High-resolution imaging of biological tissue with full-field optical coherence tomography

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ABSTRACT

A new full-field optical coherence tomography system with high-resolution has been developed for imaging of cells and tissues. Compared with other FF-OCT (Full-field optical coherence tomography, FF-OCT) systems illuminated with optical fiber bundle, the improved Köhler illumination arrangement with a halogen lamp was used in the proposed FF-OCT system. High numerical aperture microscopic objectives were used for imaging and a piezoelectric ceramic transducer (PZT) was used for phase-shifting. En-face tomographic images can be obtained by applying the five-step phase-shifting algorithm to a series of interferometric images which are recorded by a smart camera. Three-dimensional images can be generated from these tomographic images. Imaging of the chip of Intel Pentium 4 processor demonstrated the ultrahigh resolution of the system (lateral resolution is 0.8 μm), which approaches the theoretical resolution 0.7 μm × 0.5 μm (lateral × axial). En-face images of cells of onion show an excellent performance of the system in generating en-face images of biological tissues. Then, unstained pig stomach was imaged as a tissue and gastric pits could be easily recognized using FF-OCT system. Our study provides evidence for the potential ability of FF-OCT in identifying gastric pits from pig stomach tissue. Finally, label-free and unstained ex vivo human liver tissues from both normal and tumor were imaged with this FF-OCT system. The results show that the setup has the potential for medical diagnosis applications such liver cancer diagnosis.

Keyword imaging system; FF-OCT; phase-shifting interferometry; biological tissue

1. INTRODUCTION

Currently, optical coherence tomography (OCT) has been demonstrated as an effective noninvasive imaging technique for getting high-resolution tomographic images in scattering media such as biological tissues [1]. However, the typical axial and transversal resolutions of such OCT systems lie between 5 and 30 μm, which is not enough to distinguish cellular-scale structures, and furthermore, the scanning mode is by-point scanning which has the drawback of slower imaging speed [2].

A new OCT technique, the so-called full-field optical coherence tomography (FF-OCT) is an approach in which 2D en-face images are directly recorded with CCD detector array [3]. Light source with a broad spectrum (typically a halogen source) is employed to achieve micron-level resolution along the depth direction. The lateral resolution is improved by using objective lenses with the high numerical aperture (NA). FF-OCT is enabling for high-resolution over the full-field of observation. As we know, biology applications of FF-OCT technology with an isotropic resolution around 1μm will be very helpful for clinicians and surgeons. Up to now, FF-OCT has been used in studies in developmental biology and cancer diagnosis. Examples include non-invasive 3D sub-cellular live imaging of preimplantation mouse embryos with no need of dye labeling for quantitatively measuring the factors that relate to early patterning and polarity in preimplantation embryonic
development[4], obtaining en-face images of in vivo human lip[5], and observing the blood vessel in dermis and tracing the flowing of the red blood cells[6].

The light source is a key component in a FF-OCT system. Some research groups use broadband output power after coupling into multi-mode fiber to light sample[7], but the size of fiber limits the area of sampling. Some use Köhler illumination directly, but it is expensive. SLD also can be used as the light source[8], but the spectrum bandwidth is more narrow compared with the tungsten halogen lamp and more expensive. Our Linnik-based FF-OCT system uses the tungsten halogen lamp as light source and a pair of microscope objectives to image sample. Sample area can be free-space by adjusting the field stop. The aperture stop can control the light intensity incident on the sample.

This research aims at acquiring high-resolution en-face images of biological tissue which can replace the frozen-sectioning during the surgery. In this paper, we present a low cost and simple FF-OCT system which provides resolutions of 0.7 µm and 0.5 µm respectively in lateral and axial directions. We first describe the principle and performance of our FF-OCT setup, and then present en-face images of the chip of Intel Pentium 4 processor, cells of onion details in surface and different depths, gastric pits on surface of pig stomach, human normal and tumor liver tissue.

2. EXPERIMENTAL SETUP AND ACQUISITION SYSTEM

The system is based on the Linnik interference microscope geometry (shown in Fig. 1) with a 20W tungsten halogen lamp, which is coupled in an improved Köhler illumination system. The central wavelength and spectral half-width of the tungsten halogen lamp spectra are 550 and 200 nm, respectively. A pair of identical microscope objectives (20x, 0.5 numerical, Olympus) are placed in both arms. The polished surface of a YAG crystal rod provides a reference surface with a reflectivity of 8%. The interference images are digitized by a CCD camera (Matrox Iris GT300, 640 pixel × 480 pixel, pixel size is 7.4µm × 7.4µm) working at a maximum rate of 110 frames/s. The signal to be extracted is the interference fringe intensity corresponding to the light which is backscattered from a particular slice inside the sample.

With broad-spectrum illumination, interference occurs only when the optical path lengths of two arms are nearly equal. The interference signal contrast varies according to a coherence function and drops off rapidly when the optical

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Fig.1 The system of FF-OCT. BS-beam splitter; MO-microscope objective
path difference exceeds the coherence of the illumination source. The coherence length is inversely proportional to the spectrum width of the illumination source. The coherence signal, which is proportional to the amplitude of the light returning from the sample which interferes with the light reflected by the reference surface, corresponds to an *en-face* tomographic image of the reflecting and scattering sample structures. The depth of the tomographic image is determined by the reference arm length.

The image formed on the CCD detector array contains the interference information of the image of the sample with the uniform image of the reference mirror, upon which the incoherent light from reflections and backscattering from different depths in the sample and unwanted reflections in the microscope itself are superimposed. Without phase modulation, two arms are in coherence and the intensity $I$ at any pixel $(x, y)$ of the CCD array can be expressed as

\[ I_d(x, y) = I_0(x, y) + A(x, y) \cos[\varphi(x, y)] \]  

(1)

Here $I_0(x, y)$ is the average intensity, $\varphi$ is the phase difference between the object and the reference wavefronts; $A(x, y)$ is the amplitude of the interference signal. Five sequential interference images captured by the camera can be described as follows\[10]\:

\[ I_n(x, y) = I_0(x, y) + A(x, y) \cos[\varphi(x, y) - (n - 3)\delta] \]  

(2)

The phase shift between one interferograms and its next is $\delta=\pi/2$. According to the Hariharan algorithm\[11]\, *en-face* OCT image $\gamma$ without the influence of nonuniformity of light intensity distribution can be obtained from the following equation\[12]\:

\[ \gamma = \frac{A(x, y)}{I_0} \approx \frac{3[4(I_1(x, y) - I_2(x, y))^2 + (I_1(x, y) + I_5(x, y) - 2I_4(x, y))^2]^{1/2}}{2(I_1(x, y) + I_2(x, y) + I_3(x, y) + I_4(x, y) + I_5(x, y))} \]  

(3)

### 3. PERFORMANCE

With broad-spectrum illumination, interference occurs only when the optical path lengths of the two arms are nearly equal. Taking it into consideration and the coherence length $l_c$ is:

\[ l_c = \frac{\lambda_0^2}{\Delta \lambda} = 1.51 \mu m \]  

(4)

Which has the same order of magnitude with the phase shift range (each phase is $\pi/2$), that is 5-step length is $5\lambda/4 \approx 0.69 \mu m$. However, it must be pointed out that the interference signal contrast varies according to envelope and drops off rapidly when the optical path difference exceeds the coherence length of the illumination source. The coherence length is inversely proportional to the spectrum width of the illumination source. As shown in Fig. 2, the black frame contains the coherence volume inside the sample, the blue volume represents the right phase-shifting way while the yellow
one represents the wrong phase-shifting way of the reference mirror. During experiments, we should make sure the initial phase is far enough away from the edge of coherence volume.

![Graph showing the relationship between coherence length and phase shift range](image)

**Fig. 2 Relationship between the coherence length and the phase shift range**

### 3.1 Axial resolution

Within a Gaussian line shape for the light source, the axial resolution is generally half of the coherence length, but this paper uses ultra-wide bandwidth light source, so we need to redefine the axial resolution. We have discussed axial resolution in detail before, here is the formula of axial resolution $d_z$ \cite{13,14}

$$d_z = \frac{1.78\pi}{\Delta \kappa_z (1 + \cos \theta_0)}$$

(5)

$$\Delta \kappa_z = 2\kappa_0 (1 - \cos \theta_0) + 2\Delta \kappa' = 2\kappa_0 (1 - \cos \theta_0) + \Delta \kappa (1 + \cos \theta_0)$$

(6)

Here $\kappa_0 = 2\pi / \lambda_0$, represents wavenumber, $\lambda_0$ is center wavelength, $\theta_0$ represents incidence angle, $\Delta \kappa$ denotes the bandwidth of wavenumber.

In air ($n = 1$), with NA = 0.5 and a wavelength $\lambda_0 = 550 nm$, the theoretical resolution is $d_z = 0.5 \mu m$.

### 3.2 Lateral resolution

The lateral resolution of an imaging system is commonly defined as the FWHM of the point-spread function. In a conventional diffraction-limited optical system, PSF can be expressed as the well-known Airy function, which depends on optical wavelength and the NA, so the lateral resolution $d_x$ is:

$$d_x = \frac{0.61\lambda_0}{\text{NA}}$$

(7)

In air ($n = 1$), with NA = 0.5 and a wavelength $\lambda_0 = 550 nm$, the theoretical resolution is $d_x = 0.7 \mu m$.

### 4. RESULTS

Initially, we imaged inside the Intel Pentium 4 processor to prove the high-resolution imaging capability of this system. Fig.3 (a) presents common microscopic image of the sample, Fig.3 (b)-(f) show 5-steps interferograms of the sample, Fig.4 gives *en-face* image of sample. After calculating, the width of the line pointed by the little white arrow in Fig.4 is 0.8 $\mu m$ and the area of entire chip is 75$\mu m \times 75\mu m$. Although light rays cannot go through the bottom of the chip, which means that it cannot realize the so-called ‘tomography’, the ups and downs of chip surface can be displayed by...
Then, we acquired images of biological tissues. *En-face* images of the onion cells are shown in Fig. 5. We can easily find the cell nucleus of onion cells inside the area marked by the white dotted frame in Fig 5(a), but as it came to deeper areas, nucleus disappeared in Fig 5(e)-(f) which indicated that, actually they were in different depths in micrometers and ‘tomography’ has been realized. If we capture stacks of *en-face* image in different depths, the 3D image with high-resolution can be realized.
Then, we focused on the junctions between onion cells. Fig.6 presents *en-face* image around the junction. We can see many choroids beyond the onion surface. The result demonstrates that our system takes advantage of high-resolution. 

Next, we obtained the images from unstained swine gastric pits in vitro. In the tissue, we can easily recognize the typical gastric pits of different depths within the white dotted frame of Fig.7 (a) and (b). In biomedical sciences, the identification of gastric pits would contribute to the diagnosis of common gastric mucosal lesions including atrophy, intestinal metaplasia and dysplasia\[^{15}\]. This research will definitely help doctors do quick diagnosis during emergency surgery.
Although FF-OCT has been previously utilized to assess histological features of ex vivo tissues, to our knowledge, there is no report about en face tomographic images of human liver cancer tissue, the death rate of liver cancer in Asia is very high due to the Asians’ food habits and customs. Unprocessed formalin-fixed ex vivo liver tissue sample from human, which contain both of normal and tumor parts, was imaged with our system. The goal was to differentiate the microstructures of liver cancer cells. The diameter of normal liver cells is about 20-30 \( \mu m \) with the shape of polygonal. However, hematomas of tumor cells have the feature of invasive growth, which makes the liver cells hard to distinguish \cite{16}. En-face images of the liver cells are shown in Fig 8. The shape of polygonal inside in the area marked by the white dotted frame can be seen clearly in Fig 8(a). The size of normal liver cell is 25 \( \mu m \) after calculation and it is consistent with the above description. The en-face images of hematomas of liver tumor in different depths are shown in Fig 8(b)-(c). It can be seen from Fig 8(b)-(c) that the microstructures of hematomas are almost in uniform distribution compared with the one in the normal cells with the shape of polygonal. The results demonstrates that our system has the capacity of distinguishing normal and cancer liver tissue.

Fig. 8 En-face images of liver tissue. (a) en-face image of normal liver; (b),(c) en-face images of hematomas of tumor cells in different depths
5. CONCLUSION

FF-OCT is a promising biomedical diagnosis methods without the need of staining, paraffining or frozen sectioning. Also it can be used to realize the non-invasive imaging. Due to its fast speed and high-resolution imaging, it has great significance for selecting the optimal treatment regimen.

We have designed and constructed a Linnik-type interference microscope which can obtain tomographic images. With high-NA objectives, the axial resolution of system is better than 0.5 μm and the lateral resolution is better than 0.7 μm which are similar to those of a confocal microscope and significantly higher than the resolution of conventional OCT scanners. Tomographic imaging is possible within scattering media such as biological tissues. The specificity of our system is that the acquisition of a complete en-face image is done in parallel with full-field illumination, without the need of scanning. We chose Intel Pentium 4 processor, onion cell, stomach and liver as tissues. The corresponding results verify the high-resolution capacity of FF-OCT and its potential in stomach clinical diagnosis such as atrophy, intestinal metaplasia, dysplasia and liver cancer. We foresee its application as an adjunct tool in tissue selection during biopsy procedure and bio-banking.

6. ACKNOWLEDGMENTS

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7. REFERENCE


