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神戸大学博士論文

Optical Tomography and Its Biological Application

光トモグラフィと生体への応用

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Chapter 1

Introduction

When our own fingers or palms are placed under a flashlight, we may see a translucent red glow through our skin. This fact tells us the existence of light-component passing through these tissues even if the component is merely little. Today researchers are attempting to exploit this simple phenomenon to deduce beneficial information in human body. To reveal fine structure, density, or even physiological processes, they rely on the way due to absorbing, deflecting or scattering phenomena of light propagating through living tissues¹⁾. Usually near infrared light is available in comparison with visible light because these tissues act as a weak absorption medium. By measuring the intensity of transmitted or reflected light from the living tissues and sending the measured data into a computer, the fine structure of internal organs is imaged three-dimensionally. This novel technology, called an optical tomography,²⁻⁸⁾ has a diagnostic potential for visualizing optical properties of tissues *in vivo*.

The most reliable way to diagnose disease is to detect characteristic changes in interesting part of tissues, however the patient have to suffer from pain during a biopsy. Adaptation of the optical method can embody locally imaging of interesting parts in the body without the need of biopsy. The optical tomography promises a noninvasive imaging technology with the safe to patients and without the risk.⁵⁻⁹⁾

Furthermore, compact and low-cost systems are in demand for medical diagnostic tools. Such demand is due to examine the disease in early stage. In comparison with the other noninvasive biological imaging, such as ultrasound and x-ray computed tomography (CT) or magnetic resonance imaging (MRI), the optical method offers the ability of compact and

low-cost. These characteristics are possible to monitor the activities and functions of organs in daily life. In this regard, the optical imaging technology has a potential future in clinic medicine.

To realize the optical imaging in clinic applications, there is a fundamental problem to be solved. In contrast to x-rays, the near-infrared light does not cross the medium on a straight line from a source to a detector. The light is strongly scattered by the tissue.^{10,11)} As a result of multiple scattering process, the incident light finally scatters in a random direction. Such scattered light is called a diffuse light. The occurrence of intense diffuse light degrades severely the image quality. If the problem associated with the scattering can be overcome, the optical imaging technology can achieve high resolution imaging. Moreover, utilizing spectroscopic properties in the optical method may reveal more about active processes of organs, such as in the brain.

The light emerging from a scattering medium consists of two components in a model, the ballistic and the diffuse lights, as shown in Fig. 1-1. The ballistic photons are a major contributor to the signal because the photons propagate as same as in free space except for diffraction phenomenon. The number of photons, however, decreases inverse-exponentially with the propagation distance. On the contrary, the diffuse photons disturb directly imaging operation, and behave as a background noise which gives rise to lose imaging capabilities. For example, it is well known that the human tissue is a weak absorption and strong scattering medium for the near infrared light.¹²⁾ Even if the medium is non-absorption, the transmittance of the ballistic light is dramatically small and is of the order of 10^{-8} even for a short propagation distance of 2.0 mm. Note that such selection of the propagation distance is equivalent to measuring a round-trip depth 1.0 mm for a reflection-type system. The others are translated to both reflected and transmitted diffuse lights. Very weak signal of the

ballistic light must be detected from strong background noise of the diffuse light. So it is seen that the tomographic imaging in the human tissue is remarkably difficult.

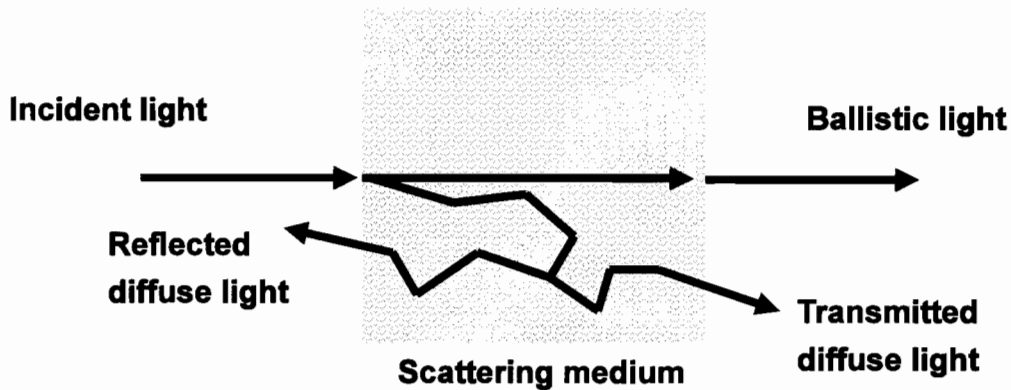


Fig. 1-1 Trajectories of photons in a random medium, showing the ballistic and diffusive components.

In this thesis, three types of optical tomography are investigated.

The first is confocal system. Although the optical tomography must provide 3D measurements, we may allow the point measurements. In the confocal process, the illumination light focuses on a desired point in the medium, and only the light emerging from the point is detected by using the optically conjugating system. At the optical system, the ballistic light can be detected without any loss. The diffuse light emerging in the medium beside the point may be rejected because the diffuse light does not keep the propagation direction.

An alternative approach to reject the diffuse light is called an optical coherence tomography (OCT). The ballistic and diffuse photons differ in their photon-pathlengths

passing through the medium. It is clear that the unscattered photon (ballistic photon) travels straightforward, and the pathlength between two points is shortest. If the diffuse photon travels between the two points, the optical path is like zig-zag, and the pathlength becomes longer than that of ballistic light. The pathlength difference can be measured with an interferometric technique. For the OCT system, we do not only utilize a confocal system, but also a low coherence interferometry to resolve the pathlength of the propagation light.

We called the third one as full-field OCT. Two techniques mentioned above reject the diffuse light at the sacrifice of optically image formation, and require the scanning system to acquire the imaging data. This optical tomography is to detect the *en face* cross section as an image. Usually the propagation direction of a plane wave is related to the spatial frequency. If the ballistic light has a zero spatial frequency, the other diffuse light with different propagation direction is distributed over the broad spatial frequency except for the zero frequency. Then, the diffuse light may be rejected by using the optically spatial filter in full-field OCT system.

This thesis consists of five chapters. In chapter 1, the background, problem and motivation of this study are described along the performance to be required to optical tomography.

In chapter 2, a fiber-based confocal system, which is a simplest technique of rejecting the diffuse light, is introduced. Since the confocal system is the point detection, the performance of the system is estimated by the resolution in lateral and axial directions in the medium. We have investigated the dependence of the resolution on a core size of the fiber to be used. Furthermore, the ability to reject the diffuse light is numerically estimated by means of Monte Carlo technique. A high signal-to-noise ratio is obtained owing to select the appropriate core size of the fiber.

Although the confocal system can reduce tremendously the amount of the diffuse light, the signal-to-noise ratio is not enough to achieve the penetration depth of millimeters. A prominent biomedical imaging technique of optical coherence tomography (OCT) is proposed in chapter 3. It works through the principle of a low-coherence interferometry. The scattered light is further rejected by means that we rely on the pathlength difference through interference except for the confocal technique. Thus, the dynamic range of OCT is high enough to offer the penetration depth of millimeters in human tissue. In addition, a new application to epidural anesthesia of OCT along the axial direction is introduced. A needle-fiber depth-resolved OCT system is established and the preliminary experiment is performed. Here we use Monte Carlo simulation to evaluate the performance of the proposed confocal OCT system and simulate the behavior of light transportation in biological tissues. Based on the simulation, we analyze the influence of focal length in strongly scattering medium, and give a suggestion to expand the ranging distance for practical application.

In chapter 4, a full-field OCT system is developed. It presents the different performance from the conventional OCT system described in chapter 3. To detect a small target in a scattering medium, a variability of the axial resolution (or longitudinal resolution) is realized using a wavelength-scanning laser source. An optically spatial filter system is usually applied to the full-field OCT system to eliminate the diffuse light. Moreover, as an example to show the usefulness of resolution changing, we demonstrate the searching for a nucleus in onion cells by low-resolution imaging and derive tomographic images of the nuclei by high-resolution imaging.

Finally in chapter 5, we summarize the results obtained in this study.

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Chapter 2

Tomographic properties of fiber-based confocal system

2.1 Introduction

Since the first confocal microscopy was published in a patent in 1961,¹⁾ it has been extensively studied and applied in biological tissues.²⁻⁴⁾ Comparing with the conventional optical system, the confocal system presents superior resolution and a strong optical-sectioning effect. Therefore, it provides a powerful means of achieving 3-D imaging (tomographic imaging) in a thick specimen.

A new form of confocal system, a fiber-based optical confocal system, has been proposed in the early 1990s.^{5,6)} This implementation has many advantages for probing the biological tissues. First, it differs from the conventional confocal system with a finite-sized pinhole owing to behaving as a coherent imaging system by using an optical fiber instead of the pinhole. Furthermore, using the optical fiber as transmission parts makes the system very compact and easy to adjust. Finally, it is simple and convenient for operating imaging modality in comparison with the other technique such as X-ray CT and MRI (Magnetic Resonance Imaging) etc. These features are attractive to expand its application in clinical medicine. Recently a fiber-optical confocal system is designed to a flexible endoscope to detect changes with cervical precancer.^{7,8)}

Tomographic imaging for biological tissues usually suffers from background noises arising from diffusely scattered light.⁹⁾ The main characteristic of the fiber-based confocal system is that the amount of the scattered light can be tremendously reduced by using a finite dimension of fiber and leading to images of a high signal-to-noise ratio. The signal

transformation of the fiber-optical confocal system has been researched a decade ago.¹⁰⁾ However, properties of scattered light and the rejection rate depending on an optical system have not been studied. Since the light source is a finite cross-section at a tip end of the optical fiber (fiber core), the imaging properties with fiber differs from those in confocal system with a point source and a point detector, and the tomographic resolution and noises due to scattered light may be affected by the finite core size of the fiber.

The present chapter is to investigate the role of the core size of fiber in the resolution of the fiber-optical confocal system. Moreover, the amounts of scattered light to be detected by the confocal system and non-confocal system are numerically calculated and analyzed, respectively. As a result the rejection rate of scattered light between the confocal and non-confocal systems has been revealed with respect to the core size of fiber.

This chapter is organized as follows. In section 2.2, the basic principle of the fiber-based confocal system is described. Based on the description, we study the effective point spread function in lateral direction and axial direction. In section 2.3, we construct a simulation model to calculate the scattered light levels of the fiber-optical confocal system and non-confocal system. Section 2.4 is devoted to the simulation results and discussion. Finally, conclusions are given in section 2.5.

2.2 Basic principle of fiber-based confocal system

2.2.1 General properties

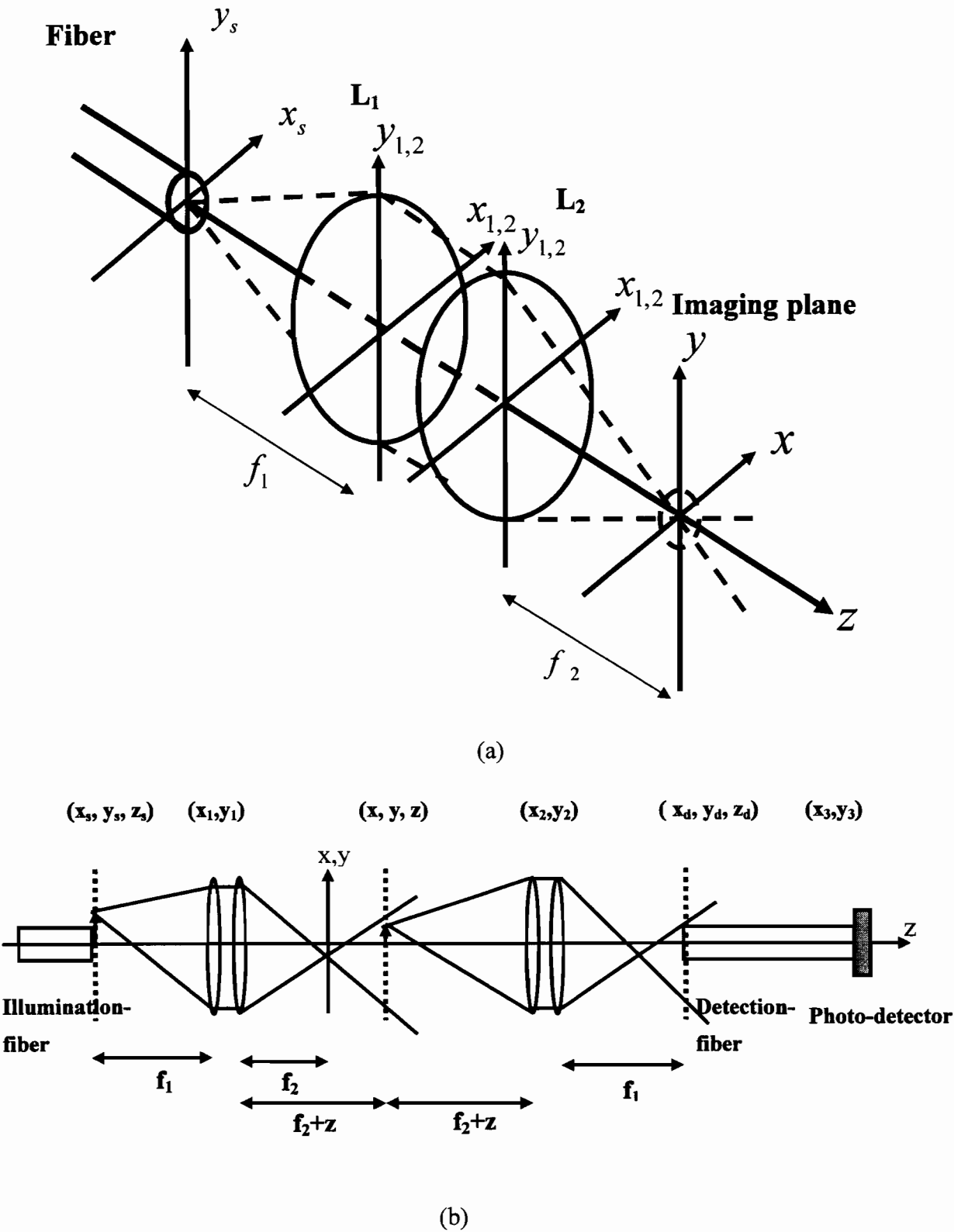


Fig. 2-1 The geometry of a fiber-based confocal system and its expansion along light propagation.

A schematic diagram of fiber-based reflection-type confocal system is presented in Fig. 2-1(a), where a same fiber is used for light-guiding for both illumination and detection. In order to analyze the performance, the system is extended along light propagation as shown in Fig. 2-1 (b). The source at $\bar{x}_s$ -plane (an output end of illumination-fiber) is imaged in an object space by a condenser lens at $\bar{x}_1$ -plane. The object plane (x, y, z) locates at a distance z from the imaging plane ($z = 0$). The backscattered light emitted at any point (x, y, z) is collected by a collector lens in $\bar{x}_2$ -plane and is injected into a detection-fiber at $\bar{x}_d$ -plane. The output light from the fiber is detected at the $\bar{x}_3$ -plane by a photo-detector. For the reflection-type confocal system as described above, the amplitude profile at $\bar{x}_d$ -plane is the same as that at $\bar{x}_s$ -plane. Here we define in this work that the optical fiber to be used is a single-mode fiber.

Let us define that $h_1(\bar{x}_s; \bar{x}, z)$ is the amplitude point spread function from $\bar{x}_s$ - to $\bar{x}$ -planes and $h_2(\bar{x}, z; \bar{x}_d)$ is that from $\bar{x}$ - to $\bar{x}_d$ -planes. The optical field of illumination light on the position $(\bar{x}, z)$ in an object space is given by

$$u_o(\bar{x}, z) = \int g_s(\bar{x}_s) h_1(\bar{x}_s; \bar{x}, z) d\bar{x}_s, \quad (2-1)$$

where $g_s(\bar{x}_s)$ is the amplitude mode-profile of the single-mode fiber, which is equivalent to an optical field at the output end of the illumination-fiber.

To obtain 3D amplitude reflectance $r(\bar{x}, z)$ in the object with the confocal scanning system, the object is scanned by $\bar{x}_{obj}$ in a lateral direction and by z_{obj} in an axial direction. Then, the optical field at the position $\bar{x}_d$ is given by superposing the fields reflected from all positions in the object space as

$$u_d(\bar{x}_d) = \int u_o(\bar{x}, z) r(\bar{x} - \bar{x}_{obj}, z - z_{obj}) h_2(\bar{x}, z; \bar{x}_d) d\bar{x} dz. \quad (2-2)$$

The field is detected through the detection-fiber whose amplitude mode-profiles are defined

by $g_d(\vec{x}_d)$ for input end and $g_3(\vec{x}_3)$ for output end. Since the field injected to the fiber is given by $\int g_d(\vec{x}_d)u_2(\vec{x}_d)d\vec{x}_d$, the output field propagating through the fiber is allotted to a rate of the output mode-function. So, the amplitude of output field can be expressed as:

$$u_3(\vec{x}_3) = g_3(\vec{x}_3) \int g_d(\vec{x}_d)u_2(\vec{x}_d)d\vec{x}_d. \quad (2-3)$$

The output intensity from the detection-fiber is detected by a large area detector with a uniform sensitivity as

$$I_{conf} = \int |u_3(\vec{x}_3)|^2 d\vec{x}_3. \quad (2-4)$$

Consider the detector acts as a spatially incoherent detection, the detected intensity is

$$\begin{aligned} I_{conf} &= |u_{conf}|^2 \\ &= \int |g_3(\vec{x}_3)|^2 \left| \int g_d(\vec{x}_d)u_d(\vec{x}_d)d\vec{x}_d \right|^2 d\vec{x}_3 \\ &= \left| \int g_d(\vec{x}_d)u_d(\vec{x}_d)d\vec{x}_d \right|^2. \end{aligned} \quad (2-5)$$

Here we have assumed $\int |g_3(\vec{x}_3)|^2 d\vec{x}_3 = 1$ because it is a constant for a given single mode fiber. As a result, we can find that the introduction of the single mode fiber does not change the coherence of the image system.

For the simplicity, we define the function

$$u_T(\vec{x}, z) = \int g_d(\vec{x}_d)h_2(\vec{x}, z; \vec{x}_d)d\vec{x}_d. \quad (2-6)$$

When a point source is placed at position $(\vec{x}, z)$ in the object space, the function u_T represents the total field injected to the detection-fiber. The optical field contributing to the detection is given by using Eq. (2-5) as

$$\begin{aligned} u_{conf} &= \int g_d(\vec{x}_d)u_d(\vec{x}_d)d\vec{x}_d \\ &= \int u_o(\vec{x}, z) r(\vec{x} - \vec{x}_{obj}, z - z_{obj})u_T(\vec{x}, z)d\vec{x}dz. \end{aligned} \quad (2-7)$$

In general, the spatial variation of the reflectance in the object is statistically independent. So,

it is considered that the reflection light from the object space is spatially incoherent. Then the condition of $\delta(\vec{x} - \vec{x}', z - z')$ is usually adopted. The detected intensity is

$$\begin{aligned}
 I_{conf}(\vec{x}_{obj}, z_{obj}) &= \left| u_{conf}(\vec{x}_{obj}, z_{obj}) \right|^2 \\
 &= \int u_o^*(\vec{x}', z') r^*(\vec{x}' - \vec{x}_{obj}, z' - z_{obj}) u_T^*(\vec{x}', z') d\vec{x}' dz' \delta(\vec{x} - \vec{x}', z - z') \\
 &\quad \times \int u_o(\vec{x}, z) r(\vec{x} - \vec{x}_{obj}, z - z_{obj}) u_T(\vec{x}, z) d\vec{x} dz \\
 &= \int |u_o(\vec{x}, z)|^2 |u_T(\vec{x}, z)|^2 R(\vec{x} - \vec{x}_{obj}, z - z_{obj}) d\vec{x} dz.
 \end{aligned} \tag{2-8}$$

The form is given by a convolution integral between the reflectance distrution $R(\vec{x}, z) = |r(\vec{x}, z)|^2$ and $|u_o(\vec{x}, z)|^2 |u_T(\vec{x}, z)|^2$. Therefore, the confocal system is characterized by the effective point spread function (effective PSF) as

$$|h_{conf}(\vec{x}, z)|^2 = |u_o(\vec{x}, z)|^2 |u_T(\vec{x}, z)|^2. \tag{2-9}$$

Now we deduce analytically the point spread functions $h_1(\vec{x}_s; \vec{x}, z)$ and $h_2(\vec{x}, z; \vec{x}_d)$ with according to Fig. 2-1. A pupil function $p(\vec{x}_1)$ of a condenser lens is located in the $\vec{x}_1$ -plane, and a pupil function $p(\vec{x}_2)$ of a collector lens is located in the $\vec{x}_2$ -plane. The functions satisfy the relation $p(\vec{x}_1) = p(\vec{x}_2)$ because of the same lens in the reflection-type confocal system. Furthermore, light in free space obeys the law of Fresnel propagation and two lenses with focal lengths of f_1 and f_2 are closely contacted. Then,

$$\begin{aligned}
 h_1(\vec{x}_s; \vec{x}, z) &= \frac{f_2}{f_2 + z} \exp[ik(f_1 + f_2 + z)] \\
 &\quad \times \int p(\vec{x}_1) \exp\left[ik\left[\frac{(\vec{x}_1 - \vec{x}_s)^2}{2f_1} - \frac{1}{2}\left(\frac{1}{f_1} + \frac{1}{f_2}\right)\vec{x}_1^2 + \frac{(\vec{x} - \vec{x}_1)^2}{2(f_2 + z)}\right]\right] d\vec{x}_1 \\
 &= h_2(\vec{x}, z; \vec{x}_s)
 \end{aligned} \tag{2-10}$$

is deduced. Since both fibers of illumination and detection are the same single mode fiber, we can define the relation

$$g_s(\vec{x}_s) = g_d(\vec{x}_d). \quad (2-11)$$

From Eqs. (2-1) and (2-6), therefore, the relation

$$u_o(\vec{x}, z) = u_T(\vec{x}, z) \quad (2-12)$$

is obtained. This shows the reversible property of light propagation in a reflection-type confocal system.

In the followings, we use several definitions. The subscripts of the functions are, respectively, omitted as $g(\vec{x}_s)$ and $h(\vec{x}_s; \vec{x}, z)$. The symbol $\otimes$ denotes the convolution operation as

$$g(\vec{x}) \otimes h(\vec{x}) = \int g(\vec{x}_s) h(\vec{x} - \vec{x}_s) d\vec{x}_s \quad (2-13)$$

and the symbol $\tilde{\otimes}$ is defined as

$$g(\vec{x}) \tilde{\otimes} h(\vec{x}) = \int g(\vec{x}_s) h(\vec{x}_s; \vec{x}, z) d\vec{x}_s. \quad (2-14)$$

Under such definitions, the performance of fiber-based reflection-type confocal system is given as follows:

$$\begin{aligned} I_{conf}(\vec{x}_{obj}, z_{obj}) &= \int |u_o(\vec{x}, z)|^2 |u_T(\vec{x}, z)|^2 R(\vec{x} - \vec{x}_{obj}, z - z_{obj}) d\vec{x} dz \\ &= R \otimes [|g_s \tilde{\otimes} h_1|^2 |g_d \tilde{\otimes} h_2|^2] \\ &= R \otimes [|g \tilde{\otimes} h|^4]. \end{aligned} \quad (2-15)$$

The effective PSF can be written as:

$$PSF = |g \tilde{\otimes} h|^4. \quad (2-16)$$

2.2.2 Effective PSF and resolution

We adopt a Gaussian mode-profile with a radius a which is equal to the core size of the illumination-fiber as

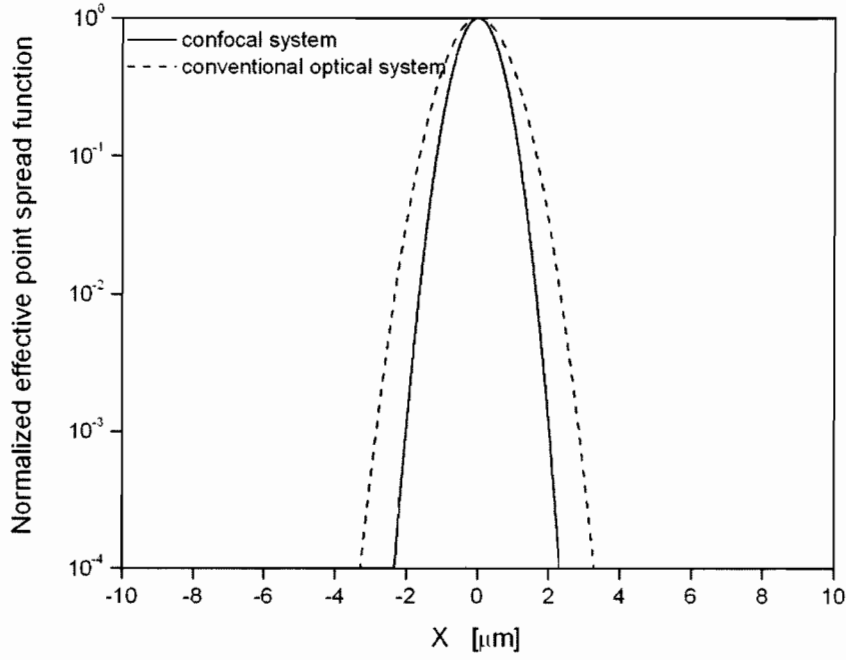
$$g(\vec{x}_s) = \exp\left[-\frac{\vec{x}_s^2}{2a^2}\right], \quad (2-17)$$

and assume that the pupil function of the lens L_1 and L_2 also presents a Gaussian profile with a radius b as

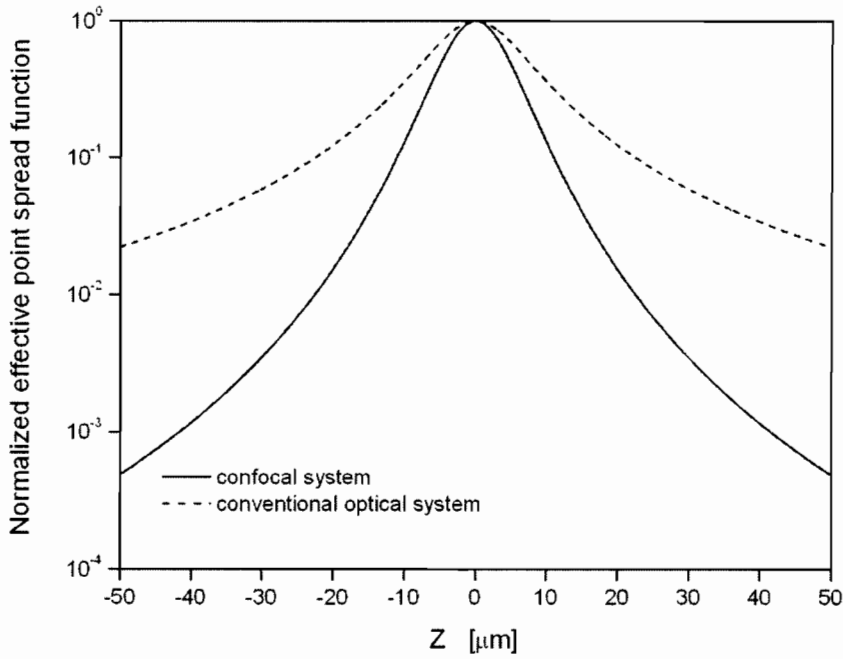
$$p(\vec{x}_1) = \exp\left[-\frac{\vec{x}_1^2}{2b^2}\right]. \quad (2-18)$$

The focal lengths of lens system are $f_1 = 18$ mm and $f_2 = 6.2$ mm so the magnification factor is approximately equal to $M = 0.34$.

Under the conditions of $b = 3$ mm and $a = 3$ μm , the effective PSF in lateral and axial directions are shown in Fig. 2-2. Here the dashed curves correspond the property of the conventional optical system. Let us consider the effective PSF of the conventional optical system. It is obvious that the conventional optical system only include the detection process because the illumination is done by a plane parallel light. Thus the effective PSF is given by $|u_T(\vec{x}, z)|^2$. For the confocal system, however, the imaging process divides into two stages: illumination and detection, then the effective point spread functions is expressed by $|h_{conf}(\vec{x}, z)|^2 = |u_o(\vec{x}, z)|^2 |u_T(\vec{x}, z)|^2$ as described in Eq. (2-9). Therefore, the confocal system presents a superior optical sectioning property because the out-of-focus light is almost eliminated through the two stages. This characteristic is demonstrated in Fig. 2-2.



(a)



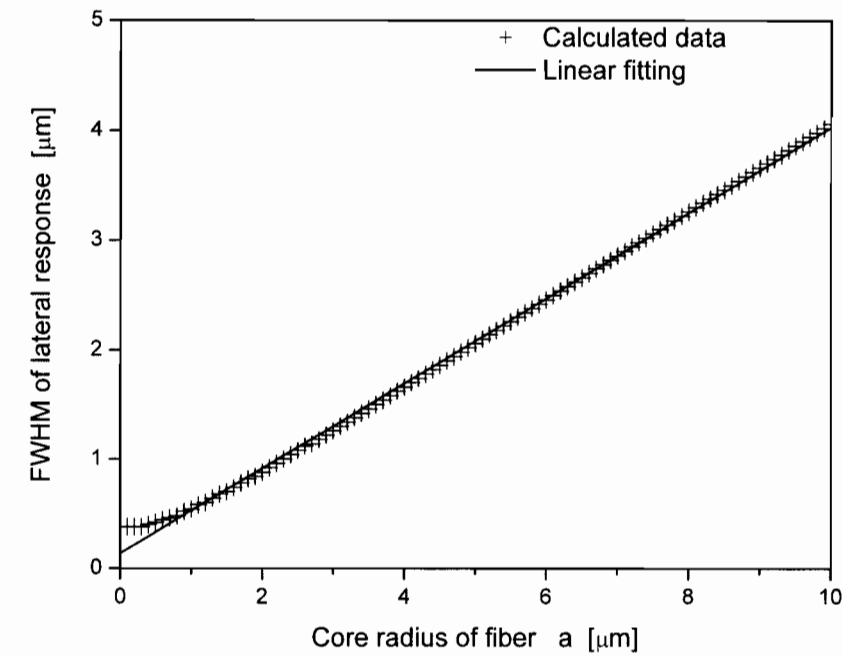
(b)

Fig. 2-2 The effective point spread function, normalized to unity at $x = 0$ and $z = 0$. (a) Lateral direction (x-axis) at $z = 0$; (b) Axial direction (z-axis) at $x = 0$.

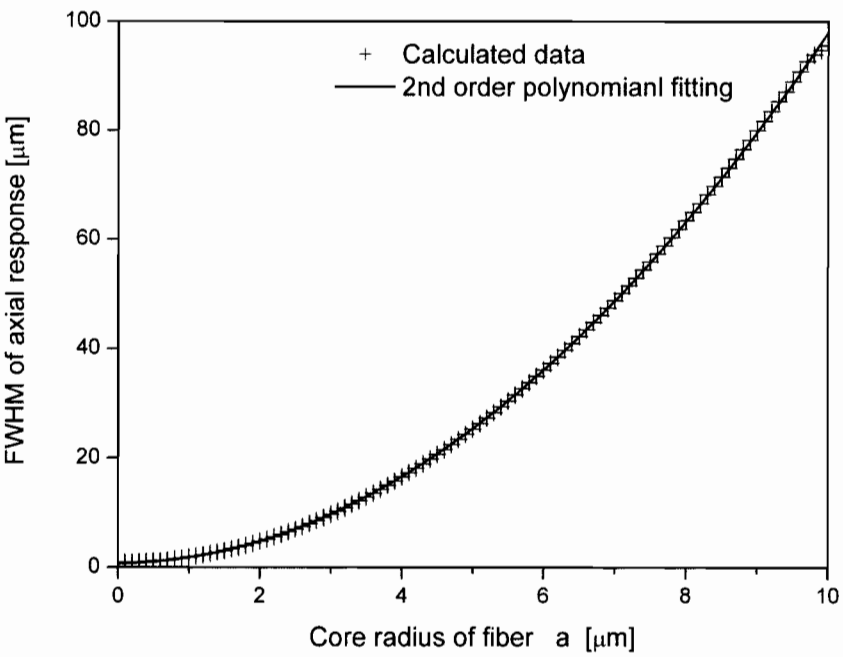
For the case of $b = 3 \text{ mm}$, the FWHM (Full Width at Half Maximum) of lateral and axial responses with different core size are illustrated in Fig. 2-3. It is seen that the lateral resolution varies linearly with the core size of fiber, and the corresponding fitting line in Fig. 2-3(a) demonstrates it agrees with the calculated data very well. It suggests that the lateral direction obeys the law of geometric optics, and its resolution is linearly related to the core size of fiber. For the axial direction, the resolution is more sensitive to the core size of the fiber than that of lateral direction. A suitable second-order polynomial fitting is performed as shown in Fig. 2-3(b). As a result we find that the second-order polynomial fitting is appropriate, the axial resolution alters as a second-order polynomial function with the core size.

When the core size of fiber is $a = 3 \text{ }\mu\text{m}$, the lateral and axial resolutions are obtained as $1.26 \text{ }\mu\text{m}$ and $9.66 \text{ }\mu\text{m}$, respectively.

If the value b (the radius of a pupil in the lens system) is changed, the behaviors of lateral and axial resolutions are illustrated in Fig. 2-4. The resolutions improve as the radius of pupil increases. At small region of b the resolutions change significantly, and tend to become larger with the pupil size of the lens system decreasing. This is because when the pupil size is small, the illuminating area of the light at the lens plane is larger than the pupil size, thus a part of illuminated light is truncated, and the resolutions turn bad. When the pupil size becomes larger than the illuminating area at the lens plane, the incident light at the lens plane becomes to pass through the lens system without truncation, so the resolutions approach a constant irrespective of changing the pupil size. For the parameters as mentioned above, it is found that the pupil of $b = 3 \text{ mm}$ tends to optimal design.

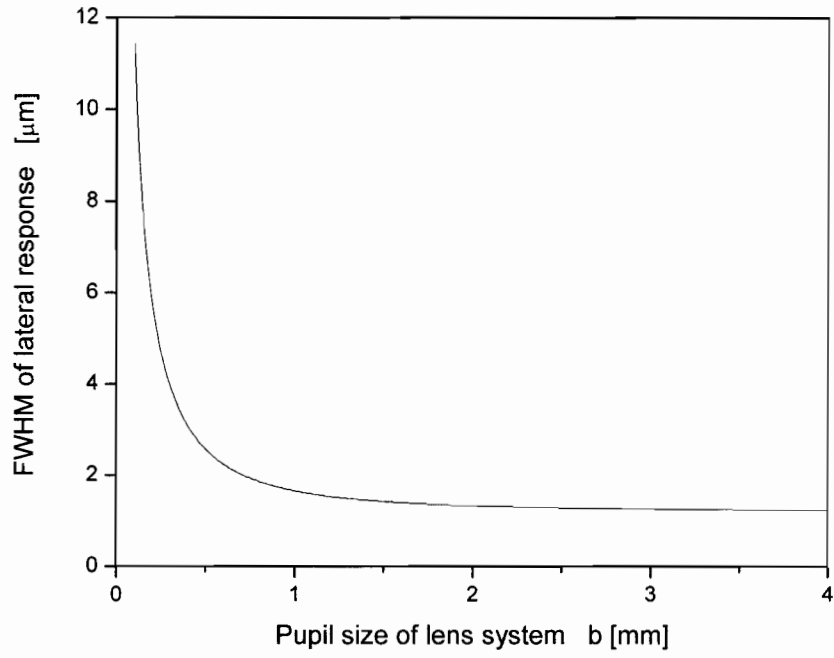


(a)

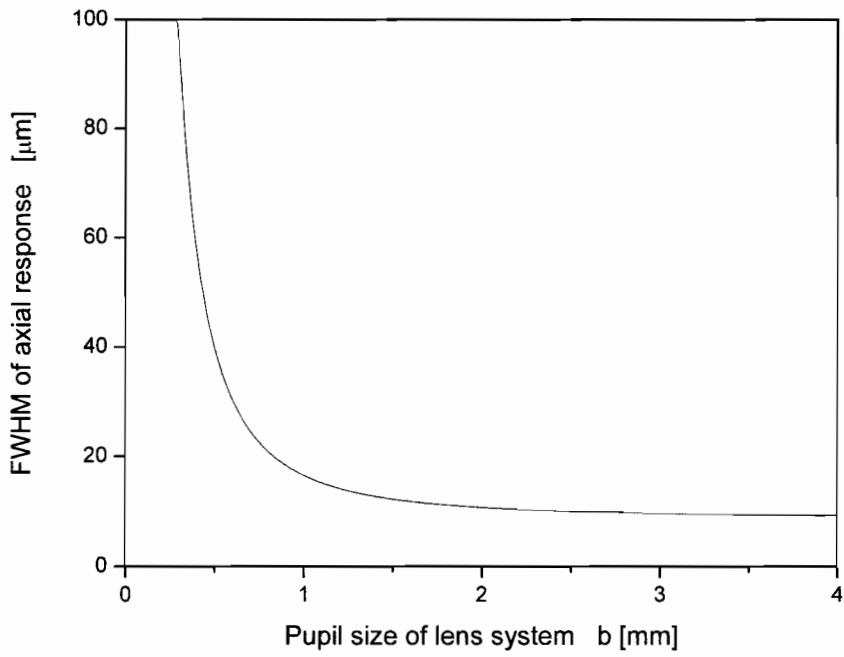


(b)

Fig. 2-3 Resolution of the fiber-based confocal system as a function of the core size a of the fiber: (a) Lateral resolution (x-axis) and (b) Axial resolution (z-axis).



(a)



(b)

Fig. 2-4 Resolution of the fiber-optical confocal system as a function of the radius b of pupil. (a); Lateral resolution (x-axis) and (b); Axial resolution (z-axis).

To test our calculation, we adopt a fiber-optical confocal system with parameters: $a = 3 \text{ } \mu\text{m}$, $b = 2.5 \text{ mm}$, $f_1 = 18 \text{ mm}$ and $f_2 = 6.2 \text{ mm}$. A ground glass plate is used as a scattering medium, and it moved along the axial direction. The detected light is shown in Fig. 2-5. The FWHM is about $9.25 \text{ } \mu\text{m}$. From the calculated data of Fig. 2-3, the calculated value of FWHM is $9.66 \text{ } \mu\text{m}$. The experimental result matches up the calculated data very well, and it proves that our calculation is correct. The data have a 4% difference between each other. This maybe the dimensions of fiber and lens are not the real Gaussian defined radiuses.

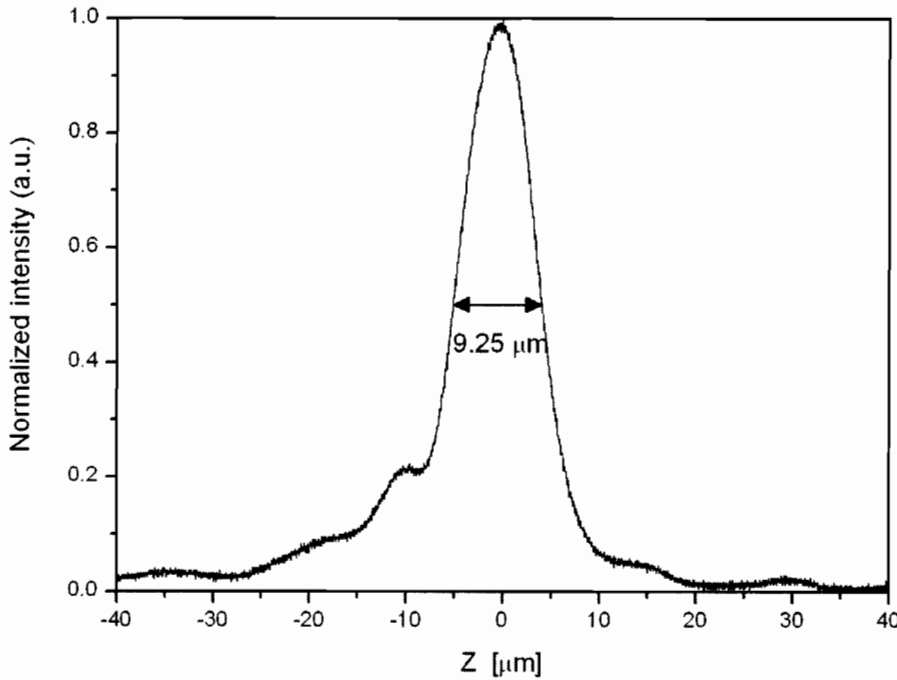


Fig. 2-5 The detected intensity for the confocal system of $a = 3 \text{ } \mu\text{m}$, $b = 2.5 \text{ mm}$, $f_1 = 18 \text{ mm}$ and $f_2 = 6.2 \text{ mm}$. A ground glass plate is used as a scattering medium, and it is moved along the axial direction.

2.3 Rejection of diffuse light

In section 2.2, we have mainly discussed the resolution of the fiber-based confocal system. To adopt the system to tomographic imaging in human tissue, the quality of detected

signal should be investigated. The human tissue is a strongly scattered medium. The light propagation in the medium undergoes multiple scattering and yields diffuse light intensely. The multiple scattering results the attenuation of signal due to ballistic light and the increase of background noise due to diffuse light. These phenomena limit the detectable depth to be needed in clinical operation and the 3-dimensional resolution discussed in section 2.2. It is well known that the confocal system has an ability rejecting the diffuse light. In this section, we investigate the rejection-performance of the diffuse light. In the past, several researchers have studied the propagation of focused light in scattering media by Monte Carlo simulation.¹⁰⁻¹⁴⁾ The simulation method provides a simple and accurate way to study the propagation in the scattering medium. We also use the Monte Carlo simulation method to simulate the behavior of light transportation in biological tissues and to evaluate the performance of the proposed fiber-optical confocal system. Based on the simulation, we give a suggestion to improve the ability to reject the diffuse light in comparison between the fiber-optical confocal system and the conventional non-confocal system.

2.3.1 Monte Carlo simulation model

Figure 2-6 shows our Monte Carlo simulation model of the reflection-type fiber-optical confocal system viewing the inside of a scattering medium. The light emitted from the tip of fiber reaches at the lens system with a Gaussian profile of radius b_{eff} . Here we assume to be $b_{eff} < b$ where b is the radius of pupil in the lens system. Since the lens system is composed of closely contacted two lenses, L_1 and L_2 , for simplicity of investigation, we define that the focal lengths are identical such as $f = f_1 = f_2$. The light from the lens system is focused at a depth z_d below the surface of the scattering medium and then the

surface of medium is illuminated over a radius r_i . The illumination area on the medium surface is determined by the relation:

$$b_{eff} = r_i + \frac{n(r_i / z_d)(f_2 - z_d)}{\sqrt{1 - (n^2 - 1)(r_i / z_d)^2}} . \quad (2-19)$$

The incident light undergoes multiple scattering process in the medium and re-emitted from the medium surface as diffuse light. The diffuse light is collected by the lens system, and enters into the fiber to be detected by a photodiode.

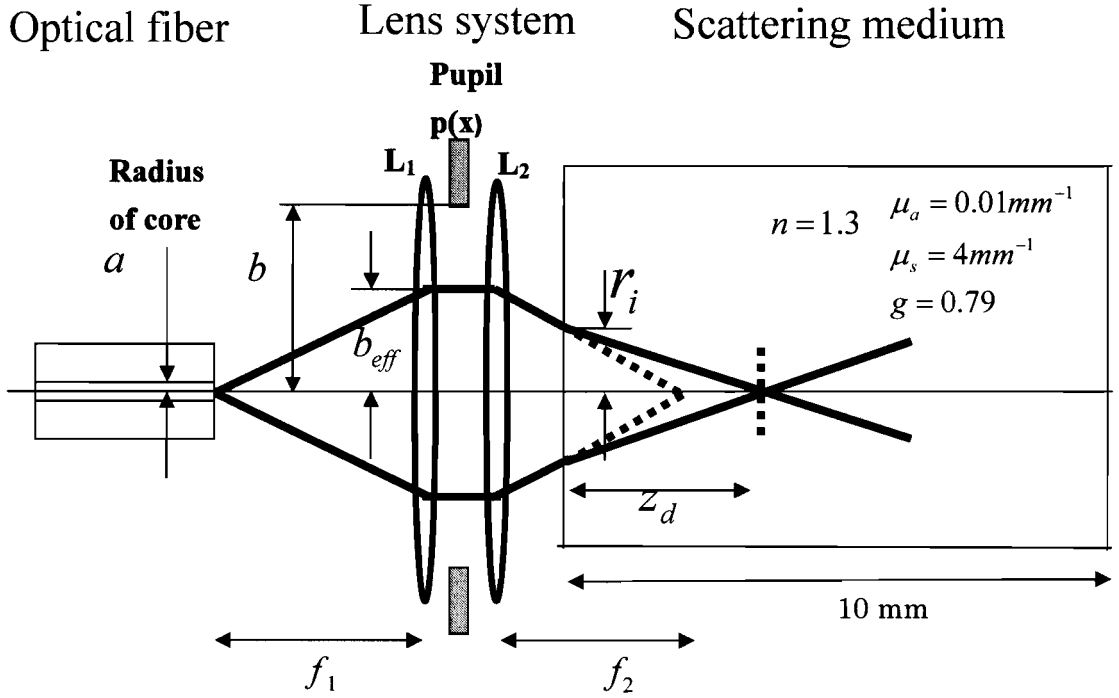


Fig. 2-6 Model of a reflection-type fiber-based confocal system. The light emerges from the tip of the fiber with a Gaussian profile, and passes through the imaging lens system to focus at a depth z_d below the surface of the scattering medium.

We take a skin as an example of biological tissue. The hypodermic tissue presents the useful information on metabolism, such as blood content. The important portion to be

investigated locates in the dermis layer which lies at a depth from 0.06 mm to 3 mm. In our simulation, the focal depth in the scattering medium varies from $z_d = 0.5$ mm to 2.0 mm by a step of 0.5 mm. The refractive index of the scattering medium is $n = 1.3$ for the tissue phantom. The scattering properties vary with wavelength of illumination light. The wavelength in our simulation is assumed to be approximately 1 μm , which provides good penetration for the human tissue. For spectral region of the near infrared, it is well known that the scattering dominates the absorption in light-tissue interaction. The optical condition of the tissue is set to the absorption coefficient of $\mu_a = 0.01 \text{ mm}^{-1}$, the scattering coefficient of $\mu_s = 4.0 \text{ mm}^{-1}$ and the anisotropy parameter of $g = 0.79$ due to the realistic application in skin tissue.¹⁶⁾ The scattering medium is infinitely wide with a thickness of 10 mm. Although the real tissue can never be infinitely wide, it is treated on the condition that it is much wider than the spatial extent of the photon distribution.

The Monte Carlo calculation of multiple scattering process is described in chapter 3. In this section we show the outline, particularly several conditions for incident and exiting photons at the surface of the scattering medium. Photons with an initial power are launched with a Gaussian probability distribution into the scattering medium. The initial input trajectory is directed towards the focal point at the imaging plane. As the scattering medium has different index of refraction with the ambient, the photon's trajectory is changed by the Snell's law on the surface boundary. A part of photon's power is Fresnel-reflected at the boundary. Once the other part enters into the medium, the photon undergoes many times interaction with scatterers in the tissue. At each interaction site, a small fraction of power is deposited in an absorption, and the rest is scattered. The scattering process is traced and terminated until either the power is below a preset threshold or the photon escapes from the tissue.

2.3.2 Confocal and non-confocal conditions

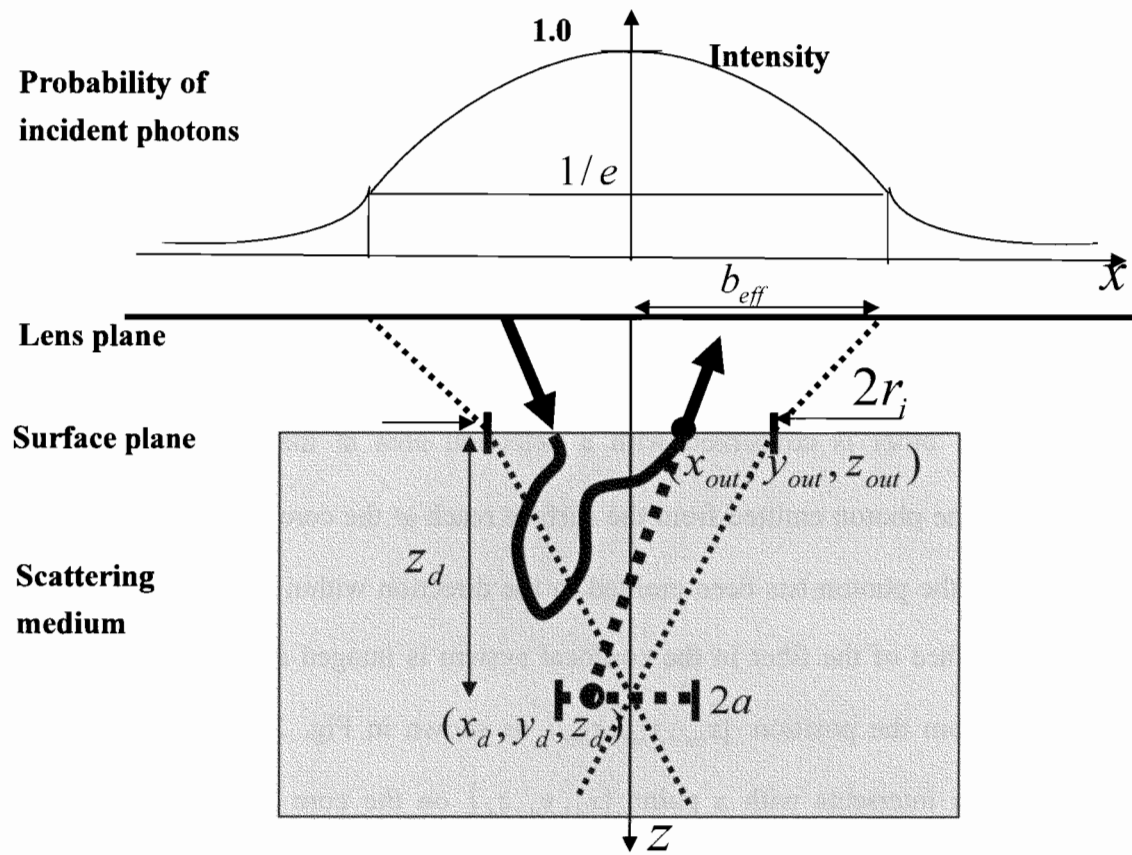


Fig. 2-7 A schematic of the exiting photon of position $\{x_{out}, y_{out}, z_{out}\}$ at surface plane and its virtual point $\{x_d, y_d, z_d\}$ on z_d -plane (imaging plane of the fiber tip).

Our aim of simulation is concerned mainly with determining how many diffuse-photons are collected by the photodetector. Confocal system is attractive as a result of providing tomographic images of high quality even for scattering medium. Such superior property will be caused by rejecting the diffuse light. On this basis, we simulate the diffuse light to be detected by the non-confocal system and the confocal system. Here the non-confocal system is a conventional optical system such as usual microscope.

To analyze quantitatively the ability of removing the diffuse light, we derive the dependence of the core size a of fiber, the focal depth z_d in the scattering medium, and the effective numerical aperture $(NA)_{lens}$ due to the lens system. To simplify the analysis, we adopt a confocal system with a magnification of 1 as described as before. According to the symmetry of system, the imaging and the tip of fiber behave conjugately and are identical to the same size of a . For the confocal process, the backscattering light to be collected by the fiber satisfies the following two conditions. One is that the position $\{x_{out}, y_{out}, z_{out}\}$ of photon emitted from the surface is located within the illumination area of the radius r_i . The other is concerned with a detection area at the input plane of the detection-fiber. If the photon emitted from the surface reach at the core area of fiber, it may be considered that the photon has been emitted in the direction within an acceptable angle. Note that the end face of the fiber in the confocal system is imaged at z_d -plane. When a photon emerges from the position $\{x_{out}, y_{out}, z_{out}\}$ as shown in Fig. 2-7, and a reciprocal path of the photon intersects with a point $\{x_d, y_d, z_d\}$ on the core image, then we can recognize that the photon is emitted within the acceptable angle, and is detected through the detection-fiber. Therefore, the two conditions can be described as:

$$\begin{cases} \sqrt{x_{out}^2 + y_{out}^2} < r_i, \\ \sqrt{x_d^2 + y_d^2} < a. \end{cases} \quad (2-20)$$

The non-confocal process is typically characterized by the infinite detection-area at the input plane of fiber. This condition is equivalent to the situation that the point $\{x_d, y_d, z_d\}$ is not restricted within the image of the fiber core. However, such photon needs to pass through lens system. So, the non-confocal process satisfies the condition that the position $\{x_{out}, y_{out}, z_{out}\}$ of exiting photon should be located within the illumination area on the medium surface.

2.4 Numerical results and discussions

In Fig. 2-8 we plot the detected diffuse light $N_{non-conf}$ versus the core size of fiber when the system is in a non-confocal condition. It is obvious that the noise due to the diffuse light decreases as the core radius of fiber increases. When the illumination light emerges from the end face of the fiber, the propagation in free space expands widely the beam radius by diffraction phenomenon. For a Gaussian beam with beam radius of a , the beam at the propagation distance f_1 extends approximately as:

$$b_{eff} = \frac{1}{2\pi} \frac{f_1 \lambda}{a}. \quad (2-21)$$

On the other hand, the fiber has a numerical aperture, $(NA)_{fiber}$. The numerical aperture of the fiber is defined as the sine of the maximum angle of an exiting beam with respect to the fiber axis. Considering the average effect, we assume that the beam will be diverged with a coefficient of 0.5 of the numerical aperture and the two optical phenomena overlap with each other. Then, the effective beam radius on the lens plane in the lens system may be described as

$$b_{eff} = \frac{1}{2\pi} \frac{f_1 \lambda}{a} + \frac{(NA)_{fiber} f_1}{2}. \quad (2-22)$$

In this work, the lens L_2 has an identical focal length with the lens L_1 , and then the numerical aperture due to the lens system is deduced as following:

$$(NA)_{lens} = \frac{b_{eff}}{f_2} = \frac{1}{2\pi} \frac{\lambda}{a} + \frac{(NA)_{fiber}}{2}. \quad (2-23)$$

In general, the numerical aperture of the lens system can be given by two definitions:

$(NA)_{lens} = b/f$ and $(NA)_{lens} = b_{eff}/f$. The former gives the maximum value in the system,

and the latter shows the effective value for a given illumination condition. So, we call the description of Eq. (2-23) the effective numerical aperture $(NA)_{lens}$. The solid curve in Fig. 2-8 displays the resulting variation for the effective numerical aperture of lens system. The behavior of the solid curve is similar to the numerically calculated results in the Monte Carlo simulation. It is found that this dependence stems from the effective numerical aperture. Usually the optical system of a large numerical aperture can collect the diffuse light with wide exit-angle. Moreover, the diffuse light is emitted from the surface as an isotropic scattering. Therefore, the decrease of the detected light with the core radius is caused by decrease of the numerical aperture. It is suggested that these calculated results are appropriate.

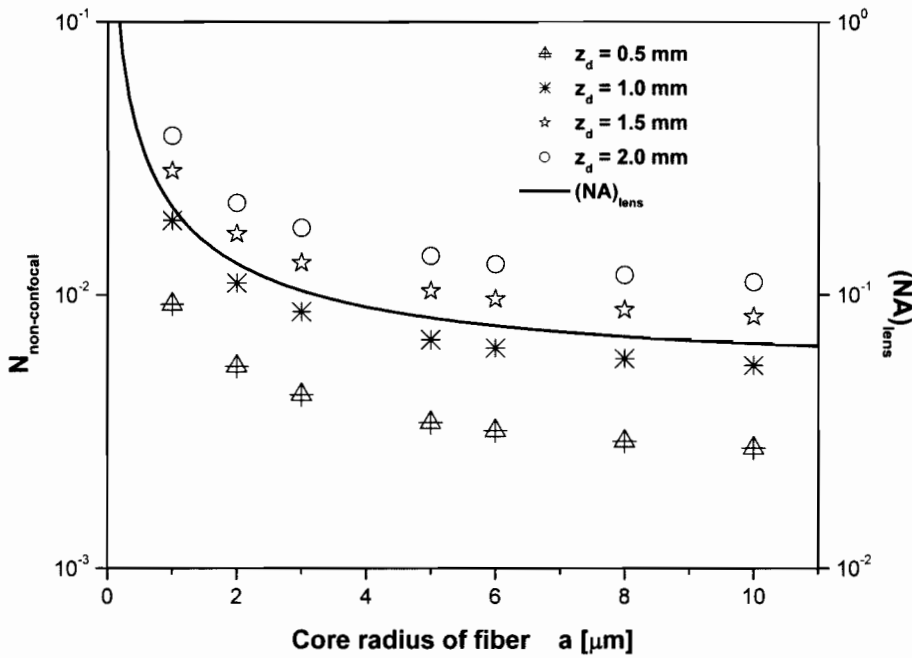


Fig. 2-8 The simulated noise in the non-confocal process by use of Monte Carlo technique. The focal depth in the scattering medium is varied from 0.5 mm to 2.0 mm. The solid curve indicates the tendency of effective numerical aperture in the lens system.

The curves in Fig. 2-8 denote that the noise due to collected diffuse light increases with increasing of the focal depth z_d in the scattering medium. By increasing the focal depth, the radius r_l of the illumination area on the surface plane becomes larger, so, the more multiple scattered light will be returned to the detector.

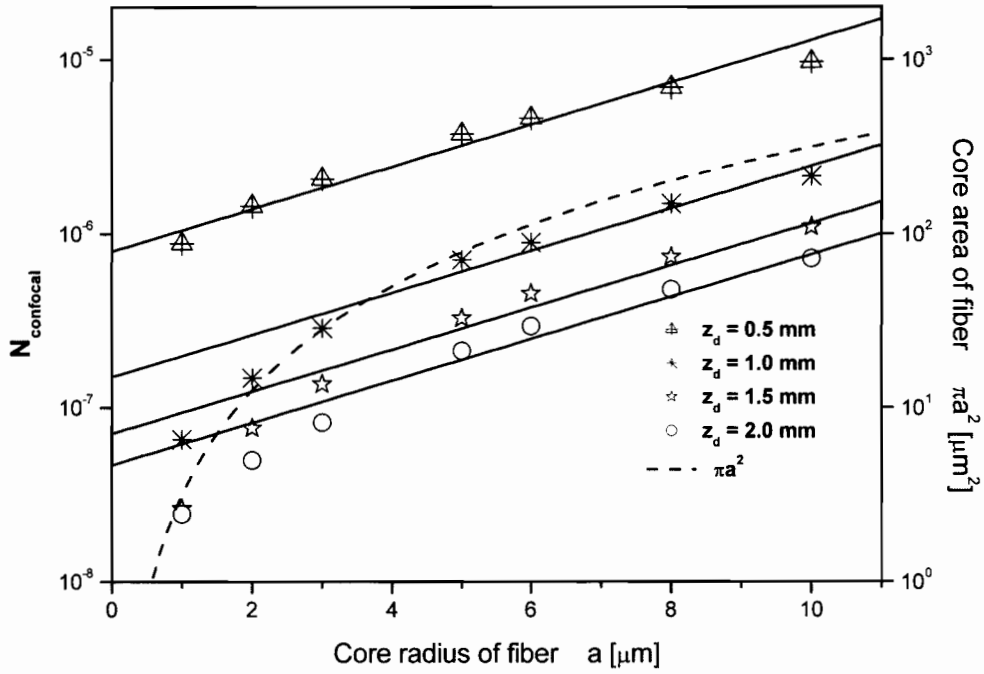


Fig. 2-9 The simulated noise in the confocal process by use of Monte Carlo technique. The focal depth in the scattering medium is varied from 0.5 mm to 2.0 mm. The solid lines are the linear fittings of the simulated results for $a > 3 \mu\text{m}$. The dashed line indicates the dependence on the core area of fiber.

For the confocal system, the diffuse light to be detected, N_{conf} , is marked in Fig. 2-9. It is denoted that the detected diffuse light (noise component) increases significantly as the core radius increases. The variation eventually takes linearly at the region of a large core radius. The linear fittings have been undergone as shown as the solid lines in Fig. 2-9. We observe

that the fitting lines at the different focal depths are parallel with each other. It implies that the noise rate of the different focal depth is fixed even for the different core radius. When the focal depth goes deep into the medium, the collected noise decreases. For the same step of 0.5 mm in the depth, the amount of noise decreases with increasing of the focal depth. The detected noise decreases as a beam incident on the medium penetrates deeper, because the scattering light diffuses widely over the detectable region in severe multiple scattering.

The detected diffuse light is collected by the entrance tip of detection-fiber (detected plane). The core area of the fiber is given by πa^2 . The dotted curve in Fig. 2-9 illuminates the core area as a function of the core size of a . It is seen that, in the range of $a > 3 \mu\text{m}$, the slopes of the lines are almost in parallel even if z_d is changed. This fact shows that the diffuse light reaches at the entrance tip of fiber with spatially uniform intensity distribution. Therefore, the decrease of the core size shows the decrease of the detected diffuse light. In the range of $a < 3 \mu\text{m}$, moreover, the slope is more rapid. This phenomenon may be caused by the effect due to the numerical aperture in the lens system.

Finally, the noise rejection rate due to the confocal system is obtained as shown in Fig. 2-10. We have defined that the rate is a ratio of $N_{\text{conf}} / N_{\text{non-conf}}$. So, the diffuse light is extremely rejected by decreasing of the core radius. The effect appears as same property even for changing the focal depth in the scattering medium. If the focal length in the scattering medium is fixed, for example, $z_d = 2 \text{ mm}$, the confocal noise drops below 10^{-7} . Furthermore the noise almost approaches to 10^{-6} than that of non-confocal process, if we can use a small-sized fiber of $a = 1 \mu\text{m}$. However, the noise rejection only achieves a degree of 10^{-5} for the case of $a = 5 \mu\text{m}$. This illuminates that the small-sized fiber is suitable for cutting off the noise and improving the signal-to-noise ratio for the confocal

system.

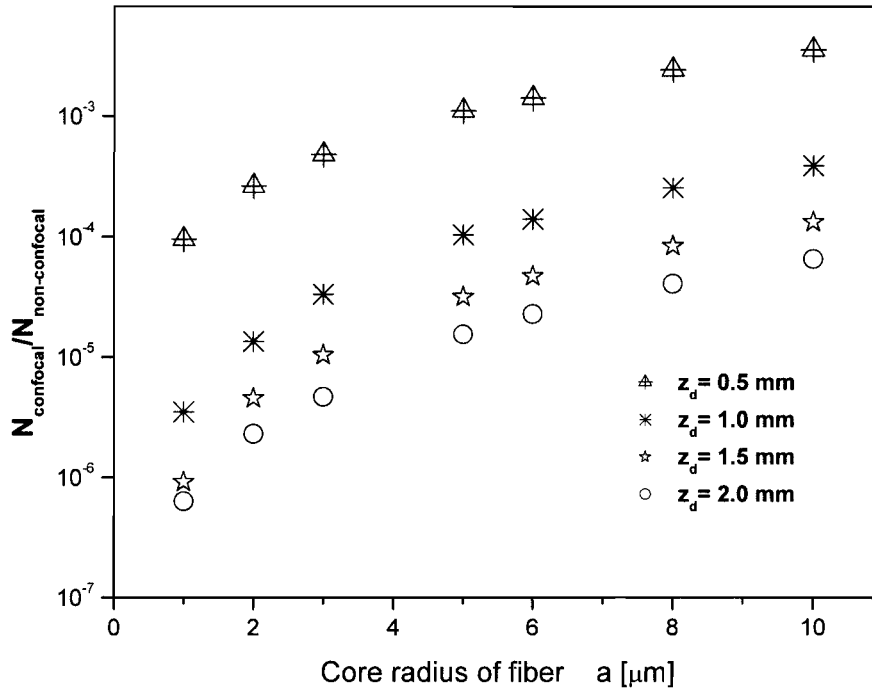


Fig. 2-10 The noise-ratio of diffuse light collected by confocal system and conventional system. The focal depth in the scattering medium is varied from 0.5 mm to 2.0 mm.

The fiber-based confocal system has superior advantage that the diffuse light is dramatically rejected and as a result the high resolution is effectively achieved even for tomographic imaging. However, the performance of the optical system is not enough to image the reflectance distribution in strong scattering medium such as human tissues. Usually the scattering phenomenon reduces greatly the signal of ballistic light, and the diffuse light is remarkably intense which is of the order of 10^8 in comparison with the signal light. Therefore the confocal system is used only for tomographic imaging in the weak scattering medium.

2.5 Conclusion

We have presented the performance of a fiber-based confocal system by means of analytically deducing the effective PSF. It is found that FWHM of the effective PSF depends on the core size of the fiber. The resolutions improve as the decreasing of core size of the fiber. A preliminary experiment using a ground glass plate as scattering medium is performed, and the experimental result agrees with the calculation very well. It denotes that our calculated data is correct and implies that we can design the required system in advance.

The main benefits of confocal system include the superior resolutions together with rejection of the diffuse light. In this chapter, we focus on investigating rejection performance of a fiber-based reflection type confocal system. To keep the high resolutions in the scattering medium and yield a high signal-to-noise ratio, we have studied the properties of the diffuse light with Monte Carlo simulation method. Since the diffuse light acts as noises for this system due to decreasing of the resolutions and the signals, the ability rejecting the diffuse light has been studied and analyzed. It is found that the numerical aperture of the lens system influences the amount of the collected diffuse light. Furthermore, the core size of the fiber affects the rejection-ability significantly. A miniature size of fiber is feasibility to introduce a high scattered light rejection. For the case of $a = 1 \text{ } \mu\text{m}$, the rejection between the confocal and non-confocal systems can approach to 10^{-6} .

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Chapter 3

Depth-resolved optical coherence tomography system

3.1 Introduction

3.1.1 Overview of OCT and its applications

Optical coherence tomography (OCT)¹⁻³⁾ is a fundamentally new type of optical imaging modality. It is a noncontact and noninvasive imaging technology using the near infrared light source, and is performed *in situ* and nondestructively without the need to excise a specimen as required for conventional biopsy. On the basis of a low coherence interferometry, OCT performs the high resolution in tomographic imaging, achieves sufficient sensitivity to probe weakly backscattering structures, and allows the localization of reflecting sites beneath the surface of biological tissues. In addition, OCT can be manufactured to be compact and low cost using the fiber-optic components. These unique features make OCT attractive for a broad field of clinical medicine application.

OCT was initially demonstrated for applying in ophthalmic imaging,¹⁾ that is partly because the available sources of light could only be used in nearly transparent tissue. Following it, OCT with a high resolution enhances early diagnosis and objective measurement for tracking progression of ocular diseases, as well as monitoring the efficacy of therapy.⁴⁾

With an advance in technology, OCT imaging can now be performed in nontransparent tissue, and opens up a wide variety of biomedical applications. For example, OCT provides extensive applications related to endoscopy. In contrast to conventional biopsy that presents hazards to patients, the endoscopic OCT is a safer and instantaneous *in situ* decision tool

during the screening of early detection of cancers.⁵⁾

The feasibility of compact and high speed OCT imaging system suggests a broad field of future clinical applications. In the next section, we will introduce a new application of fiber-based OCT system.

3.1.2 Motivation

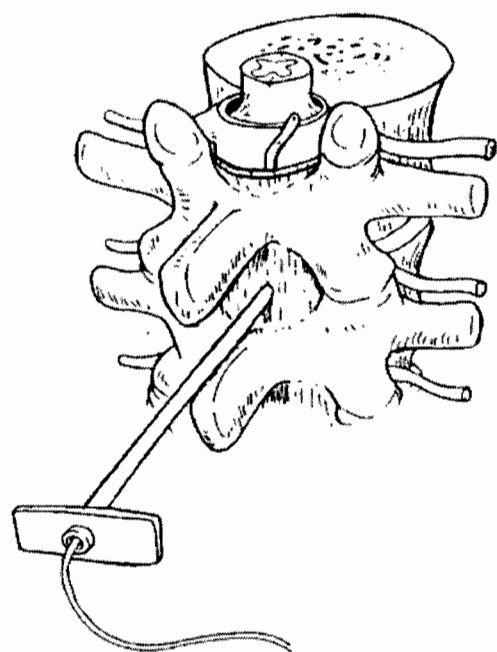


Fig. 3-1 The operation of epidural anesthesia. A puncture needle should be advanced progressively into the epidural space.

Epidural anesthesia is one way of taking away the pain of labor in birth. Like most medical treatments, it has risks. In the operation of epidural anesthesia as shown as Fig. 3-1, a puncture needle should be advanced progressively into the epidural space. Then medication is injected into the epidural space. The space is very shallow (about 2~3 mm in cervical, 4~5 mm in thoracic, and 5~6 mm in lumbar), so it is too little room for positioning

adequately the tip of the needle. The successful injection is related to accurately judge the position of epidural space. Note that the excessive advancement of the needle beyond the space and an inaccurate shot of medicine will introduce medical accident. At present, the insertion relies on anesthetist's skill.

We plan to solve this problem by OCT technique, which monitors the axial information in front of the needle. In this chapter, we show a needle-fiber OCT system.⁶⁾ An optical fiber attached to a focusing lens is put into a puncture needle and a fiber-based OCT system is built. The reflected signal is captured by confocal OCT system that can improve the signal-to-noise ratio. By choosing the appropriate focal lens, the distance of several millimeters from the tip of the needle to the target can be controlled.

The present chapter is organized as following: We first demonstrate the basic principle of OCT system in section 3.2. To focus on the application in epidural anesthesia, in section 3.3 a needle-fiber depth-resolved OCT system is established. The experiment of a mirror in *tofu* as strong scattering medium is performed in section 3.4. Based on the experimental data in *tofu*, we construct a simulation model that represents a confocal system probing scattering media, and discuss the simulated results in section 3.5. A preliminary experiment in an animal is presented in section 3.6. Finally, Conclusions are given in section 3.7.

3.2 Basic principle of depth-resolved OCT system

OCT is based on the classic optical measurement technique of low coherence interferometry or white light interferometry. Optical coherence tomography performs high resolution imaging of the internal microstructure in highly scattering media. This is done by measuring the time delay and magnitude of optical echoes reflected at different positions. However, the time delay of OCT imaging is not possible to be directly detected by an

electronic technique. The optical echoes of backscattered light can be measured by scanning a reference mirror in the interferometer. The scanning makes the reference light Doppler-shift, so that the backscattered optical signal is modulated by the Doppler frequency because the interferometer performs a heterodyne detection.

In this section, the principle of low coherence interferometry and heterodyne detection of signals with Doppler frequency are described.

3.2.1 Low coherence interferometry

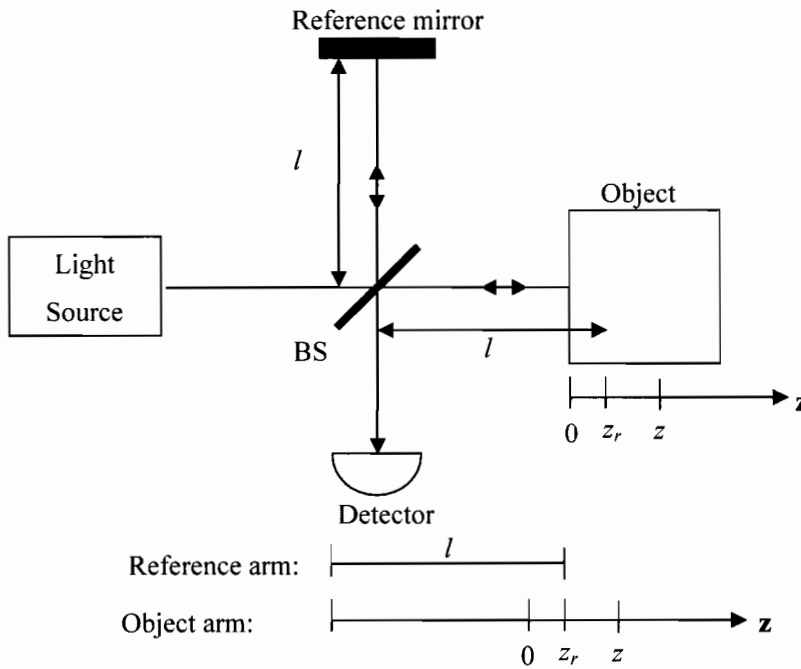


Fig. 3-2 Schematic diagram of a low coherence interferometer.

A schematic diagram of low coherence interferometry is shown in Fig. 3-2. A low coherence light is coupled into a Michelson interferometer, and divided by a beam splitter.

One beam is directed to a reference mirror and the other beam propagates through the object. The lights backreflected from reference mirror and backscattered from the object meet at the beam splitter again and generate an interference signal, which is detected by a photodetector.

The electric field with a broad spectrum can be described as

$$E(t) = a(t) \exp[ik \cdot r - \omega_c t + \phi], \quad (3-1)$$

where $a(t)$ is the amplitude of wave train at time t , k is the wave number, ω_c is the central angular frequency of the light, and ϕ is the initial phase. We define the light is emitted from the beam splitter at time $t = 0$ and the path-length between the beam splitter and the reference mirror is l . Then the reference light is

$$E_r(t) = a(t - \tau_0) \exp[i(2kl - \omega_c t + \phi)], \quad (3-2)$$

where $\tau_0 = \frac{2l}{c}$ is the time delay due to the round-trip path-length $2l$, and c is the light speed. Let us define that the z-axis is taken along the object arm as shown in Fig. 3-2, where the surface of object locates at $z = 0$. The equal position of reference mirror lies at z_r . Then, the object field backscattered at the position z is

$$E_o(t) = a(t - \tau_0 - \tau) r(z) \exp\{i[2k(l + z - z_r) - \omega_c t + \phi]\}, \quad (3-3)$$

where $\tau = \frac{2(z - z_r)}{c}$ is the time delay due to the path-length difference of the two beams, and $r(z)$ is the amplitude reflectance at the position z . Note that the object field is consisted of backscattered lights from different positions along the z-axis. Then the object field can be integrated as

$$E_o(t) = \int a(t - \tau_0 - \tau) r(z) \exp\{i[2k(l + z - z_r) - \omega_c t + \phi]\} dz. \quad (3-4)$$

The light from a source consists of many wave trains, and these wave trains are statistically independent to each other. So, the intensity to be measured should be time-averaged under a stationary condition. The time-averaged intensity of two beams on the photo-detector is

$$\begin{aligned}
I(t) &= \left\langle |E_r(t) + E_o(t)|^2 \right\rangle \\
&= I_{DC} + 2 \operatorname{Re} \left[\int r(z) \langle a(t - \tau_0) a(t - \tau_0 - \tau) \exp[i2k(z - z_r)] \rangle dz \right],
\end{aligned} \tag{3-5}$$

where $I_{DC} = I_r + I_o = \langle |E_r(t)|^2 \rangle + \langle |E_o(t)|^2 \rangle$ is the mean (dc) intensity independent of τ , $\langle \dots \rangle$ is the time average within the detector's response time. The second term represents the interference signal.

According to the complex coherence function $\Gamma(\tau)$, it follows as

$$\begin{aligned}
\Gamma(\tau) &= \langle a(t - \tau_0) a(t - \tau_0 - \tau) \exp[i2k(z - z_r)] \rangle \\
&= \langle a(0) a(\tau) \rangle \exp(i\omega_c \tau) \\
&= \Gamma_{env}(\tau) \exp(i\omega_c \tau),
\end{aligned} \tag{3-6}$$

where $\Gamma_{env}(\tau)$ is the envelope coherence function at the time delay τ between the two beams. If the amplitude reflectance $r(z)$ is a real function, by substituting Eq. (3-6) into Eq. (3-5), we get

$$\begin{aligned}
I(t) &= I_{DC} + 2 \operatorname{Re} \left[\int r(z) \Gamma(\tau) dz \right] \\
&= I_{DC} + 2 \operatorname{Re} \left[\int r(z) \Gamma(z - z_r) dz \right] \\
&= I_{DC} + 2 \operatorname{Re} [r(z_r) \otimes \Gamma(z_r)] \\
&= I_{DC} + 2 [r(z_r) \otimes \Gamma_{env}(z_r)] \cos(\omega_c \tau).
\end{aligned} \tag{3-7}$$

The interference signal is expressed as a convolution integral. The depth information, such as location and reflectance in the object, can be resolved using a low coherence interferometry. As seen from Eq. (3-7), the sharpness of $\Gamma_{env}(\tau)$ determines the axial resolution. Because $\Gamma(\tau)$ is related to Fourier-transformation of the power spectral density, the source with a broad spectrum is suitable for a high resolution in OCT system.

If the object is an ideal mirror at the position $z = z_{obj}$, then $r(z)$ becomes

$$r(z) = \delta(z - z_{obj}). \tag{3-8}$$

The detected intensity is given as

$$I(z_r) = I_{DC} + 2\Gamma_{env}(z_{obj} - z_r)\cos\left[2k(z_{obj} - z_r)\right]. \tag{3-9}$$

We can see that the interference intensity varies sinusoidally by sliding slowly the reference mirror of position z_r . Let us define that the coherence length of light source is l_c . The envelope coherence function takes the maximum value at $z_{obj} - z_r = 0$. Therefore, the interference signal is obtained within the range of $|z_{obj} - z_r| \leq \frac{l_c}{2}$ with the maximum value at the position $z_r = z_{obj}$.

3.2.2 Rapid scanning heterodyne detection

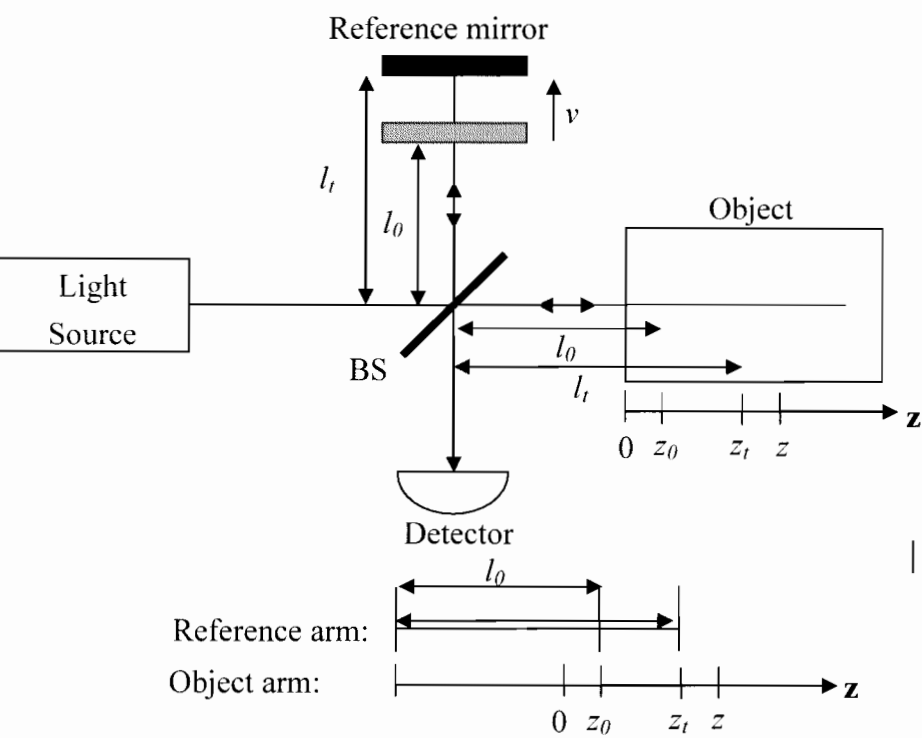


Fig. 3-3 Schematic diagram of rapid scanning heterodyne detection system.

Detecting the interference signal described in section 3.2.1 is equivalent to using a heterodyne detection in the electric engineering. The heterodyne technique is superior to detect a weak signal at a high signal-to-noise ratio. To extract only the envelope function from the interference signal, we make the reference mirror move at a constant velocity v . The reference light is Doppler-shifted, and the interference signal is modulated at the frequency.^{7,8)} Adopting the demodulation technique will extract the envelope function in Eq. (3-9). This technique facilitates the removal of both the dc background and a low-frequency noise such as $1/f$ noise.

Figure 3-3 shows a diagram of rapid scanning heterodyne detection system. The distance between a beam splitter (BS) and a reference mirror is l_0 at time $t = 0$. When the reference mirror is translated at a constant velocity v , the distance at time t is

$$l_t = l_0 + vt. \quad (3-10)$$

The each corresponding position on the z -axis is z_0 at time $t = 0$ and z_t at time t according to

$$z_t = z_0 + vt. \quad (3-11)$$

The reference and object fields on the detector at time t are respectively given as

$$E_r(t) = a(t - \tau_0) \exp[i(2kl_t - \omega_c t + \phi)], \quad (3-12)$$

$$E_o(t) = \int a(t - \tau_0 - \tau) r(z) \exp\{i[2k(l_t + z - z_t) - \omega_c t + \phi]\} dz, \quad (3-13)$$

where $\tau_0 = \frac{2l_t}{c}$ is the time delay due to the round-trip path-length $2l_t$ at time t , and

$\tau = \frac{2(z - z_t)}{c}$ is the time delay due to the path-length difference of the two beams. The

interference intensity component is given by

$$\begin{aligned} I_{\text{int}}(\tau) &= 2 \operatorname{Re} \left[\int r(z) \langle a(t - \tau_0) a(t - \tau_0 - \tau) \exp(i\omega_c \tau) \rangle dz \right] \\ &= 2 \operatorname{Re} \left\{ \int r(z) \langle a(t - \tau_0) a(t - \tau_0 - \tau) \exp[i2\pi \Delta f t] \exp[-ik(z - z_0)] \rangle dz \right\}, \end{aligned} \quad (3-14)$$

where $\Delta f = \frac{2v}{\lambda_c} = \frac{v\omega_c}{\pi c}$ is the Doppler-shift frequency, and λ_c is the central wave length of light with the angular frequency ω_c .

Next we will discuss the average bracket term during the detector's response time T . The coherence time of the light source τ_c corresponds to 0.16 ps when the coherence length is 50 μm . The heterodyne term $\exp[i2\pi\Delta ft]$ has $T_D = \frac{1}{\Delta f} = 0.125$ ms when the velocity of moving mirror is 4 mm/s. Noting that $\tau_c \ll T \ll T_D$, the heterodyne term only changes slowly during the response time of the detector. Since the term can be detected, therefore we get

$$\begin{aligned} I_{\text{int}}(\tau) &= 2 \operatorname{Re} \left[\int r(z) \langle a(t - \tau_0) a(t - \tau_0 - \tau) \rangle \exp[i(2\pi\Delta ft - \phi)] dz \right] \\ &= 2 [r(z_t) \otimes \Gamma_{\text{env}}(z_t)] \cos(2\pi\Delta ft + \omega_c\tau), \end{aligned} \quad (3-15)$$

where $\phi = \frac{4\pi}{\lambda_c}(z - z_0)$. Let us define the normalized coherence function as

$$\begin{aligned} \gamma(\tau) &= \frac{\Gamma(\tau)}{\sqrt{I_r I_o}} \\ &= \gamma_{\text{env}}(\tau) \exp(i\omega_c\tau). \end{aligned} \quad (3-16)$$

Then the detected intensity at time t is rewritten as

$$I(t) = I_r + I_o + 2\sqrt{I_r I_o} [r(z_t) \otimes \gamma_{\text{env}}(z_t)] \cos(2\pi\Delta ft + \omega_c\tau). \quad (3-17)$$

Since the reference mirror is scanned at a constant velocity, the amplitude reflectance $r(z_t)$ in the object along the depth is given by time-dependence, and the interference signal is modulated by a Doppler-shift frequency. Therefore, a bandpass filter centered at the Doppler-shift frequency is used to separate the interferometric signal from the DC term and the noise in the OCT system, and the envelope $[r(z_t) \otimes \gamma_{\text{env}}(z_t)]$ is subsequently extracted through the demodulation of detected signal.

The modulation technique with Doppler effect provides a simple method to get the interference signal components of OCT system. Furthermore, it is used to obtain internal

information of the tissues at high speed and wide dynamic range. For one scanning procedure of the reference mirror, we can measure the reflectance over several millimeters along a depth direction in the object.

3.3 Depth-ranging system

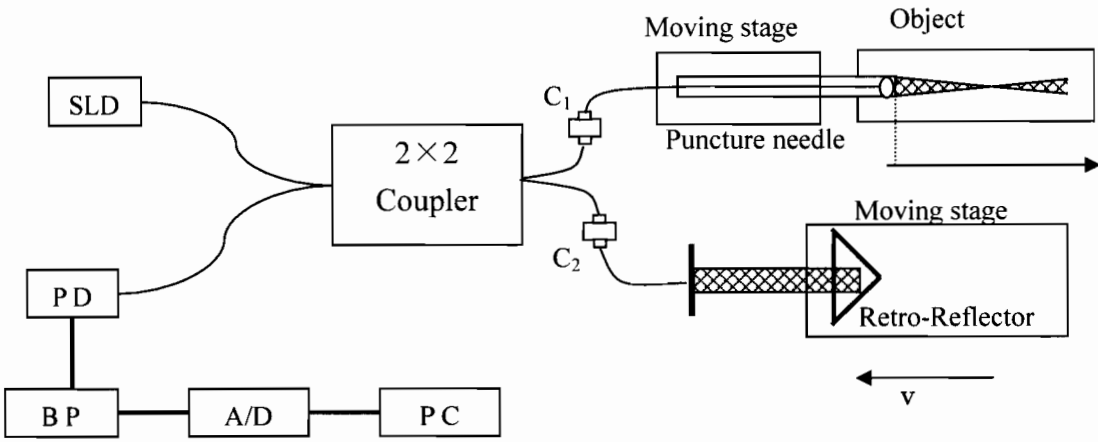


Fig. 3-4 Schematic diagram of depth-ranging system based on the optical coherence interferometer: SLD: superluminescent diode source; PD: photodiode; BP: bandpass filter; A/D: analog-to-digital converter; C_1, C_2 : fiber connector. The fiber-needle and retro-reflector are mounted at the moving stages, respectively.

The depth-ranging OCT system in the operation of epidural anesthesia consists of a fiber-optic Michelson interferometer as shown in Fig. 3-4. We use a superluminescent diode source (SUPERLUM; Model SLD-481-HP2) at central wavelength of $\lambda_c = 978$ nm with broad-bandwidth (22 nm FWHM) as a low coherence light source. Light emitted from the SLD is coupled into a single-mode fiber, and is divided into an object arm and a reference arm by a 2x2 fiber coupler. Figure 3-5 shows the fiber probe covered by a metal tube, which is placed into a puncture needle. The probe has a GRIN lens attached closely to the fiber's

tip to focus the output light. The end face of the fiber and the two faces of the lens are polished without anti-reflection coating. The focal plane is 3.0 mm from the lens face in air. Backscattered light from the object can be coupled again into the fiber by confocal system. The collected object light is combined with a reflected reference light and generates an interference signal detected by a photodiode. The reference mirror is moved with a constant velocity of $v = 4 \text{ mm/s}$ to produce interference modulation with a Doppler-shift frequency of $\Delta f = 2v / \lambda_c \approx 8 \text{ kHz}$. The interference signal passes through a band pass filter with a bandwidth from 6 kHz to 10 kHz. The output is digitized with a 12-bit analog-to-digital converter (Interface; Model PCI-3153), and the envelope is calculated as an OCT signal. The sampling period is enough shorter than the Doppler period of $T_D = 1 / \Delta f = 0.125 \text{ ms}$ to make sure to get sinusoidal functions of the interference signals.

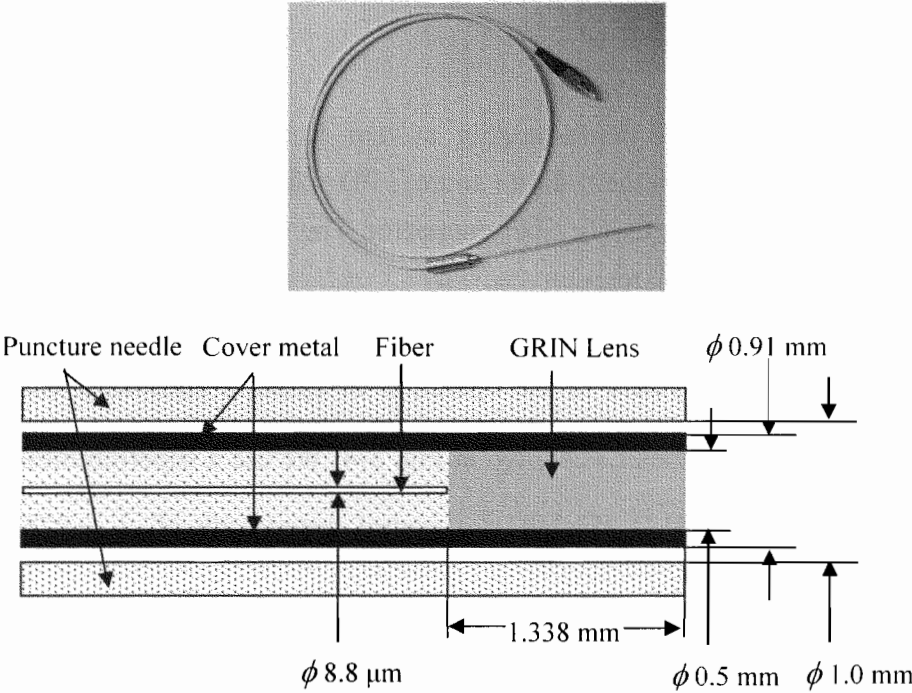


Fig. 3-5 Experimental fiber-needle system: (a) The photograph of a fiber-needle. (b) Internal structure of the confocal fiber system mounted in the needle.

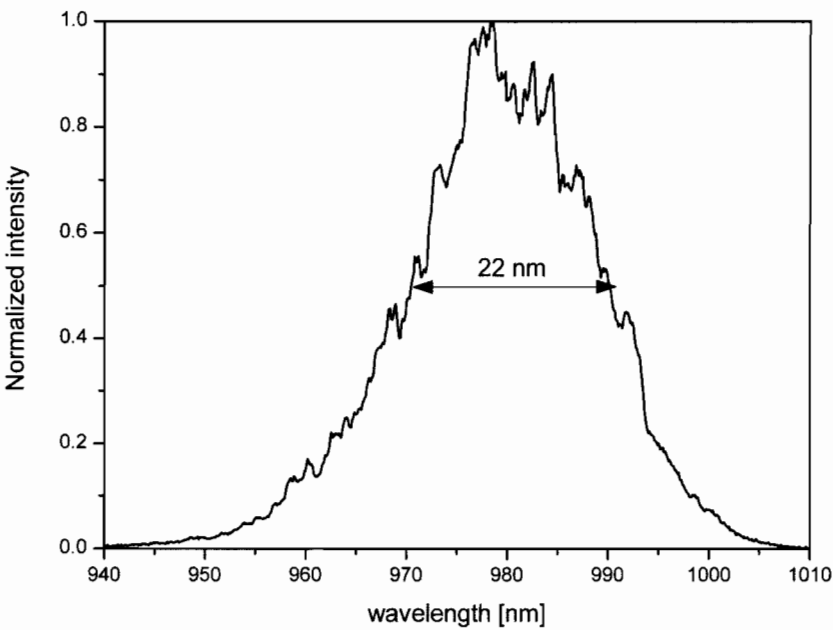


Fig. 3-6 The spectrum of SLD at a temperature of 25 °C and a driver current of 95 mA.

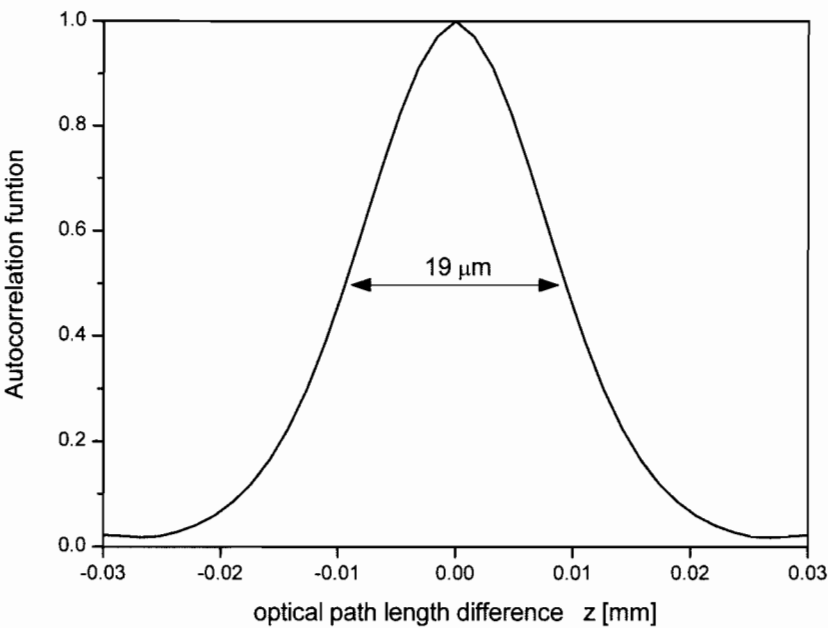


Fig. 3-7 Linear version of FFT of the measured spectrum.

The light source used in our study is a superluminescent diode (SLD) operating at 978 nm. The wavelength provides good penetration in the high scattering tissue. High power stability and broad spectrum make it the ideal choice for our application. The maximum output power is up to 20 mW. Figure 3-6 shows the power spectrum of the SLD at a temperature of 25 °C and a driver current of 95 mA. It can be seen that the central wavelength is 978 nm and the spectral linewidth is 22 nm. If a Gaussian profile is assumed, the axial resolution is inversely proportional to the spectral linewidth, $\Delta\lambda$, and proportional to the square of the central wavelength, λ_c , of the light source:

$$\Delta z = \frac{2 \ln 2}{\pi} \frac{\lambda_c^2}{\Delta\lambda}. \quad (3-18)$$

This gives an axial resolution of 21 μm . According to the Winer-Khintchin theorem, the coherence function is the Fourier transform of power spectrum. Figure 3-7 illustrates the Fourier transform of the spectrum from the SLD. The FWHM in Fig. 3-7 is about 19 μm which shows excellent agreement with the calculated 21 μm .

3.4 Experiment in an object

3.4.1 Experiment without object

First we evaluate the needle-fiber system by measuring the signal without scattering medium. Figure 3-8 shows the detected signal by scanning the reference mirror. The signals R-1 and R-2 are generated by the reflected light from the front and back faces of the lens in the object arm. Since the signal R-1 is the Fresnel reflection due to the boundary between the fiber and the lens, it keeps constant even if the needle is placed into different medium. Therefore, the detected light power is normalized by the signal R-1 in the experiment. The signal R-3 is generated by the multi-reflected light between the front and back faces of the

lens.

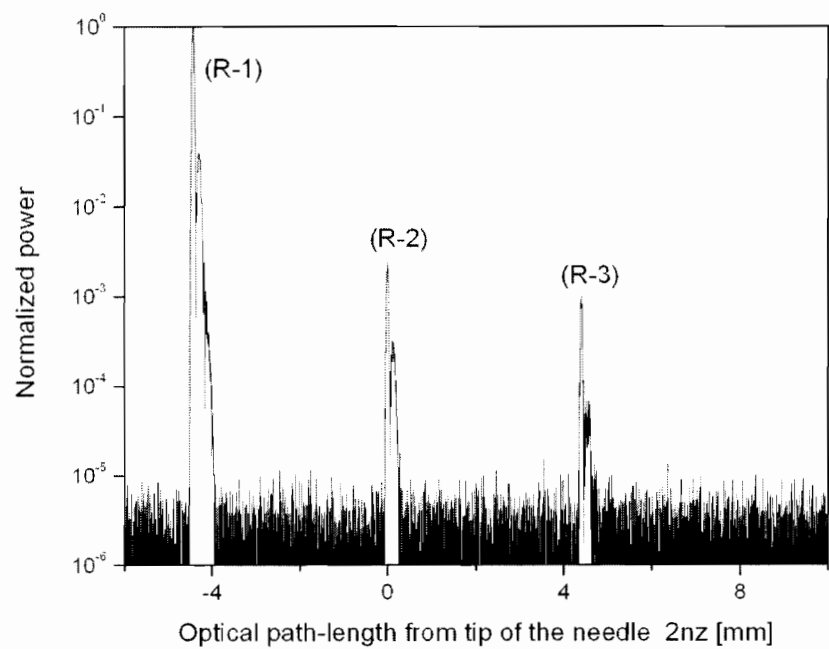


Fig. 3-8 Detected signal without scattering medium. The signals (R-1) and (R-2) are caused by front and back faces of lens and the signal (R-3) is done by multi-reflected light.

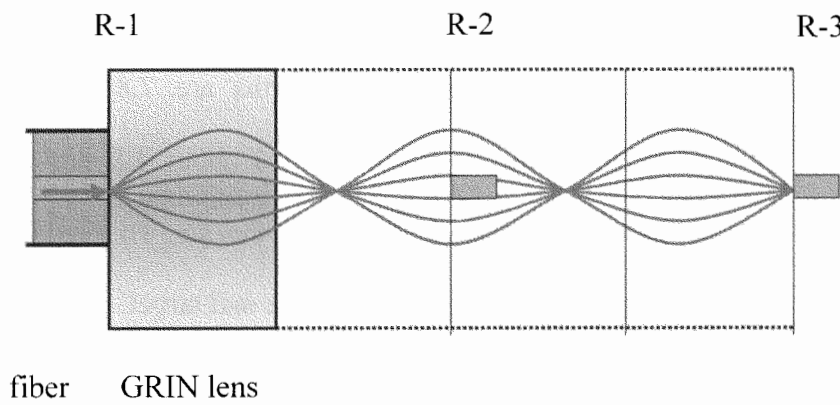


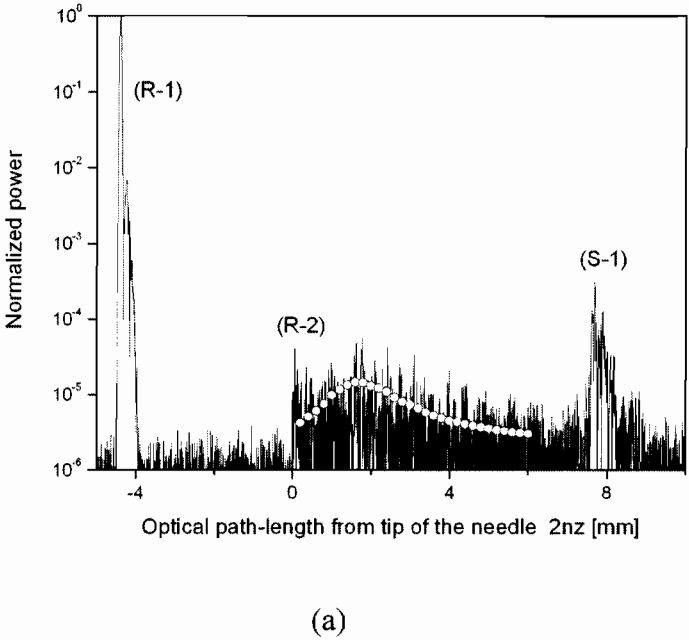
Fig. 3-9 Propagating light in GRIN lens.

The length of GRIN lens is equal to 3/8 pitch, then the broadness of propagating light at

R-1, R-2 and R-3 planes can be detail illustrated in Fig. 3-9. If the front and back faces of the lens produce specular reflection and refraction as normal phenomenon, the strength of R-1, R-2 and R-3 in Fig. 3-8 will decrease continuously at a terrible rate. As seen from Fig. 3-9, however, the passing area of propagating light at R-2 plane is fully extended over a cross section of GRIN lens, but that at R-3 plane just focuses at a point. Only a small part of propagating light will be accepted at the R-2 plane whereas most of propagating light will be accepted at the R-3 plane. Therefore, the accepted signal at R-3 plane has almost the same strength as that at R-2 plane.

3.4.2 Experiment in a *tofu*

In the next experiment, a small mirror is embedded into a plastic cell ($35 \times 22 \times 15$ mm) filled with homogeneous scattering medium: *tofu*, which is gel-like protein of beans. A hole is opened at a sidewall of the cell to make the puncture needle insert into the scattering object. When the puncture needle is moved progressively towards the mirror embedded in the *tofu*, Fig. 3-10 is obtained. Here n is the refractive index of the medium. The measuring time is performed within 2 second. The signals S-1 and S-2 in Figs. 3-10 (a) and 3-10 (b) are reflected by the object mirror whose optical path-lengths are 7.8 mm and 3.0 mm, respectively. It shows that the puncture needle is moved about 4.8 mm in the optical path-length. FWHM of the axial resolution is 21 μm in the air. This axial resolution is enough to measure the distance between the tip of the needle and target.



(a)

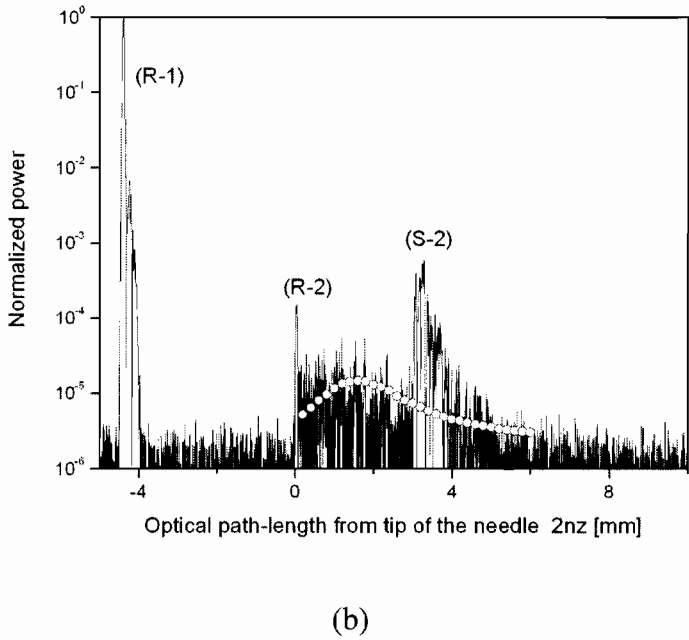


Fig. 3-10 Detected signal when the needle moves forward toward a high reflective mirror in a strong scattering medium. Signals (S-1) and (S-2) are caused by reflected light from the mirror, whose optical path-lengths from the puncture needle are 7.8 mm in (a) and 3.0 mm in (b), respectively. The open circle curve denotes the averaged background of the experimental data which is caused by diffused light.

The background level at the position of $z < 0$ is a constant due to shot noise. The background at $z \geq 0$ is roughly denoted by an open circle curve on an average, which has a peak at the position of $2nz = 1.6$ mm. When the light transmits through a medium such as the *tofu*, substantial scattering has occurred. A few part of the collected diffuse light propagates through the single mode fiber, and is interfered with the reference light.^{9,10)} The fact, for example, appears in long tails of target-signals S-1 and S-2 in Fig. 3-10. In our needle-fiber OCT system, only the detected light from the target is signal, the other detected components act as background noise. The background level of the interfered diffuse component determines a detectable minimum value of a target-signal. Under assumption that the ratio of the interfered component to total diffuse light is constant, the background noise except for shot noise is analyzed numerically in the next section.

3.5 Numerical evaluation of backscattered noise

3.5.1 Monte Carlo simulation method

To investigate the performance of confocal properties and evaluate the background noise by a well-known Monte Carlo simulation method,¹¹⁻¹⁵⁾ the needle-fiber system is modeled as shown in Fig. 3-11. For simplicity, we assume that photons from a focused Gaussian beam are injected into the scattering medium from the different positions at $z = 0$ plane (a back plane of GRIN lens). Each photon has initial coordinates:¹⁶⁾

$$\begin{cases} x_0 = r_s \sqrt{-\ln(1-\xi)} \cos \alpha, \\ y_0 = r_s \sqrt{-\ln(1-\xi)} \sin \alpha, \\ z_0 = 0, \end{cases} \quad (3-19)$$

where ξ is a uniform random number between 0 and 1, α is also a uniform random number between 0 and 2π , and r_s is the beam radius where the intensity is $1/e$ value of

the peak at $z = 0$ plane. The initial direction at each position is given by the condition that the light is going to focus at a point $(0, 0, z_f)$. The components of initial directional cosines for each photon are given by a function of the starting coordinates:¹⁶⁾

$$\begin{cases} u_{x,0} = -\frac{x_0}{\sqrt{x_0^2 + y_0^2 + z_f^2}}, \\ u_{y,0} = -\frac{y_0}{\sqrt{x_0^2 + y_0^2 + z_f^2}}, \\ u_{z,0} = \frac{z_f}{\sqrt{x_0^2 + y_0^2 + z_f^2}}. \end{cases} \quad (3-20)$$

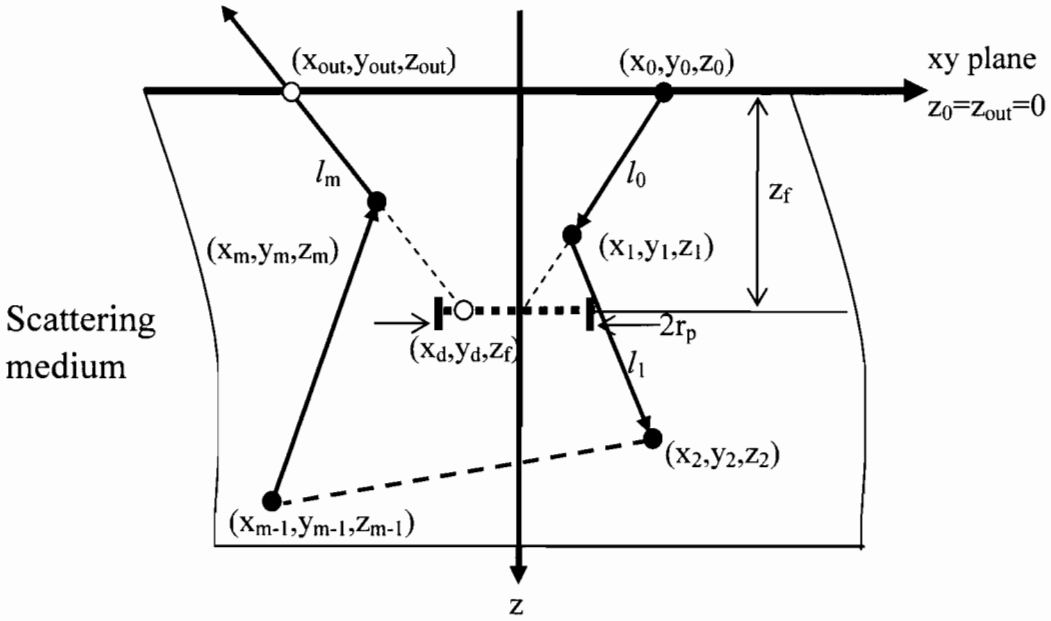


Fig. 3-11 Model of a confocal system probing a scattering medium. The thick arrow lines show an example of the trajectory of a photon traced into the scattering medium. The core end face of the fiber is imaged on the focal plane of the depth z_f over the radius r_p .

Each photon with an initial input trajectory and initial coordinate is launched into the medium by a path-length l_0 , which can be given by:

$$l_0 = -\frac{\ln \xi}{\mu_s}, \quad (3-21)$$

where μ_s is the scattering coefficient of the medium. The m -th event is set by the sets

$$\{u_{x,m}, u_{y,m}, u_{z,m}, l_m\}.$$

Once the photon has reached at an interaction site, a fraction of the photon weight (the initial weight is $w = 1$) is absorbed and is updated by:

$$w_m = w_{m-1} \exp(-\mu_a l_{m-1}), \quad (3-22)$$

where μ_a is the absorption coefficient. The propagation direction of the scattered photon is described by scattering angle θ and azimuthal angle φ . In the simulation, the scattering angle, which is determined with the random number ξ , is governed by the Henyey-Greenstein phase function. For a given scattering event the azimuthal angle φ is selected from a uniform distribution between 0 and 2π . Once the deflection and azimuthal angles are chosen, the new direction of the photon can be expressed:¹¹⁾

$$\begin{cases} u_{x,m} = \frac{(u_{x,m-1}u_{z,m-1} \cos \varphi_{m-1} - u_{y,m-1} \sin \varphi_{m-1}) \sin \theta_{m-1}}{\sqrt{1-u_{z,m-1}^2}} + u_{x,m-1} \cos \theta_{m-1}, \\ u_{y,m} = \frac{(u_{y,m-1}u_{z,m-1} \cos \varphi_{m-1} + u_{x,m-1} \sin \varphi_{m-1}) \sin \theta_{m-1}}{\sqrt{1-u_{z,m-1}^2}} + u_{y,m-1} \cos \theta_{m-1}, \\ u_{z,m} = -\sqrt{1-u_{z,m-1}^2} \sin \theta_{m-1} \cos \varphi_{m-1} + u_{z,m-1} \cos \theta_{m-1}. \end{cases} \quad (3-23)$$

If the angle is too close to the z-axis, the following formulas should be used to obtain the new photon directions:

$$\begin{cases} u_{x,m} = \sin \theta_{m-1} \cos \varphi_{m-1}, \\ u_{y,m} = \sin \theta_{m-1} \sin \varphi_{m-1}, \\ u_{z,m} = \frac{u_{z,m-1}}{|u_{z,m-1}|} \cos \theta_{m-1}. \end{cases} \quad (3-24)$$

The path-length l_m determined by the scattering coefficient has the same probability distribution as l_0 .

In the multiple scattering process, the photons, which are satisfied with two conditions, are collected by the fiber. One is that the position $\{x_{out}, y_{out}, z_{out}\}$ of scattered photon should be located within the area of GRIN lens. The other is that the exit angle of scattered photon is within the acceptance angle of fiber. Let us assume that the end face of the fiber in the confocal system is imaged at z_f -plane under the magnitude of 1. When a photon emerges from the position $\{x_{out}, y_{out}, z_{out}\}$ and a reciprocal path of the photon intersects with a point $\{x_d, y_d, z_f\}$ on the imaging plane, the relation

$$\begin{cases} x_d = x_{out} + z_f \frac{u_{x,m}}{u_{z,m}}, \\ y_d = y_{out} + z_f \frac{u_{y,m}}{u_{z,m}} \end{cases} \quad (3-25)$$

is given. Then, the two conditions can be described as:

$$\begin{cases} \sqrt{x_{out}^2 + y_{out}^2} < r_s, \\ \sqrt{x_d^2 + y_d^2} < r_p, \end{cases} \quad (3-26)$$

where r_p is the radius of pinhole in the confocal system, which is corresponding to the core radius of fiber in the system.

If the photon escapes from the scattering medium or its weight is below the threshold (10^{-4} of the initial weight in this simulation), we will stop tracing the current photon packet. When the photon is backscattered and can be detected by the detector, the weighted strength of the photon and the optical path-length accumulated from input to output are recorded.

3.5.2 Results and discussion

The detectable ranging distance is mainly related to two factors. One is the light propagation property in scattering medium which is typically characterized by the transport mean free path-length,¹⁴⁾ $L_t = 1/[\mu_a + \mu_s(1 - g)]$. The other is the focal distance z_f in the confocal system, which determines both the strength of target-signal and the background noise due to interfered diffuse component. The background sets a limit to detect weak signals. We investigate numerically the detected background as functions of L_t and z_f when there is no target in a scattering medium. In our Monte Carlo simulations, several conditions are commonly used as followings: The optical condition of sample is set to the absorption coefficient of $\mu_a = 0.01 \text{ mm}^{-1}$, the scattering coefficient of $\mu_s \geq 5.0 \text{ mm}^{-1}$ and the anisotropy parameter of $g = 0.9$ due to the realistic application in biological tissues.^{17,18)} In the numerical calculations, the radius at $z = 0$ plane of Gaussian beam illumination is fixed at $r_s = 0.25 \text{ mm}$ because the radius of the focal lens to be used in our experiment is $r_L = 0.25 \text{ mm}$. To get signals with high quality under a low computation cost, the radius of pinhole in the confocal system is selected to $r_p = 10 \text{ }\mu\text{m}$ which is nearly twice larger than the core radius in the fiber ($4.4 \text{ }\mu\text{m}$) to be experimentally used.

In Fig. 3-12, we have plotted the intensity of the background noise versus the optical path-length for various focal distances z_f in relatively weak scattering medium. The scattering coefficient is assumed as $\mu_s = 5 \text{ mm}^{-1}$, which is corresponding to $L_t = 2 \text{ mm}$. It can be clearly seen that, if the focal length is much less than the transport mean free path-length, the background due to the diffuse light has a relatively sharp peak. We note that the peak position approximately is given by $2z_f$. This shows the confocal system works well. For example, the peak is located at 0.6 mm for $z_f = 0.3 \text{ mm}$, whereas the peak is

located at 0.8 mm for $z_f = 0.4$ mm. The background noise after peak position decreases monotonically as increasing of the path-length and finally becomes insensitive to the focal length z_f . Figure 3-13 shows the simulated results in strong scattering medium, which means that the focal length is comparable with or longer than the transport mean free path-length. The changing of focal distance from $z_f = 0.5$ mm to 1 mm diminishes the strength of detected light, but the peaks are located at the same position which is related to the transport mean free path-length, that is, the peak in strong scattering medium does not depend on z_f .

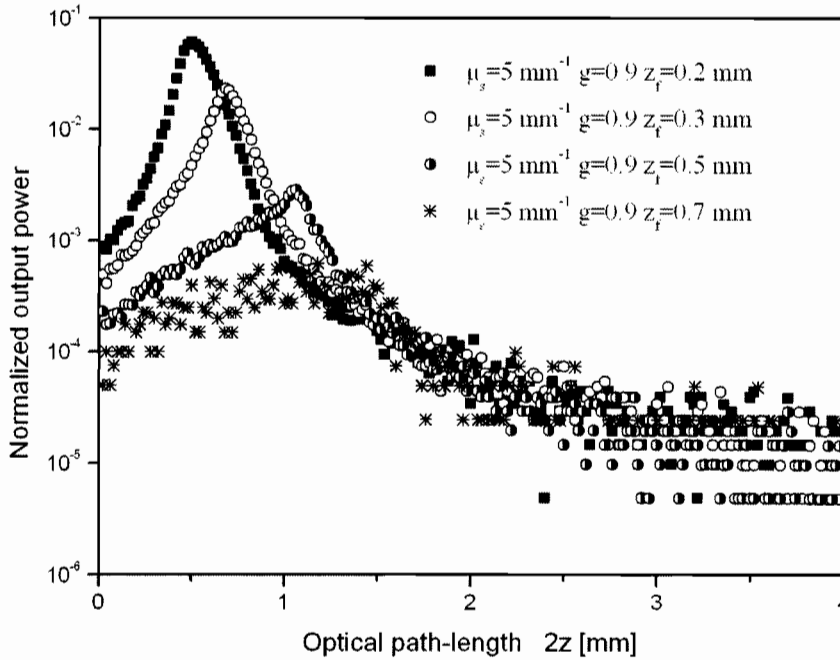
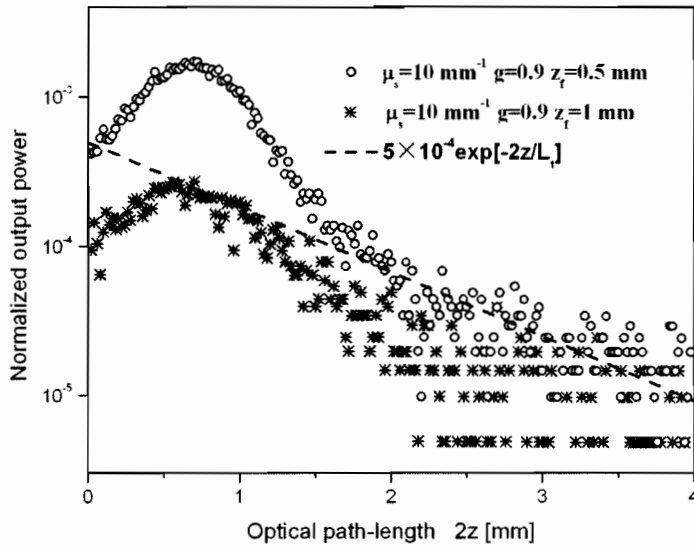
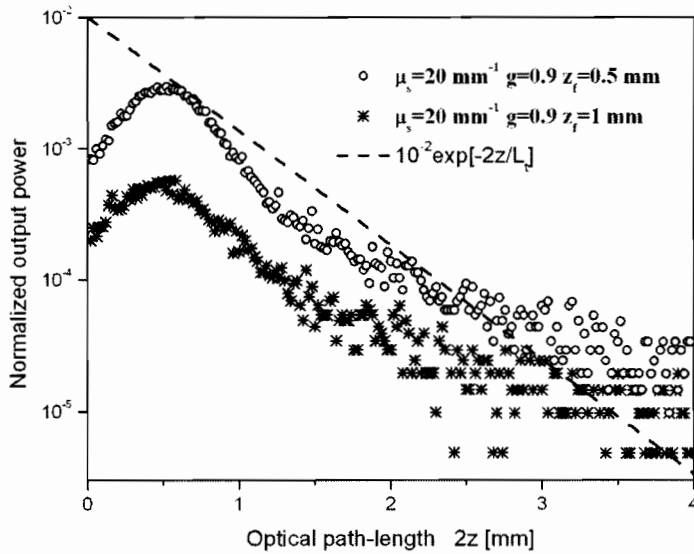


Fig. 3-12 The simulated optical path-length distribution by use of Monte Carlo technique. The radius of pinhole in the confocal system is selected as 10 μm , and the radius at $z = 0$ plane of Gaussian beam illumination is fixed at 0.25 mm. The transport mean free path-length is 2 mm.



(a)



(b)

Fig. 3-13 The simulated optical path-length distribution by use of Monte Carlo technique. The radius of pinhole in the confocal system is selected as $10 \mu\text{m}$, and the radius at $z = 0$ plane of Gaussian beam illumination is fixed at 0.25 mm . The transport mean free path-length is varied from (a) 1 mm to (b) 0.5 mm . The dashed lines indicate the tendency of signal attenuation.

Therefore, we can deduce that focusing has a substantial influence on the background noise and the maximum of detected background noise is located at $2z_f$ for $2z_f \ll L_t$ in weak scattering medium. In strong scattering medium, however, the background noise becomes insensitive directly to the focal length, and the peak of detected background noise is located around L_t for $2z_f \geq L_t$. Increasing of z_f makes the detected diffuse light degrades continuously although the confocal effect is reduced.

The experimental curve at the region of $z > 0$ in Fig. 3-10 shows that the open circle curve of background noise has a single peak. Comparing with the simulated results, this tendency is similar to that of the simulated diffused light in strong scattering medium. Note that the peak position in Fig. 3-10 is around the location of $2nz = 1.6$ mm, it is far less than twice of our focal length $z_f = 3.0$ mm, so this indicates that the *tofu* is a strong scattering medium. Assuming that the refractive index of the *tofu* is $n = 1.45$, we can predict that the transport mean free path-length of the *tofu* is about 1.1 mm.

The detectable minimum signal of OCT is determined by the background noise and the amount of signal. In the strong scattering medium, the peak of background noise is located at around the mean free path-length. The background noise at a long path-length decreases inverse-exponentially as the optical path-length increases. The amount of signal is related to the products of three terms: the attenuation decay due to scattering effect,¹⁹⁾ the intensity of illumination light on the target, and the reflectance of the target. If it is assumed that the signal in propagation decreases exponentially with the attenuation coefficient of $1/L_t$ under non-focusing illumination such as a plane wave, the decay rate of the signal is almost same as or faster than that of the background noise as shown as a dashed line in Fig. 3-13. The reflectance of the target is intrinsic to a given medium. The intensity of illumination light

along the optical axis is decided by the focal length in the non-scattering medium, and has the maximum value at the focal distance. For providing a long ranging distance in the strong scattering medium, therefore, it should be designed so that the focal distance is set to the longest distance to be needed.

As seen from Fig. 3-10, the reflected signal of S-1 has approximately the same height as the signal S-2 even for twice propagation distance in strong scattering medium. Since the focusing point is set to $z_f = 3 \text{ mm}$ in this system, the signal S-1 is detected under the optimal optical system. If we want to inject medication into a 2 mm epidural space during the operations of epidural anesthesia, the focal depth of nearly 2 mm in the medium maybe the best choice if the scattering coefficient is the same as that used in the calculation and the amount of signal is enough to be detected. Then we can detect effective signals from the membrane in the tissue over the range.

3.6 Preliminary experiment in a white mouse

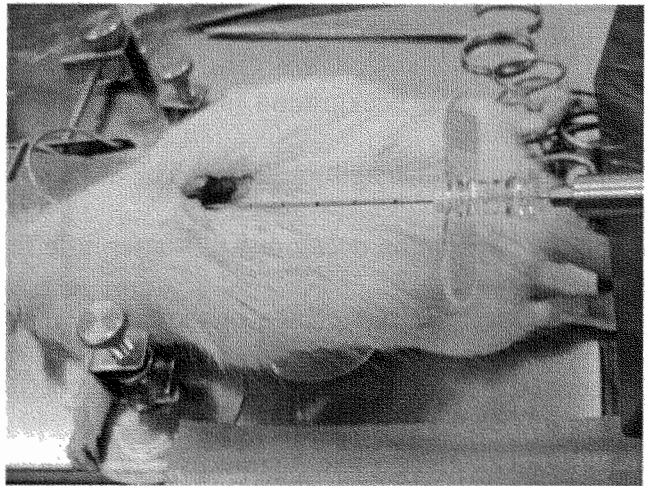


Fig. 3-14 A puncture needle is inserted into the internal belly part of white mouse.

To examine the feasibility of *in vivo* imaging in a biological tissue, experiments were done with the needle-fiber system. Figure 3-14 shows the anesthetized specimen: a white mouse. The mouse is placed on the experimental platform, the fur and skin of its belly are cut and the internal tissue is visible. The puncture needle is inserted into the tissue.

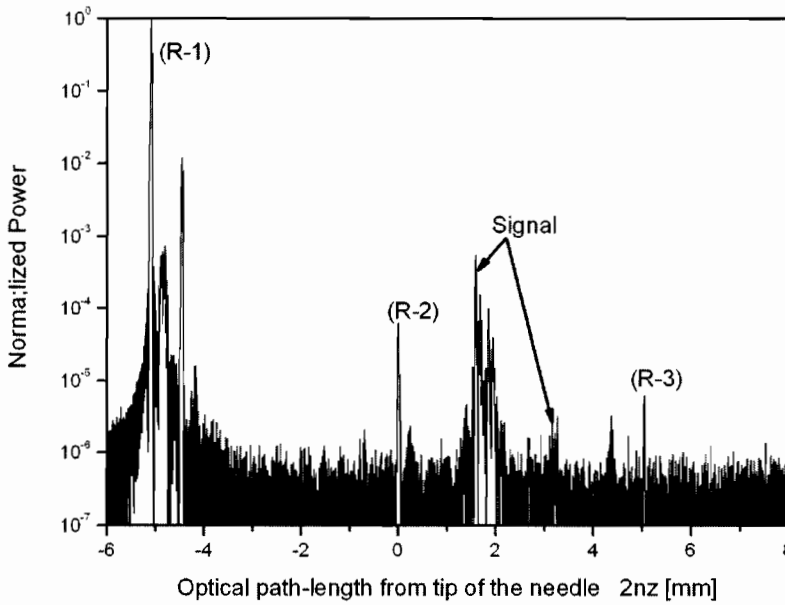


Fig. 3-15 Detected result when the needle is injected in a white mouse.

When the reference mirror is scanned at a constant velocity of 4 mm/s, Fig. 3-15 is obtained using a white mouse as a scattering object. The signals at 1.6 mm and 3.2 mm can be visible. The remarks of signals also illuminate that the penetration depth of 1~2 mm can be easily realized. The fact means that the needle-fiber system may be applicable to living tissues in the epidural anesthesia.

3.7 Conclusion

We propose a new application of OCT in the operation of epidural anesthesia. To avoid a medical accident, it is important to monitor the axial position of the tip of the needle with several millimeters in front of the needle. In this work, a fiber-needle system has been presented using OCT technique for ranging measurement. The obtained axial resolution of $21\text{ }\mu\text{m}$, which is not as fine as the super resolution level of $1\text{ }\mu\text{m}$, is about 1% of ranging distance and enough to measure the distance between the tip of the needle and target. In the OCT system for a given light source, the detectable ranging distance depends on the elimination of the diffused light and the high sensitive detection of the target-signals. From this point of view, the confocal system is used by mounting a lens at the tip of the fiber and consequently the focal distance in the system has influence on the quality of the OCT signals. By the Monte Carlo simulation technique, the background noise of a confocal OCT system is evaluated. The numerical results indicate that the background noise decreases exponentially after the maximum value on the transport mean path-length whereas the target-signal also decreases as the almost same rate because of the scattering effect. Therefore, the performance of the ranging distance may be improved by setting the focal length to the distance of a few millimeters to be needed in strong scattering medium. The fact gives a suggestion for design of OCT system in complicated biological tissue. Based on above analysis, we undergo the preliminary experiments using the *tofu* and a white mouse as scattering mediums. The signals are proved to be detected under the optimal condition, and 1~2 mm penetration depth is easily realized. The results predict that our needle-fiber OCT system is suitable for applying in epidural anesthesia.

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Chapter 4

Full-field optical coherence tomography system with wavelength-scanning laser source

4.1 Introduction

4.1.1 Background

Recently, various methods for biological measurement have been investigated in medical operations. Tomographic imaging of biological tissues is one of the most important techniques in medical science and engineering. For such a situation, optical coherence tomography (OCT) has used.¹⁾ As described in chapter 3, OCT has some attractive features for a broad range of biological application. On the basis of a low coherence interferometer, OCT performs high resolution in tomographic imaging and achieves sufficient sensitivity to probe weakly backscattering structures, and allows the localization of reflecting sites beneath the surface of biological tissues.²⁾ Povazay *et al.* have reported a longitudinal resolution of 0.75 μm using a sub-10 fs Ti:sapphire pulse laser.³⁾ Also, the use of a halogen illuminator achieves a resolution of 0.9 μm .⁴⁾

A conventional OCT system is a point detection system combined with focused illumination, and needs the transverse scanning of the light spot to obtain a cross-sectional image. To measure the internal biological structure in a real time and at high speed, the full-field OCT with an area sensor has been developed to obtain sliced images.⁴⁾ However, the full-field OCT has some problems that should be solved. For example, biological tissues

are highly scattering media, even though a near infrared light (NIR) is employed as a light source. The multiple scattered light (i.e., diffuse light) does not contribute to the interference signal (OCT signal), but yields background noise. The noise prevents us from accurate measurement. In the point detection of the conventional OCT, a confocal system is available for the elimination of the diffuse light. However, a full-field OCT system cannot be adapted to the confocal system, and a spatial filter system is usually used, although the elimination performance is not sufficient.⁵⁾ Another problem is that the full-field OCT requires a scanning system in the axial direction to obtain three dimensional information. In general, many sliced images are required. For the tomographic imaging of a small target in a scattering medium, therefore, the selection of the measuring area containing the target and the estimation of the depth of the target are important for accurate measurements.

To solve the latter problem, the variability of longitudinal resolution should be implemented in a single system. We have developed a full-field OCT system in which a wavelength-scanning laser source is used.^{6,7)} As described in chapter 3, longitudinal resolution depends on the coherence length of the light source. Therefore, we should develop a method to adjust the width of the spectrum of light emission. In the system, a low-coherence interferometer is driven by the synthesis of the coherence function. However, the effectiveness of the system has not yet been described quantitatively. In this study, therefore, we evaluated our system in detail. First, the tunable range of the longitudinal resolution in our system is evaluated experimentally. Moreover, as an example to show the usefulness of resolution changing, we demonstrate the searching for a nucleus in onion cells by low-resolution imaging and derive the depth of the nuclei by high-resolution imaging.

4.1.2 Chapter structure

In section 4.2, we briefly explain the theoretical procedure of tomographic measurement based on the synthesized coherence function. The experimental setup of the full-field OCT system using a wavelength-scanning laser source has been introduced in section 4.3. In section 4.4, we show experimental results to estimate the relationships between the wavelength-scanning range of the light source and the longitudinal resolution or the dynamic range, and we discuss the characteristics of our system. The imaging of a plant cell under both high- and low-resolution conditions is focused on in section 4.5. At last, Conclusions are given in section 4.6.

4.2 Basic principle of full-field OCT system based on a synthesized coherence function

The properties of the interferometer with a wavelength-scanning laser source are denoted by the notation shown in Fig. 4-1. When the CCD is used as a photodetector, the input intensity within a frame time is integrated as electronic charges. Because different frequency fields sequentially appear in one frame time by frequency sweeps of the LD, let us define the angular frequencies of the two optical fields on the detector to be ω and ω' at time t . Then, the integrated reference field E_r and the integrated object field E_o to be detected by the CCD are given by

$$E_r = \int a(\omega) \exp[i(2kz_r - \omega t + \phi_r)] d\omega, \quad (4-1)$$

$$E_o = \iint r(z) a(\omega') \exp\{i[2k'(z_r + \Delta z) - \omega' t + \phi_o]\} d(\Delta z) d\omega'. \quad (4-2)$$

In these equations, $a(\omega)$ is the amplitude of the optical field with frequency ω at time t , k and k' are the wave numbers, ϕ_r and ϕ_o are the initial phases, $r(z) = r(z_r + \Delta z)$ is the amplitude reflectance at any position z on the z -axis along the object arm, and z_r is

the position on the z -axis that is equal to the reference arm length. Amplitude reflectance is usually distributed along the axial direction. Therefore, the object light is composed of fields reflected at each position along the z -axis while the reference mirror is maintained at the conjugate position z_r . Since $2\Delta z$ is the optical path-length difference between the interferometer arms, the time delay τ due to this difference is

$$\tau = \frac{2\Delta z}{c}, \quad (4-3)$$

where c is the speed of light.

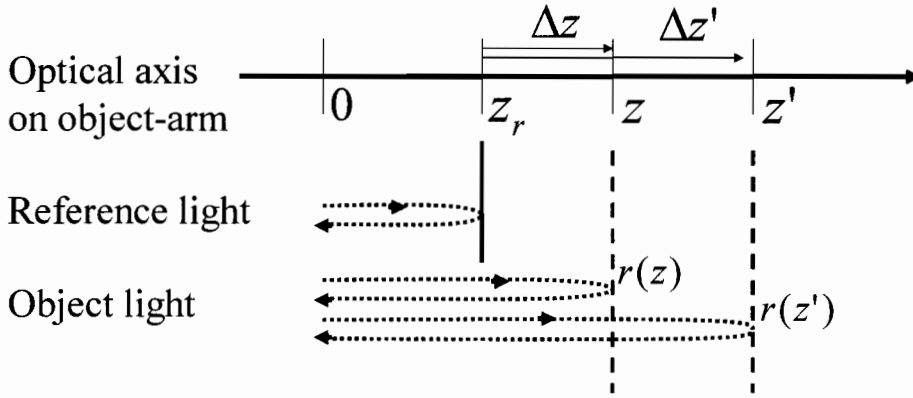


Fig. 4-1 Schematic diagram used to analyze OCT using synthesized coherence function.

On the detector, the accumulated intensities of the reference and the object lights, I_r and I_o , respectively, are denoted by

$$I_r = \int |a(\omega)|^2 d\omega, \quad (4-4)$$

$$\begin{aligned} I_o &= \iiint |a(\omega)|^2 r(z) r^*(z') \exp[i2k(\Delta z - \Delta z')] d(\Delta z) d(\Delta z') d\omega \\ &= \int \left| \int a(\omega) r(z) \exp(i2k\Delta z) d(\Delta z) \right|^2 d\omega. \end{aligned} \quad (4-5)$$

The normalized power spectrum of the source is represented by

$$S(\omega) = \frac{|a(\omega)|^2}{\sqrt{I_r I_o}}. \quad (4-6)$$

Let us assume that two optical fields with different frequencies do not interfere with each other, because light of different frequencies is emitted at different times in the present system. Interference fringes are formed by two fields of the same optical frequencies. Therefore, the fringes at every frequency can be independently summed. Then, the detected interference component under the conditions of $\omega = \omega'$ and $k = k'$ is given by

$$\begin{aligned} E_r^* E_o + c.c. &= \iint |a(\omega)|^2 r(z) \exp[i(2k\Delta z + \phi)] d\omega d(\Delta z) + c.c. \\ &= \sqrt{I_r I_o} \int r(z) \exp(i\phi) \int S(\omega) \exp(i\omega\tau) d\omega d(\Delta z) + c.c. \\ &= \sqrt{I_r I_o} \int |r(z)\gamma(\tau)| d(\Delta z) \exp[i(\phi + \theta)] + c.c. \\ &= 2\sqrt{I_r I_o} \int |r(z)\gamma(\tau)| d(\Delta z) \cos(\phi + \theta). \end{aligned} \quad (4-7)$$

Here, $c.c.$ is the complex conjugate of $E_r^* E_o$, ϕ is the phase difference between the two optical fields, θ is the phase of $r(z)\gamma(\tau)$, and $\gamma(\tau)$ indicates the coherence function of an optical field obtained using the Wiener-Khintchine theorem. By scanning the wavelength of coherent light within a frame time of the CCD, the coherence function becomes equivalent to that of a low-coherence light source, which is called a synthesized coherence function.^{8, 9)} This light source has an advantage that the optical field is spatially perfectly coherent and temporally low coherent. These coherence properties are useful for practical interference experiments.

Therefore, the integrated intensity of the m -th frame is represented by

$$I_m = (I_r + I_o) + 2\sqrt{I_r I_o} \int |\gamma(\Delta z)r(z_r + \Delta z)| d(\Delta z) \cos(\phi_m + \theta). \quad (4-8)$$

where the coherence function has been represented as a function of Δz using Eq. (4-3).

Because the coherence function is symmetric, i.e., $\gamma(\Delta z) = \gamma(-\Delta z)$, the reflectance

information is given by a convolution integral with the coherence function. To extract the amplitude of the cosine function in the second term in our system, the phase difference ϕ_m is changed at every frame time using the PZT actuator, as shown in Fig. 4-2. In Eq. (4-8), therefore, the phase difference at the m -th frame is represented by ϕ_m instead of ϕ in Eq. (4-7). The phase step is set to $\Delta\phi = \phi_m - \phi_{m-1} = 2\pi p/M$, so that p fringes are measured within the measuring time of M frames. Here p is an integer.

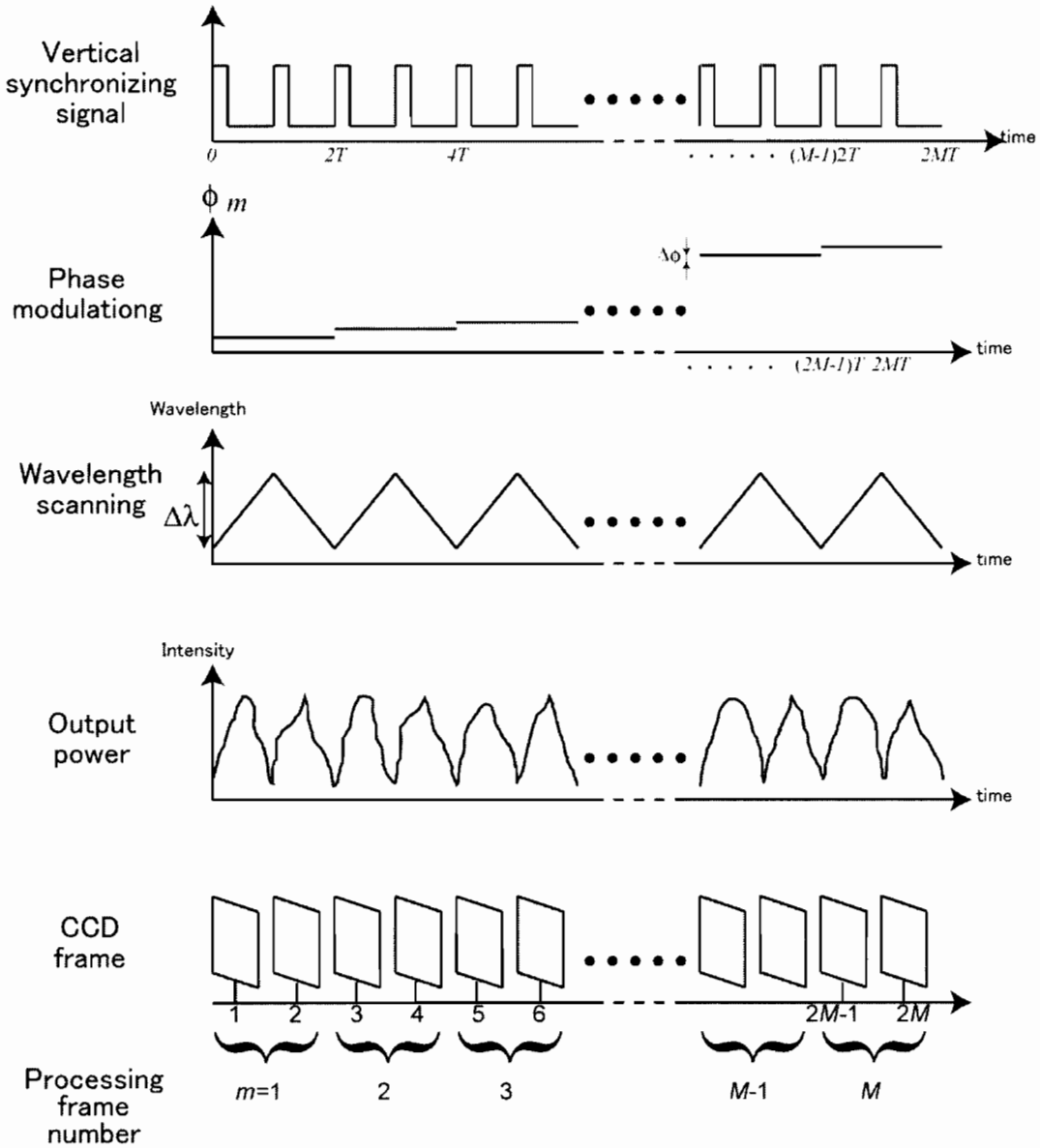


Fig. 4-2 Timing chart of components controlled by vertical synchronizing signals of CCD. T is the frame time of the CCD and m is the frame number for processing.

Let us acquire data for $2M$ frames using the CCD. Here, note that we treat M frames ($m = 1, 2, \dots, M$) in the processing because two successive sets of frame data are summed as shown in Fig. 4-2. If I_o and I_r are known in advance, the reflectance is derived using the following signal processing equation.¹⁰⁾

$$\begin{aligned} I_{proc}(m) &= \frac{I_m - (I_o + I_r)}{2\sqrt{I_o I_r}} \\ &= [|r(z_r) \otimes \gamma(z_r)|] \cos(\phi_m + \theta), \end{aligned} \quad (4-9)$$

where the symbol $\otimes$ denotes a convolution integral. As noted in Eq. (4-9), the processed intensity $I_{proc}(m)$ varies sinusoidally with increasing m because $\phi_m = (2\pi p/M)m$. Therefore, we can extract the amplitude of the cosine function using a general Fourier transform theorem. Let us define the obtained amplitude to be A_{exp} . We adjust the phase step to $\Delta\phi = 2\pi p/M$ in order to obtain A_{exp} accurately. Such digital processing of M frames of data gives

$$|r(z_r) \otimes \gamma(z_r)| = A_{exp}. \quad (4-10)$$

In biological measurements, let us assume that the reflection yields spatially incoherent light, because the boundaries with a refractive index difference in tissue are usually composed of optically rough surfaces. Under this assumption,

$$|r(z_r) \otimes \gamma(z_r)|^2 = |r(z_r)|^2 \otimes |\gamma(z_r)|^2 \quad (4-11)$$

is satisfied.

The intensity distribution over all pixels of the CCD gives a sliced image of reflectance information at $z = z_r$ in the object. To generate one sliced image, therefore, a measuring time of $2MT$ seconds is required, where T is the frame time. In a full-field OCT, the three dimensional information can be obtained by moving mechanically the object along the

z -stage, i.e., scanning the position z_r .

For the condition $|\gamma(\Delta z)| = \delta(\Delta z)$, the energy reflectance $R(z_r) = |r(z_r)|^2$ is obtained from Eqs. (4-10) and (4-11). This shows that the reflectance is ideally measured using the temporally incoherent light. Also, when the object is a mirror placed at the position $z_m = z_r + \Delta z$, i.e., $|r(z)| = \delta(z_m)$, Eq. (4-10) gives the squared coherence function as

$$|\gamma(\Delta z)|^2 = |A_{\text{exp}}|^2. \quad (4-12)$$

Since Δz corresponds to the optical path difference between the object mirror and the reference mirror, this formula can be used for obtaining the coherence function experimentally.

4.3 System architecture

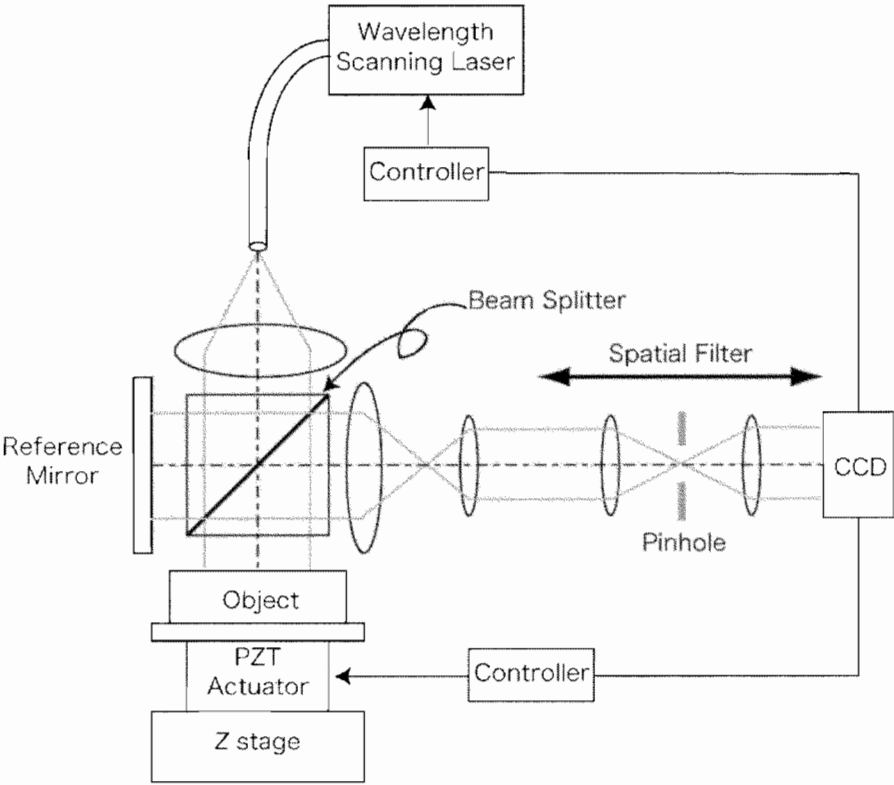
A schematic diagram of the full-field OCT system is shown in Fig. 4-3(a). It consists of a Michelson interferometer, a spatial filter, and a charge coupled device (CCD). In the interferometer, a wavelength-scanning laser diode (Environmental Optics Sensors, ECU-2001A), which is shown in Fig. 4-3(b), is employed for the light source. In this emitter, the optical cavity consists of a laser diode (Environmental Optics Sensors, DMD810-015, 15 mW), a grating (1,800 grooves/mm) and a retro-reflector (triangular prism) mounted on a galvanometer optical scanner (Cambridge Technology, Model 6450). The wavelength is selected by the angle of the reflector. Because the angle is proportional to the input voltage applied to the galvanometer, it is easy to control electrically the oscillating wavelength. The laser light guided by a single-mode fiber is transformed into an extended plane wave and illuminates the object and a reference mirror. The object is placed on a multilayered piezoelectric transducer (PZT) to modulate the phase components of the object light field.

The reflected reference and object fields are superposed on a beam splitter in the interferometer. The interference signal passes through the spatial filter and is detected by the CCD (Hamamatsu Photonics, Model C4880-82, 14 bit, 656×494 pixels) in which the pixel size is 9.9×9.9 μm . The detector typically works at an acquisition rate of 25 frames/s for 100×100 pixels. Once the image size is set to be small, we can increase acquisition rate.

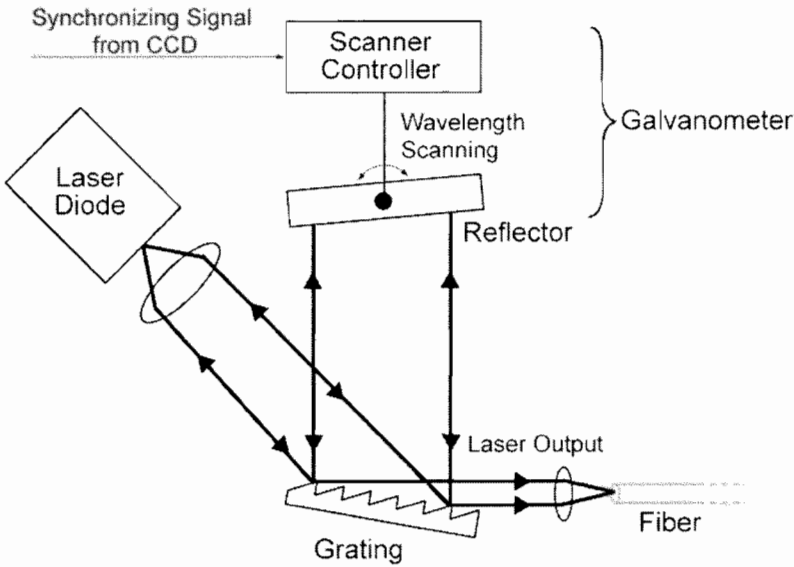
The *en face* cross section of the object to be detected is imaged on the photoelectric plane of the detector. In the interferometer, the optical arm length of the reference mirror is adjusted to be equal to that of the cross section. In the object light, the diffuse light backscattered from out-of-focus planes is predominantly contained. To eliminate such undesired light, the spatial filter is inserted. It is composed of two lenses and an aperture. The cutoff spatial frequency is characterized by the hole radius of the aperture. The diffuse light can be significantly decreased by making the cutoff frequency as low as possible. The lower the cutoff frequency, the more diffuse light can be eliminated. In this case, high-frequency components in the acquired image are lost. Therefore, the lateral resolution of images detected using this system is mainly determined by the broadness of the optical transfer function of the spatial filter. If the effective cutoff frequency for the elimination of diffuse light is comparable or equal to the maximum spatial frequency limited by the period of the CCD pixels, our OCT system can acquire an image without significant loss of lateral resolution.

In the setup, the operations for wavelength scanning of the laser diode (LD), the phase modulation of the PZT, and the image data acquisition are synchronized by synchronizing signals of the CCD. The timing chart is shown in Fig. 4-2. The galvanometer scans up and down the wavelength linearly during one frame time of the CCD. Since the output power is slightly different between upscan and downscan, we generate a tomographic image from the

sum of two successive sets of frame data by the subsequent image processing.



(a)



(b)

Fig. 4-3 System configuration of a full-field OCT system with variable resolution.

4.4 Controllable longitudinal resolution

4.4.1 Synthesized coherence function and sidelobes

To analyze the synthesized coherence function, we investigate the spectral shape of our system. Figure 4-4(a) shows the measured results of four types of power spectrum. The scanning width of wavelength is electrically controlled by the amplitude of the triangle wave voltage applied to the galvanometer, during which, the injection current of the laser diode is maintained at 75 mA and the central wavelength λ_c is set to approximately 820 nm. In the experiments, the FWHM of the spectral shape can be varied from $\Delta\lambda = 2$ to 40 nm during one frame. At the maximum scanning range, we can obtain a broad band spectrum from 796 to 846 nm. When scanning at the narrow bandwidth, the shape of the spectrum is approximately rectangular, whereas when scanning at the wide bandwidth, it becomes asymmetric with distorted convexity. These spectral shapes are due to the relationship between the broadness of the gain curve in the laser cavity and scanning bandwidth.

The squared coherence functions $|\gamma(\Delta z)|^2$ are shown in Fig. 4-4(b), and are obtained by the Fourier transform of the spectra shown in Fig. 4-4(a). Many sidelobes appear in the squared coherence functions. Figure 4-5 shows the squared coherence functions obtained using two types of power spectrum with the same FWHM, i.e., the Gaussian form and rectangular form. The characteristics of the sidelobes in the coherence functions depend on the form of the power spectrum. Such sidelobes generate ghost images that appear as noise in an OCT image.³⁾ In particular, high-quality imaging requires a narrow coherence function due to broadband scanning. Then, the wavelength-scanning system used in the proposed method gives a convex spectrum. The broadband scanning is required to suppress the sidelobes.

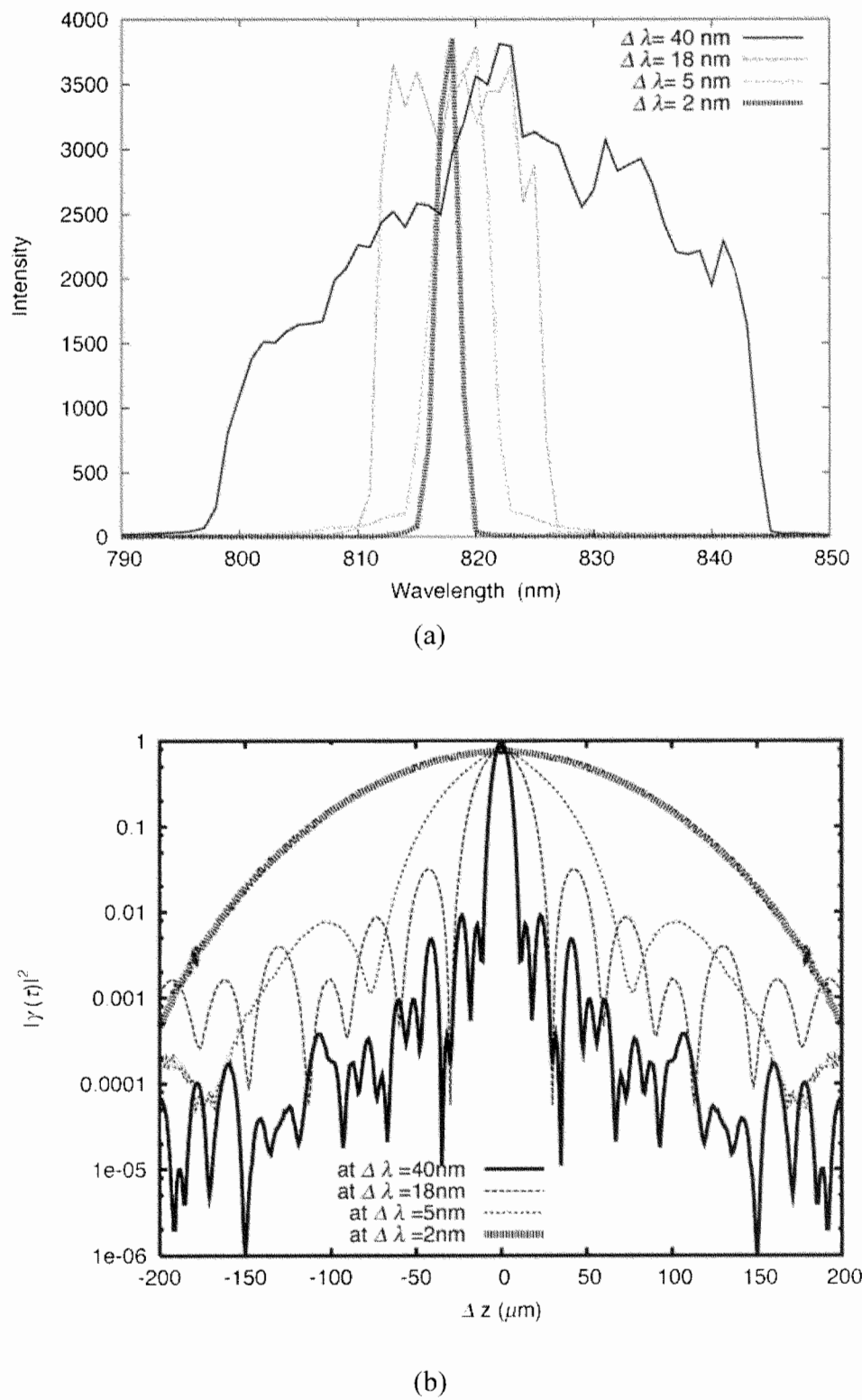


Fig. 4-4 Characteristics of wavelength-scanning laser source. (a) the power spectra and (b) the synthesized coherence functions obtained by the Fourier-transform of (a).

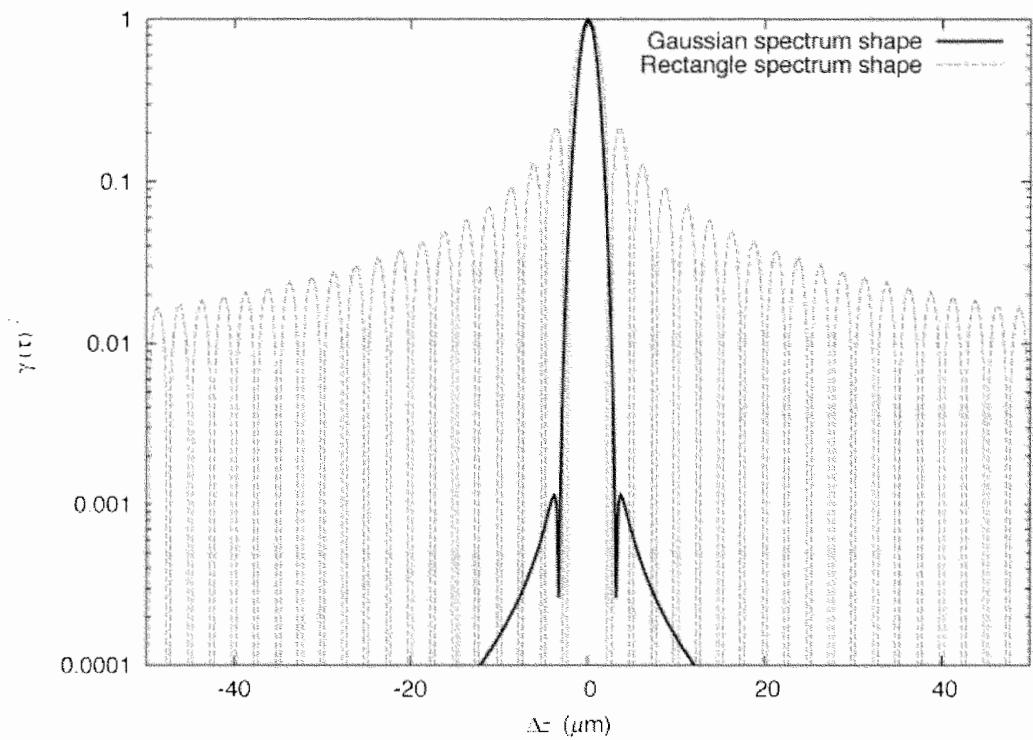


Fig. 4-5 Coherence functions of rectangular and Gaussian spectra with same FWHM.

4.4.2 Resolution and dynamic range

Since the reflectance is given by a convolution integral with the coherence function as noted in Eq. (4-10) or (4-11), the squared coherence function is called the axial point spread function (PSF) of the OCT system. The axial PSF can be directly obtained from an interferogram, as given in Eq. (4-12). In the experiments, a reflection mirror is placed on the object stage with a small tilt. Figure 4-6 shows the axial PSF for four types of $\Delta\lambda$ (FWHM of squared spectrum). To determine the resolving power of our OCT system, we define the longitudinal resolution ΔR as the FWHM of a PSF profile. As shown in Fig. 4-6, we obtained $\Delta R = 6\ \mu\text{m}$ at $\Delta\lambda = 40\ \text{nm}$ and $\Delta R = 120\ \mu\text{m}$ at $\Delta\lambda = 2\ \text{nm}$. These results are in good agreement with the FWHM shown in Fig. 4-4(b). As an example of comparison between

Figs. 4-4(b) and 4-6, the case of $\Delta\lambda = 40 \text{ nm}$ is shown in Fig. 4-7. Here, the solid curve shows the overall characteristic of the OCT system, and the dashed curve shows that of the wavelength-scanning source. The two curves coincide in the important range of $|\Delta z| \leq 40 \text{ }\mu\text{m}$. Therefore, the present interferometer system using the wavelength-scanning laser source is suitable for low-coherence tomography. We have confirmed that the light source can control the longitudinal resolution.

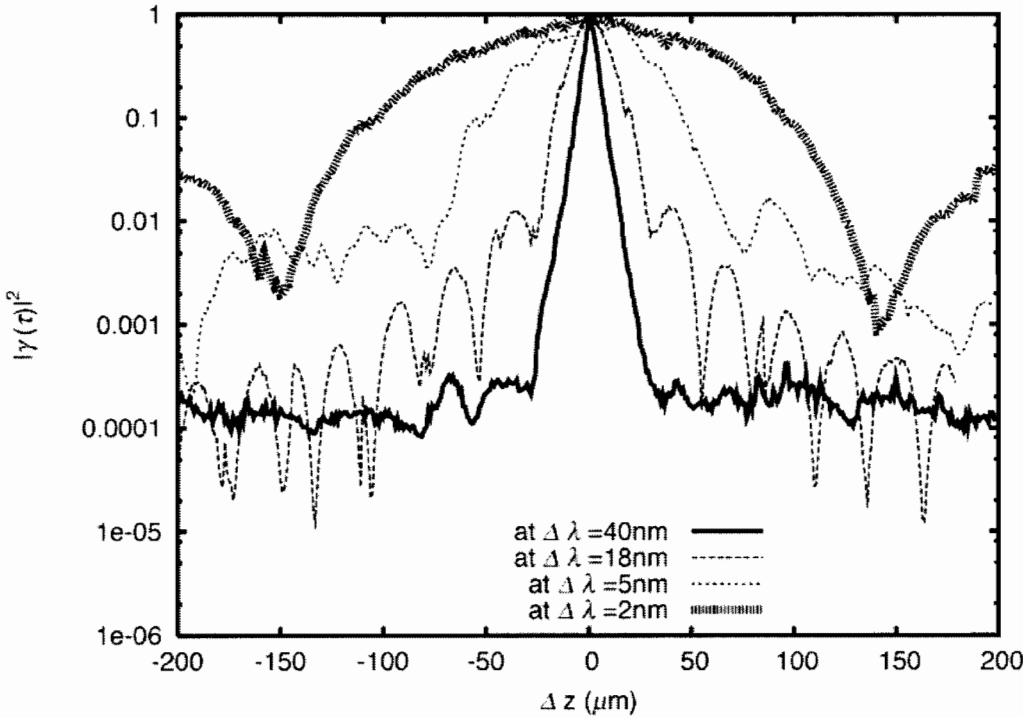


Fig. 4-6 Axial point spread function measured by present system.

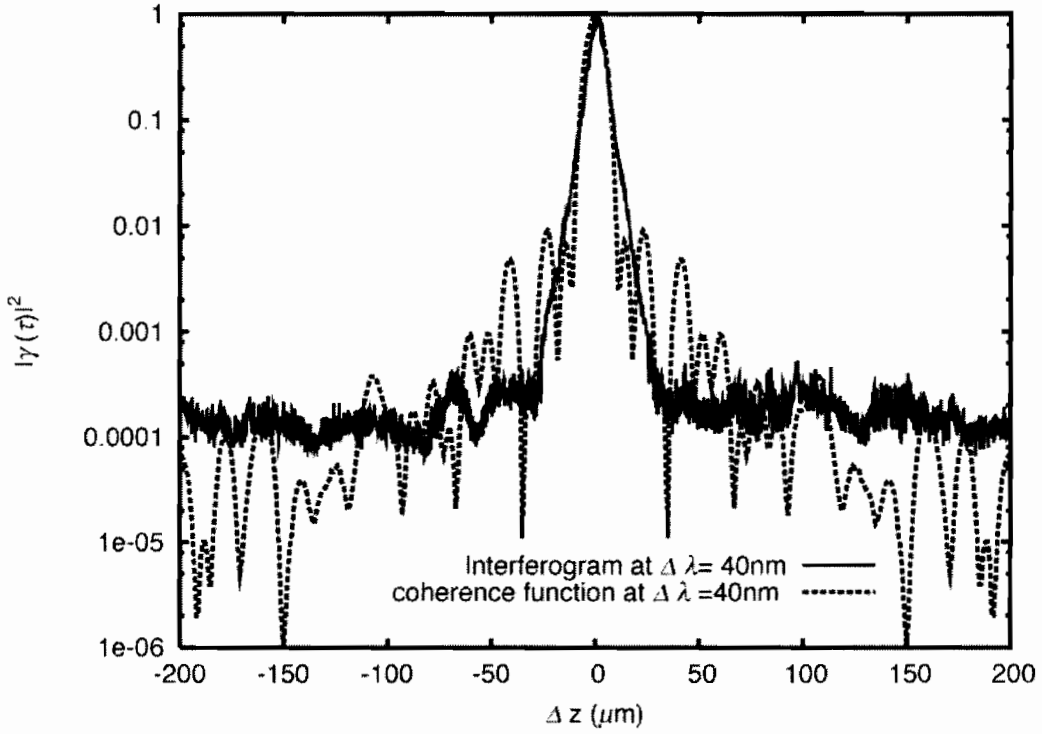


Fig. 4-7 Comparison between direct measurement of interferogram (solid curve) and Fourier transform of power spectrum of light source (dashed curve).

The solid curve in Fig. 4-7 shows approximately a constant value of 1.7×10^{-4} for $|\Delta z| \geq 40 \text{ } \mu\text{m}$. The constant level is on the same order as the sidelobes shown by the dashed curve, but if the level is equal to that of the sidelobes, it must decrease as $|\Delta z|$ increases. Therefore, the constant level may be considered to be a noise depending on the total intensity. On the basis of a proposal by Laude et al.,⁹⁾ the level has a shot noise limit in the CCD detection that obeys Poisson statistics. From these results, the constant level at the curve of $\Delta\lambda = 40 \text{ nm}$ as shown in Fig. 4-6 is assigned to be a shot noise. To widen the dynamic range by decreasing the noise level, it is necessary to increase the number of acquisition frames. As noted from the conditions of $T = 110 \text{ ms}$ and $2M = 256$, the acquisition of one

sliced image requires a long measuring time of 28.2 seconds. This is a disadvantage of using the present CCD in the full-field OCT. However, if the photoelectron saturation of CCD used is much larger than that of our CCD (40,000 electrons/pixel), a wider dynamic range will be obtained even for a few frames for accumulation or a short measuring time.

4.5 Observation of onion cell

Tomographic measurements are frequently used to obtain a detailed structure of a small target in living tissues. In this case, we are confronted with the difficulty in determining whether or not the target is contained in a large volume. When searching for the target at a high resolution, much time is required for measurement because many sliced images must be acquired.

Under the low-resolution condition, the image quality of the sliced image is low. However, the sliced image provides volume information, which contains reflectance information over a wide range (ΔR) in the axial direction. Therefore, the low resolution is suitable for searching for the target in the object, because the whole region can be observed using fewer images. Once the target has been found, high-quality images can be obtained by changing the wavelength-scanning range in the LD. From the viewpoint of practical use, the technique of both high- and low-resolution imaging has the advantage of decreasing the measuring time. Therefore, in the present system, the controllable resolution is important.

We observe plant cells to show the usefulness of variable-resolution imaging. As the object, onion cells are selected. A very thin layer of onion, which nuclei is stained by acetic acid, is attached to a glass plate. In following experiments, $2M = 256$ frames are adapted for acquiring a sliced image. First, Fig. 4-8 shows the experimental result of imaging using the low-resolution condition of $\Delta R = 40 \text{ } \mu\text{m}$. Two nuclei of different depths and the cell walls

are observed on the selected image, which is the same as a micrograph at a long depth-of-focus. From the result, it is found that the observed area is selected successfully because the normal nuclei of the target are contained in the area.

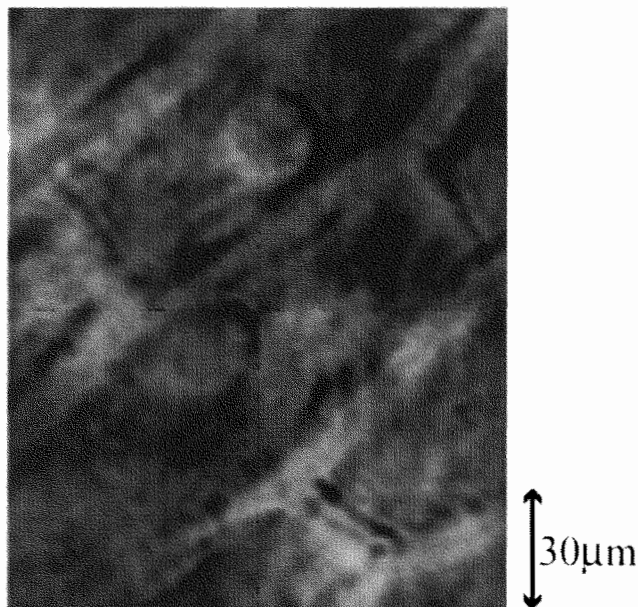


Fig. 4-8 Sliced image at low resolution of $\Delta R = 40 \mu\text{m}$.

Next, high-resolution images at different axial positions for the selected area in Fig. 4-8 are shown in Figs. 4-9(a) and 4-9(d). In this case, the longitudinal resolution is set to $\Delta R = 6 \mu\text{m}$. Axial positions of these images are $z_r = -6, 0, 6$, and $12 \mu\text{m}$, respectively, where the original position of $z_r = 0$ is selected near the object surface. In Fig. 4-9(a), neither cell walls nor nuclei are observed. From the image in Fig. 4-9(b), cell walls and two nuclei can be recognized. Signals from the lower left nucleus are clearer than those from the upper right nucleus. In Fig. 4-9(c), on the other hand, the signal intensity of the upper right nucleus is more intense than the lower left nucleus, although both nuclei are almost the same in size.

Finally, in the image of Fig. 4-9(d), signals of the nuclei are very weak or nonexistent.

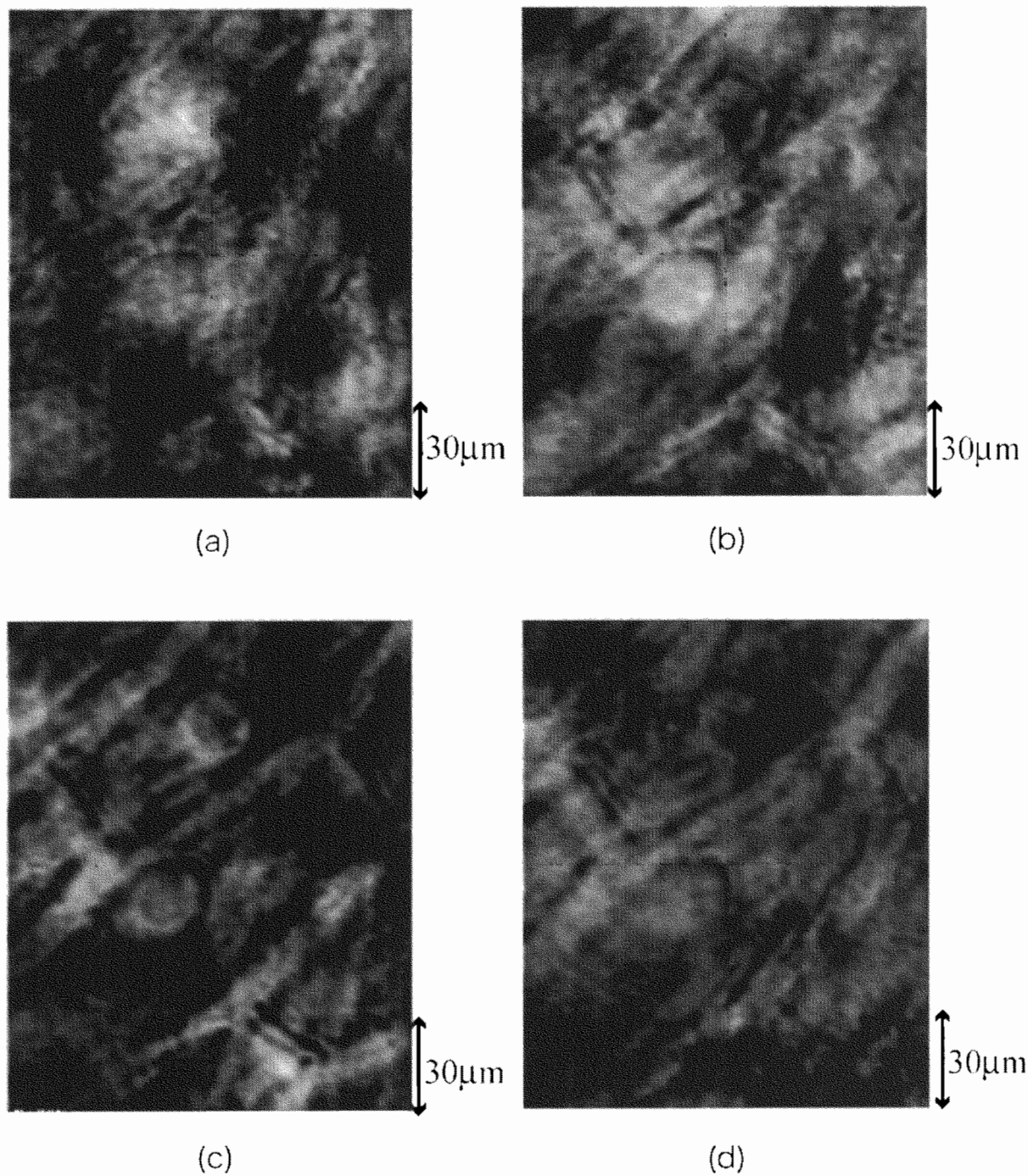


Fig. 4-9 Sliced images at high resolution of $\Delta R = 6 \text{ }\mu\text{m}$. The axial positions are (a) $z_r = -6$, (b) 0, (c) 6, and (d) 12 μm .

Using Figs.4-8 and 4-9, we can derive the three-dimensional structure of the target object. From the experiment described in this section, we have confirmed that the low-resolution imaging of our system is useful for searching over a wide area, and the high-resolution imaging is effective for detailed three-dimensional measurement at a selected area.

4.6 Conclusion

A full-field OCT system can obtain a sliced image of high resolution. To eliminate the undesired diffuse light, a spatial filter is used. Although the elimination performance is not sufficient, the spatial filter can significantly decrease the background noise and improve the signal-to-noise ratio.

On the other hand, for full-field optical tomographic imaging, we have proposed a method of suitable measurement, which consists of two stages, searching at a low resolution over a wide area and imaging at a high resolution in the specified region for the interesting target. To facilitate this measuring method, the present system offers controllable longitudinal resolution.

The source also acts as a temporally low-coherence source by wavelength scanning over a broad band during the detector response time. Using this characteristic, a full-field OCT system with variable-resolution imaging has been constructed. The most significant feature is the achievement of both high- and low-resolution imaging with a single piece equipment and a simple operation of the laser diode. Our system can control the longitudinal resolution between 6~120 μm . This improvement has been verified by measuring the nuclei of a target in an observed area of several onion cells.

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Chapter 5

Conclusion

Current clinical practice emphasizes the development of techniques to diagnose disease in early stages without damages. Optical tomography approaches to this promising technique for tomographic imaging in noncontact and noninvasive operation. In the past decade, its applications have emerged actively in areas such as ophthalmology and endoscope. The use of near-infrared light as a source opens the path for tomographic imaging because of weak absorption process in tissues. But the intense diffuse light due to multiple scattering deteriorates severely the image quality as a background noise. The image quality is usually determined by two factors of the resolution and the background noise. The aim of this thesis is to obtain high quality image by means of both improving the resolution and rejecting the background noise due to the diffuse light as much as possible. In the studies three kinds of optical tomography are proposed and the results are summarized below.

1. The fiber-based confocal system of a simple system is introduced in chapter 2. The characteristics of confocal system with near infrared light guided by a single mode fiber have been investigated. The important part of obtained results has been confirmed experimentally by measuring a ground glass plate of scattering sample. The important characteristic about the resolution is obtained as follows. The axial resolution is more sensitive to the lateral resolution, and tends to be high as the core size of fiber decreases. Typically our system using the fiber of core radius $3\text{ }\mu\text{m}$ achieves the axial resolution of $9.25\text{ }\mu\text{m}$, whose resolutions coincide with the results of the analytical method. The

system also can reduce tremendously the amount of the diffuse light because the light is almost eliminated through two stages: illumination and detection. The rejection rate of the diffuse light has been investigated by numerical simulation using Monte Carlo technique. It is found that the diffuse light to be detected by this system abruptly decreases as the core size becomes small, especially less than the radius of 5 μm . The diffuse light can be rejected below 10^{-6} at the radius of 1 μm . Since the system consists of fiber-based system, it is compact and flexible. The system is expected to application to remote-sensing in body such as the endoscope. To obtain the tomographic images in tissues at high image quality, however, the performance is not enough.

2. In order to further improve the rejection rate of the diffuse light, we utilize a pathlength-resolved imaging modality in chapter 3: optical coherence tomography (OCT). This is realized by the fact that the scattering light experiences a longer pathlength than the unscattered light and the pathlength is resolved by a low coherence interferometry. The OCT performs the high resolution with sufficient sensitivity to probe scattering tissues. We establish a needle-fiber OCT system for applying to the epidural anesthesia. The axial resolution is determined only by the band width of low coherence light to be used, and achieves 21 μm which is enough to range a long distance of several millimeters. The focusing of the illumination light has a substantial influence on the background noise. It is found with a Monte Carlo method that the focal length of focusing lens should be chosen at a desired distance of interesting portion. As a result, the signal-to-noise ratio is improved, and the ranging distance in tissues is done to several millimeters. The fact is confirmed by positioning the internal organs in living tissue of a white mouse. To obtain the tomographic image with the OCT system, the scanning system of the x- or y-direction must be considered to be required in general.

This experimental example shows that there is useful application without the scanning system.

3. The full-field OCT is described in chapter 4. In order to realize the 3D tomographic imaging, the scanning system for the optical system or the sample object is additionally required. In our cases, the 3-dimensional scanning of x-, y-, and z- direction must be done for the confocal system in chapter 2, and the 2-dimensional scanning of x- and y-direction must be done for OCT system in chapter 3. The full-field OCT can measure directly the image (*en face* image) along the x-and y-axis at a certain position on z-axis with imaging system. However, additional problem occurs that the *en face* image must be measured at low axial resolution. To overcome this problem, the full-field OCT system with wavelength-scanning laser source has been established. At the low resolution, searching the target, and at high resolution, 3-D measuring the target, we have realized the resolution of 6-120 μm by scanning the wavelength of the laser light over a few ten nano-meters. This operation is made electrically, and the resolution is controllable. On the other hand, to reject the diffuse light, this system uses an optically spatial filter instead of the confocal system. In this system, therefore, the diffuse light is rejected by filtering due to propagation direction (spatial filter) and the use of interferometry due to low coherence light. As an application of full-field OCT with controllable resolution, we have verified the advantage by measuring 3-dimensionally the nuclei of a small target in an observed area of several onion cells.

Optical tomography is a unique and potential technique in biomedical application. Rejecting the diffuse light is an unavoidable and challenge topic. It is anticipated that our work gives some ideas for future applications.

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List of published papers

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