed from OCT images; Right figure: 3D images reconstructed from emap atlas. 1. Optic eminence; 2. Caudal extremity of tail; 3. Pericardial region; 4. Forelimb bud; 5. Region overlaying roof of fourth ventricle.

The current demonstration is only preliminary. Fine structures in the embryo still cannot be clearly identified due to speckle noise. The speckle noise can be reduced either by using even higher axial resolution or by doing multiple angle combinations or both. Currently developed methods of speckle reduction allow only slow speed acquisition. With our ultrahigh speed system, we may have much greater freedom to do that. However, the limited axial resolution of an ultrahigh speed system should be further improved, moving towards an ultrahigh-speed system with ultra high resolution.
5.2 MAPPING FIBER ORIENTATION OF MOURINE HEARTS

The cardiac muscle plays an important role in the electrical propagation and force development throughout the myocardium [9]. The conductivity is determined by the direction of the fibers and is highest in the direction of the longitudinal axis. Fiber orientation is also important for myocardial stress and strain. Many diseases can be related to fiber orientation. For example, arrhythmia refers to disorders in the regular rhythmic beating of the heart. About 2.2 million Americas are living with atrial fibrillation, one type of rhythm problem. It is believed that myocardial fiber orientation is important in both the generation and the maintenance of reentry. The disorder of fiber orientation could affect the pattern of electrical propagation. Therefore, understanding the different fiber orientations in normal and diseased hearts is very important for diagnosis and therapy. Traditionally, the histological methods of reconstruction of fiber orientation are complex and labor intensive [10]. Mapping of fiber orientation in a large number of normal or diseased hearts is not feasible. Furthermore, fixation may also alter the structure from normal state. Recently, a new method based on diffusion tensor magnetic resonance imaging (DTMRI) was developed and shown to be viable for mapping fiber orientation [5, 11-14]. The fiber orientation was then extracted to help in modeling the electrical processes in the heart. However, this method has lower resolution as compared to histological sectioning. Typically, the in-plane resolution is about 150 µm×300 µm with a slice thickness of millimeters. At this scale, it is very challenging to achieve fiber orientation mapping \textit{in vivo} with high spatial resolution.
Further, there is a need for clinical imaging of the myocardial microstructure *in vivo* in order to assess structural remodeling during the course of life threatening diseases. In their early stages, some diseases progressively replace healthy myocardium with fat or fibrous tissue. Early detection of this process will help to identify patients at risk for sudden cardiac death and may serve as an indication for therapy. Similarly, basic research into the numerous cardiac diseases would greatly benefit from structural imaging at the cellular level, which is possible only by histology. Early detection requires imaging on the scale of a myocyte, which is approximately $100 \times 10 \times 5 \, \mu m$ ($L \times W \times H$).

OCT has the ability to image opaque tissue at high resolution and high speed. The cardiac muscle is highly scattered. The origin of this scattering may be due to the periodic sarcomere structures in the muscle, which can alter light propagation in the muscle [15]. Previously, we have demonstrated imaging and quantification of fiber orientation in the plane parallel to the wall surface. During these experiments, the right ventricular free wall of a New Zealand White rabbit was dissected, stretched, and pinned—epicardial side down—onto a silicon disk. This procedure ensured that the sample surface was relatively flat. An optical clearing procedure was conducted to enhance the visibility of the fibers within the OCT image sets. Here, time domain OCT, with a resolution around $12 \, \mu m$ in tissue, was employed and an optical clearing procedure was required for enhancing contrast, which is impossible for *in vivo* imaging. With UHR-OCT, samples maybe imaged without stretching, pinning or optical clearing procedures. In addition, for smaller hearts, like murine hearts, the
fiber will be smaller than that of rabbits. In order to map fiber orientation, high resolution is necessary. In this section, we demonstrated the mapping of fiber orientation of murine hearts with UHR-OCT without extra processes.

The OCT setup used here was exactly same as that described in the section 5.1. The protocol was approved by the Institutional Animal Care and Use Committee at Case Western Reserve University. Briefly, the mouse was anesthetized and the heart was removed and coronary was perfused with Krebs solution and then perfused with formalin on a Langendörff apparatus. The heart was stored in formalin for one day prior to imaging.

To calculate fiber orientation from en-face OCT images, multiple B-scans were compiled and reconstructed in Matlab. En-face images were obtained by re-slicing the 3-D images. A modification of the intensity-based gradient algorithm described by Karlon et al. was used and coded [16]. Within en-face OCT images, low spatial frequency changes in the background intensity were due to the uneven sample surface. These artifacts introduce another gradient within the image. A two dimensional second order Butterworth high pass filter was used under the assumption that the change in the surface height has a lower spatial frequency component as compared to visible fiber structures. The high pass filter was convolved with the en-face OCT image to reconstruct the OCT image with relatively constant background intensity to suppress variations in background intensity. Thereafter, the high pass filtered en-face OCT image was convolved with a Wiener filter for noise reduction.

Two-dimensional 3x3 Sobel filters were used to approximate the gradient. Gx
and $G_y$ are defined as the convolution of the horizontal and vertical Sobel filters with the 2-D en-face filtered. After fiber orientation measurements were assigned to each window within the image, two criteria were used to reject fiber orientation assignments. First, the algorithm identified windows with no tissue by taking a threshold on the average intensity value within the image. Second, the confidence in fiber orientation measurements produced by the automated algorithm was defined and used as a threshold to remove unwanted fiber orientation assignments.

Figures 5.3 and 5.4 show the fiber orientation of two different mouse hearts. Both images were achieved at about 5-10 µm below the surface. Fiber orientation marked in red lines was identified by the described algorithm. Some areas without obvious fibers were also identified, due to mistakes of the algorithm. However, the contrast decreased in deeper ranges. This can be attributed to lateral resolution degradation. First, the designed lateral resolution of the scanner was 7 µm, with a beam waist of about 150 µm. The beam size was expanded to about 25 µm at a point about 1 mm away from the focal point. Second, due to limitations of the hardware, the minimum step of a C-scan is 7 µm. For 7 µm lateral resolution, the step should be less than 3 µm. Last, cardiac tissue is high-scattering, so the beam was not focused as designed in the tissue. However, from the images, it is clear that UHR-OCT has the ability to map small and intact murine hearts. Further improvement is still possible.
When compared with DTMRI, the resolution of OCT is about one order higher and can provide images similar to histology slides. However, the image depth is limited to less than 2 mm. For 800 nm, the image depth cannot be more than 1 mm. It is hard to determine fiber orientation from epicardium to endocardium. Measurements of fiber orientation throughout the walls of the RV and LV have been shown with extended confocal microscopy [17]. Significant local variation in fiber orientations has been observed. However, with this method,
it is impossible to image the whole heart and is overly time consuming. For OCT, young mouse hearts may be used for imaging local fiber orientation variations if the wall is thin enough.

The degradation of the later resolution will affect imaging quality and identification of the fiber orientation. Currently, Gaussian beam is employed for scanning, which has a small beam waist. In order to keep the high lateral resolution over a long distance, an axicon lens is a possible solution. The beam waist can be kept to several hundreds micrometers without apparent degradation. However, an axicon beam is generated by interference so the beam shows serious side lobes [18]. This deleterious effect can be reduced by deconvolution. It is highly desirable to image the fiber orientation \textit{in vivo} in order to observe the variations in fiber orientation during electrical propagation or in propagation of the pattern of diseased hearts. It is very challenging for DMMRI. However, OCT can image at very high speed, which makes \textit{in vivo} imaging possible. Motion artifacts caused by beating can be eliminated by using gated OCT [7]. Here, the demonstration of mapping the fiber orientation of hearts without special care is an important step towards this goal.

5.3 Feasible Study of Imaging a Sliver Sensor Implanted in Skin

5.3.1 Introduction

Diabetes is a disease that is becoming more prevalent world-wide and is one of the leading causes of death and disability. The diagnosis and management of diabetes requires a close monitoring of blood glucose levels. Since Clark and Lyons first
proposed to use glucose enzyme electrodes in 1962 [19], tremendous efforts have been made to develop reliable devices for glucose monitoring.

The glucose sensor based on the electrochemical reaction is well suited for personal glucose testing. Current personal glucose meters are based on disposable enzyme electrode strips. The patient puts a small drop of blood on the sensor strip and reads the blood glucose concentration on the indicator. This device is cheap, portable and user friendly. However, this monitoring system is limited by the number of tests per 24 hour period. An even greater demand exists for continual and reliable monitoring of glucose concentration. This can supply diabetic patient with all the information required for optimizing the dose of their insulin injection. Furthermore, a fully automated feedback control for subcutaneous insulin delivery could be developed via portable insulin pumps.

For in vivo glucose monitoring, an implanted subcutaneous glucose sensor was developed by monitoring the levels of glucose in interstitial fluid. Several studies have shown that the levels of glucose in interstitial fluid tracks blood concentrations closely, although there is some time delay due to differing sample environments [20, 21]. Until now, clinical results of subcutaneously implanted glucose sensors have failed [22]. Although the exact reason is unclear, it is suspected that inflammatory response leads to encapsulation of the devices within a sheath of leukocytes, macrophages, fibroblasts, etc. In addition, the potential for infection could happen via the “wire” crossing the skin. This is thought to be especially pertinent for a patient outside the hospital setting for extended periods.
A novel concept of implanted sensor called a “sliver sensor” has been proposed by Dr. Gratzl [23]. Glucose can be monitored via color changes, which are induced by the production of gluconic acid in enzyme reaction. Absorption change at 625 nm can be linearly related to the variation of the glucose concentration. This will then change the color of the sensors and can be visualized or quantified by a CCD camera. Compared with previously developed implanted glucose sensors, a major advantage of this sensor would be its passive nature; that is, it would not require external power to function. In addition, this sensor must be embedded under very thin skin in order to be visible.

5.3.2 Feasibility of imaging sliver sensor with OCT

For further development of this new sensor, possible inflammation induced by the sensor itself must be studied. Previously, inflammation induced by implanted devices was identified by histological slices [24]. Although it is a gold standard, it has several serious disadvantages. Firstly, the workload of tracking inflammation at different time intervals is heavy. Second, it is difficult to keep the results consistent due to the fact that different subjects must be tested. Third, it is hard to do on human subjects, because tissue samples around sensor must be achieved from subjects.

For the sliver sensor, because it is implanted just below the skin surface at about 200 µm to 500 µm, it is very possible to achieve in vivo imaging by OCT. As the first step of monitoring inflammation induced by sliver sensors, we studied the feasibility of imaging sliver sensors implanted in mouse skin in preparation for long-time
monitoring and in order to find the optimal depth of implantation.

5.3.3 *In vivo experiments*

The OCT used for the *in vivo* experiment was a TDOCT based on rapid scanning delay line equipped with microscopy. The speed of the OCT was running at 4K A-scans per second, which can give a frame rate at around 8 frames per second. The light source centered at 1.3 μm provides an axial resolution of about 14 μm. The B-scan distance was set to 3 mm with a lateral resolution of 15 μm.

A living nude mouse was used for *in vivo* experiments. The sliver sensor was implanted into the dorsal skin of the mouse about 1 month prior to testing. No visible inflammation was observed during subsequent to this period. After anesthetizing the mouse, the mouse was fixed on a plate with a temperature control. A drop of glycerol was put on the skin to enhance the penetration and to avoid reflection from the first surface. The images were taken from different locations around the sensor, as well as on the sensor for comparison. Fig. 5.5 (f) shows the imaged taken by microscopy where the sensor was marked and clearly visible by naked eyes. Fig. 5.5 (a-e) shows the OCT images at different locations on or around the sensor. As shown in the images, the epidermis and the dermis can be clearly identified. However, we did not see the sensors in the skin. 3-D images cannot be achieved due to motion of the skin during imaging.
Fig. 5.5. \textit{In vivo} imaging of mouse skin around the implanted sliver sensor. The center one is the image of the skin on the sensor. Other figures represent the images located around corresponding positions. The color picture on the upper left panel is the image taken by microscopy. Sliver glucose sensor is visible. DE: Dermis. EDE: Epidermis.

5.3.4 \textit{In vitro experiments}

\textit{In vitro} experiments were conducted for 3-D scanning under more controllable conditions. FDOCT at 1.3 μm, with similar resolution as TDOCT, was employed for high speed imaging. Under the same condition, FDOCT have better sensitivity as compared to TDOCT. A piece of mouse skin was cut from the mouse and fixed in
formaldehyde. A sliver sensor was manually inserted into the skin and then imaged with FDOCT.

Fig. 5.6 shows the images of the 2-D cross section of mouse skin. The black square under the skin about 200 μm to 300 μm is clear visible. The thickness of the sensor is about 150 μm. However, we lost the layered structure of skin we observed in *in vivo* experiments. This can be attributed to the non-fresh tissue used here. Fig. 5.7 shows the 3-D reconstruction with Amira, the rectangular shape of the sliver sensor can be clearly identified. Three spots are separately located on the sensor. They are the glucose-sensing spots. The base plate of the sensor is uniform and has a smooth surface, so there was no direct signal from the base plate except at the boundary. The sensing spots consisted of multiple membranes that can scatter photons, which is visible in OCT.

Fig. 5.6. The OCT images of sliver sensor implanted in the mouse skin. Left figure: image along the long axis of the sliver sensor; Right figure: image of cross section of sliver sensor.
5.3.5 Discussion

The typical structure of skin is shown in Fig. 5.8 [25]. The topmost layer of the skin is the stratum corneum. The second layer is the epidermis with a thickness of 100 \( \mu \text{m} \) to 200 \( \mu \text{m} \) and consisting of cells containing keratohyalin granules, columnar cells, and other cells. The third layer is called the dermis and is primarily made up of collagen fibers with nerves and blood vessels running throughout. The dermis is sometimes divided into the papillary dermis and the reticular dermis based on the size of the blood vessels in them. The last layer is the subcutaneous layer and consists of fat. The corresponding thickness of the different layers was edited for reference [26].
In *in vivo* experiments, after the sensor was implanted for one month, no observable histological change was observed via OCT at locations on or around the sensor. One must question whether OCT has the ability to resolve the inflammation. It is also possible that either OCT lacks the resolution to distinguish inflammation or that inflammation does not induce appreciable histological changes to OCT. It is hard to answer this definitively because inflammation often occurs near the sensor. In *in vivo* experiments, the sensor seems to be implanted too deeply to be imaged by OCT. In addition, the motion artifact during the imaging also affects the ability to capture high quality images.

For answering the above question, the question of the appropriate depth for an implanted sensor, must be answered first; that is, is there a depth at which OCT can reliably image not only the tissue surrounding the sensor but also the sensor itself? To answer this, we setup *in vitro* experiments.
In *in vitro* experiments, the insertion of sensor can be well controlled. According to the images, the whole sensor can be reconstructed in 3-D and the depth of the location of the sensor was around 200 μm-300 μm, which is about the interface of the epidermis and the dermis. In addition, the upper blood plexus exits around this region, which will facilitate the glucose measurement. Due to the thickness of dermis, it is hard to track inflammation with OCT at sensors implanted subcutaneously, especially for human subjects.

Another consideration is the convenient position for OCT imaging. OCT is very sensitive to motion artifacts. In order to track the sensor for extended periods of time, the sensor must be implanted in an area where the motion artifact can be avoided so that the images can be repeated. The dorsal skin of a mouse is not an ideal position due to motion. Possible locations are the arms or legs, which can be stably anchored on the platform. In addition, precise insertion of the sensor around the interface of the dermis and the epidermis is difficult without feedback. One possible solution is to use OCT to monitor this process. Currently, real-time imaging can be achieved with OCT. This process can be easily realized with some training.

Although OCT can achieve high-resolution histology-like images, it is hard to say definitively that OCT can track the inflammation. The response of inflammation is cell adhesion and fibrous tissue encapsulation due to the body’s self defense mechanisms. It can be divided into four steps [24]: hemostasis, inflammation, repair and encapsulation. OCT can track the last step, because tissue around the implant becomes avascular, which will change the optical properties of the tissue. For the
early steps, the changes happen on the cellular or molecular level. More sensitive and even higher resolution imaging modalities are probably required. For OCT, in order to achieve high resolution in 3-D, optical coherence microscopy (OCM) maybe required. OCM can then be combined with two photon microscopy (TPM) and second harmonic microscopy (SHM). Normally TPM and SHM have resolution at the 1 μm level, with imaging depth up to 200 μm, and are sensitive to endogenous or exogenous agents. SHM is sensitive to fibrous tissues and phagocytic cells may be imaged by TPM through the injection of contrast. By combining multiple optical imaging modalities, inflammation can be tracked invasively under high resolution.

Successfully tracking the response of the tissue surrounding the implanted objects is very important and could have wide applications. For example, wireless biochips have been proposed for use in saving and identifying personal information by implanting such a chip into a human body. For active components, the biochip is driven by a power supply and has relatively strong RF electromagnetic field around the antenna. Although it is possibly weak, it is still unknown if the current and electromagnetic field will affect the microscopic environment of the surrounding tissue and eventually lead to disease. In vivo imaging of response at the cellular and histological level will provide valuable information for evaluation the inflammation.
CHAPTER 6. SUMMARY AND FUTURE WORK

6.1 SUMMARY

This thesis investigated high performance broad band light sources at above 1 µm, the feasibility of using gold nanorods as a probe for contrast sensitive OCT and some potential applications of UHR-OCT.

In Chapter 1, the background knowledge was reviewed and basic concepts were introduced. The parameters for evaluating the performance of OCT were described and the conclusion for achieving high-quality OCT imaging was attributed to the performance of the light sources used. However, currently developed light sources for UHR-OCT have been limited by noise, power or spectral shape especially at the wavelength range above 1 µm. By comparing with other imaging modalities, we find that OCT can fill the range of the imaging resolution between 1 µm to 10 µm with the ability to generate imaging at high speeds. However, OCT is sensitive only to absorption and scattering. It cannot be used directly for contrast imaging. External probes can be introduced to make OCT sensitive to contrast, which will greatly enhance the function of OCT. For this purpose, CSOCT was proposed and several different probes were investigated.

As the first part of this study, three different broad band light sources were developed, each based on different methods. In Chapter 2, multiple SLEDs at around 1.3 µm with offset central wavelengths were combined by fiber couplers to synthesize
a broad band light source. This is the most straightforward method for achieving broad band. However, it is limited by the commercial availability of SLEDs. We created this light source by combining four SLEDs from different vendors. Great care was put on the spectrum shape of the combined light sources. By choosing different splitting ratios of the fiber couplers and adjusting the current of the separated SLEDs, a FWHM band width of about 145 nm centered at 1325 nm was achieved. Axial resolution around 6.5 µm was measured in air. The advantages of UHR-OCT were demonstrated by comparing the two images with different axial resolution, but with the same setup and sample. The enhancement of visibility of fine structures is obvious.

Broad band light sources based on the SCG were developed quickly after the invention of PCFs. However, to this point, they are rarely used for practical applications due the limitations of noise, spectral modulation and lasers. In Chapter 3, we concluded by numerical simulation that PCF with flat dispersion profile near zero ZDW is best for SCG. By pumping this kind of PCF around flat dispersion areas, the spectrum will be very broad, low noise and excellent for UHR-OCT. Two different PCFs were used in our experiments. PCF1 has two closely spaced ZDWs and a flat dispersion profile at around 1 µm. By using a Nd:glass fs laser, a dual band light source was demonstrated. However, due to stimulated Raman scattering and cross phase modulation, serious spectral modulation was present. Two methods were used to optimize the spectral shape, polarization control and ultrashort pulse (<50 fs) pumping. Ultrashort pulses around 50 fs were achieved by a fiber-compressor system
that was then used for pumping. Smooth dual band light sources at 0.8 µm and 1.3 µm were generated and used for UHR-OCT imaging. In order to achieve even higher axial resolution, PCF2 with no ZDW was also pumped with 1 µm laser. Due to the lack of the negative dispersion of this fiber, the SCG was mainly attributed to SPM. The generated spectrum is smooth and low noise. The FWHM band width can reach 240 nm corresponding to the axial resolution around 2.8 µm in the air. The most valuable aspect of this light source is that the wavelength range covers from 0.9 µm to 1.3 µm, which is very advantageous for UHR-OCT above 1 µm. By considering the tissue properties and dispersion, the spectral properties of this light source maybe the best one for UHR-OCT at or above 1 µm without additional spectral filtering. The images demonstrated with this light source showed impressive performance.

Contrast sensitive OCT was proposed to push OCT to image events at the molecular level. The success of CSOCT really depends on the choice of probes and methods. For CSOCT, it is essential to use a bright field imaging method. The absorption and scattering probes have been tried for CSOCT. However, scattering probes are advantageous over the absorption probe. For this purpose, gold nanoparticles with high scattering were proposed for CSOCT. Previously, NRs have been proposed for using as a SP for CSOCT. However, the method used there is not practical for OCT imaging.

In Chapter 4, the feasibility of using NRs as a probe was studied by using the photothermal effect (PT) and spectroscopic analysis. PT was expected to modulate the scattering of NR. However, we found that the photothermal lensing effect was
dominant, which made this method difficult for CSOCT. Due to the well-defined SPR band of NRs at NIR, spectroscopic analysis can also be used. A dual band light source with different wavelength ranges was employed. One of them was overlapped to the SPR band of NRs. Two images can be achieved in one attempt by separating them in the frequency domain. By direct comparison of two images, the contrast from NRs maybe extracted. Contrast originating from the scattering and absorption of NRs was observed. However, at the size of NRs used there, the absorption is dominant. In order to achieve enough contrast from scattering, larger size of NRs will be required. For the suppression of spectral noise due to the inherent extinction, NRs also need to be further narrowed. We demonstrated that it was possible to narrow the band width of NRs down to 100 nm by photothermal melting. This will make NRs comparable to organic dyes.

In Chapter 5, three applications of OCT were demonstrated. With real time UHR-OCT at 800 nm, a 3-D mouse embryo was reconstructed by multiple B-scans. Many fine structures can be identified. However, the embryo was imaged intact without labor as intensive as histological sectioning. This study is the first step in future imaging of living mouse embryo, once ultrahigh speed and resolution OCT becomes available. With the same UHR-OCT, the fiber orientation of small mouse hearts was imaged without further processing such as optical clearing. The preliminary data proved that with UHR-OCT, it is possible to image fiber orientation of intact small hearts. This may pave the way of using UHR-OCT to image the fiber orientation of a beating heart and to relate the beats to different electrical propagation
patterns. In the last application, we studied the feasibility of using OCT to track implanted silver glucose sensors. For long-term tracking, the previous task was to ensure that OCT could reliably image silver sensors. The in vivo and in vitro images were developed using sensors implanted in mouse skin. We believe the implanting depth of silver sensor should be around the interface between dermis and epidermis, which can facilitate either imaging or function of the sensors. However, in order to tackle possible inflammation, UHR-OCT at above 1 µm is required. In addition, more information can be provided if OCT can be combined with nonlinear microscopy.

6.2 Future Work

6.2.1 Development of compact and cheap lasers with high performance at 1 µm

Currently, the axial resolution of OCT has been reported to be less than 1 µm. However, the potential upper limit of the real resolution in the tissue is still unknown. In the future, the axial resolution OCT should be kept to at least around 6 -7 µm in the air, since it is not hard to achieve resolution around 20 to 30 µm with ultrasound. However, considering dispersion, absorption, available optical components and detectors, the best axial resolution for UHR-OCT should be pursued to around 2-3 µm at the range of above 1 µm. Further enhancement of axial resolution will make the system cost-prohibitive and impractical for real applications and may even be deleterious for axial resolution. The light sources developed with PCF2 may prove to be very promising for this purpose. However, compact and cheap lasers with high
performance are still desired. Many 1 µm lasers have been developed with compact sizes that can be directly coupled into fiber, especially those of fiber lasers. The current output pulses of fiber lasers are highly chirped and inadequate for pumping. We should continue to monitor that situation and look for excellent solid state lasers in this wavelength range.

Another option should go to the swept light source (SS). However, currently SS is typically limited to relative narrow bandwidth and only performs well in the range around 1.3 µm. Broad band and high speed SS should be developed for ultrahigh resolution and speed system.

6.2.2 Construction of real time UHR-OCT at above 1 µm

The UHR-OCT we demonstrated is of relatively slow speed. For in vivo imaging, a high speed system is required. Typically, high speed Fourier domain OCT is preferred. A line scan camera at above 1 µm is still expensive and has limited pixel numbers. It will be interesting to develop technologies for using a line scan camera with 512 pixels to achieve an imaging depth of above 2-3 mm at high speed. In addition, broad band optical components should also be pursued to accommodate such broad band light sources. For example, currently available achromatic lenses are not good for this bandwidth. A consumer-designed lens group should be considered as a first priority. For endoscopic application, the ability to integrate an achromatic lens group into fiber probes represents a significant challenge. With the ultrahigh speed and resolution OCT at above 1 µm, all the application which we referred to in Chapter
Although spectroscopic analysis can extract a contrast signal, they do have ambiguity in explaining the data. External modulation should be the long term goal for CSOCT. This requires that chemists develop effective probes for CSOCT. Currently, probes for CSOCT have not attracted enough attention due to the fact that the importance of CSOCT has not been demonstrated in real applications. For differentiating from nonlinear and fluorescence microscopy, CSCOT should keep not only reasonable resolution but also reasonable depth. In this sense, scattering probes are desired. NRs are one of the choices. However, it is imperative to synthesize NRs with a modulated scattering cross section and to effectively deliver them to target areas. One of solution is to combine the gold nanorods with magnetic particles. It is well known that NR is very sensitive to the polarization. By using a external alternative magnetic field, we control the magnetic particles which will then change the orientation of the gold NRs. The biggest advantage is the extinction coefficient of NRs can be externally modulated which will avoid any ambiguity induced by the inherent background. However, it is really challenge to synthesize such kind of particles.
Chapter 1


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Chapter 2


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Chapter 4


Chapter 5


