Development of High Speed 3D Tomographic Microscope for Non-invasive Monitoring of Biological Samples

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ABSTRACT

System design and implementation of a Fourier Domain Optical Coherence Tomography (FD-OCT) for microscopic flow monitoring is presented. The system is capable of capturing flow characteristics underneath the surface of biological samples at micrometer resolution. The high speed imaging capability allows for in vivo 3D mapping of micro-structure of biological tissues as well as their microvascular system. An image resolution of 10 microns over 1 mm depth from the sample surface and across a 10 mm x 10 mm lateral field-of-view is possible. The capability of the developed system for monitoring of flow activity within the heart of an African frog tadpole is demonstrated. In addition, a progress in development of a high speed FD-OCT based on our custom built high speed spectrometer is presented.

Keywords: Carotid pulse, Push pull effect, Piezoelectric sensor, Tilt table, Biological Engineering

1. INTRODUCTION

To date, optical imaging technology plays an important role in medical diagnostics and treatments. It also has applications in guiding the biopsy and surgery. The main advantages of optical imaging are its high-resolution high-speed and noninvasive capability. A non-invasive, reliable and affordable cost optical imaging system with the capability of detecting early stage of pathology would be a valuable tool to use for screening or detecting pathology. Optical coherence tomography (OCT)[1] is an emerging technology that is capable of noninvasive high-speed high-resolution cross-sectional imaging of biological tissues [2]. OCT is based on low-coherence interferometry (LCI) that takes advantage of the short coherence length of broadband light sources, which is in the order of microns, to achieve precise depth sectioning in scattering media. Analogous to ultrasound imaging, OCT illuminates biological sample with broadband near infrared light beam and measured the amplitude and depth location of the backscattered light and uses it to construct a cross-sectional image that reveals structure beneath the sample surface [3]. To date, OCT has been proven and recognized by physicians as a potential tool for medical diagnostics and research. Particularly in the field of ophthalmology, OCT has been established for early detection of many retinal pathologies such as glaucoma, diabetes, and age related macular degeneration [4-6].

Since the invention of the OCT, there are various implementations of OCT techniques. One technique in particular that push forward the advancement of OCT is the Fourier-domain optical coherence tomography (FD-OCT) [7]. The fundamental principle of FD-OCT is based on coherence theory in the frequency domain [8]. FD-OCT captures spectral interference at the output of an interferometer, e.g. Michelson interferometer, and then Fourier transform to obtain depth-resolved reflectivity profile along the incident beam path beneath the surface of the sample under test. Sequentially, performing 2D scanning of the laser beam across the sample’s surface allows nondestructive 3D reconstruction of sample microstructure. The main advantage over the time domain counterpart is that FD-OCT obtained the whole depth profile at once without scanning of the optical path length of the reference beam. Hence its imaging speed is dramatically improved.

Besides structural imaging, OCT is also capable of functional imaging such as bidirectional flow velocity mapping. Analogous to the flow measurement technique in Doppler ultrasonography, Doppler OCT (DOCT) is capable of in vivo detection of flow activity embedded beneath the surface of a fairly thick biological sample in high resolution and wide velocity dynamic range. DOCT allows visualization of tissue structure and blood flow activity that provides important information for clinical diagnostics. For example, vessel flow property is an early indicator of many retinal pathologies such as glaucoma, diabetes, and age related macular degeneration [6]. Moreover, detailed knowledge of in vivo blood flow under the skin surface is useful for burn-depth determination and port wine stains treatment [9]. Combining Doppler detection with FD-OCT enables imaging of microscopic flow, such as in vivo blood flow in capillary network, at high speed, which is particularly useful for real time flow monitoring purpose [10-12].

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2. MATHEMATICAL DESCRIPTION OF FD-OCT

FD-OCT is built on a concept of the detection of interference pattern of low-coherence light beam in spectral domain. A commonly used detection system is a high resolution and high speed spectrometer. Considering a system as shown in Fig. 1 for instance, a complex spectral electric field in the reference arm can be expressed as

$$^\wedge E_R(k) = ^\wedge K_R^\wedge E_0(k) r_R \exp(ikl_R) \quad (1)$$

where the caret denotes a function in the frequency domain, $k = \frac{2\pi}{\lambda}$ is the wave propagation number, $^\wedge E_0(k)$ represents the spectral electric field emitted from the light source, $K_R$ is a real number representing total losses in the reference path, $l_R$ is a round-trip optical path length along the reference arm, and $r_R$ is the reflectivity of the reference reflector [13].

In the sample arm of the system, the spectral electric field is a collection of many backscattering events happening at various depths of the sample that can be modeled as

$$^\wedge E_S(k) = K_S^\wedge E_0(k) \int_{-\infty}^{+\infty} r(l_S) \exp(ikl_S) dl_S \quad (2)$$

where $K_S$ is a real number representing total losses in the sample path, $l_S$ is a round-trip optical path length along the sample arm, and $r_S(l_S)$ represents the sample reflectivity profile along the depth as a function of $l_S$ [14]. Therefore, the spectral intensity as detected at the output of an interferometer is a superposition of the two signals that can be expressed as

$$^\wedge I_D(k) = \left| ^\wedge E_R(k) + ^\wedge E_S(k) \right|^2 = \left| ^\wedge E_0(k) \right|^2 \left( 2K_RK_Sr_R \int_{-\infty}^{+\infty} r_S(l_S) \exp(ik(l_S - l_R)) dl_S \right) + \left| K_S \int_{-\infty}^{+\infty} r_S(l_S) e^{-ikl_S} dl_S \right|^2 \quad (3)$$

The $1^{st}$ term is regarded as a DC-signal that can be removed through the direct subtraction method. The $3^{rd}$ term is an autocorrelation, which is regarded as noise. Nevertheless, when imaging most biological samples, the backscattering signal from the sample is usually much smaller than the reference signal (i.e. $r_S \ll r_R$) and hence the autocorrelation term is negligible. By defining the optical path length difference $l_D = l_S - l_R$, the overall constant factor $K = 2K_RK_Sr_R$, and ignoring the DC and autocorrelation terms ($1^{st}$ and $3^{rd}$ terms), the spectral interference signal can be reduced as

$$^\wedge I_{int}(k) = K \cdot \hat{S}(k) \cdot \int_{-\infty}^{+\infty} r_S(l_D) \cos(kl_D) dl_D \quad (4)$$

where $\hat{S}(k) = \left| ^\wedge E_0(k) \right|^2$ represents the power spectral density of the light source and $r_S(l_D)$ is the sample reflectivity profile as a function of the optical path length difference. Equ. (4) represents the spectral interference pattern as detected by the spectrometer. Without losing any general description, Equ. (4) can...
be rewritten in a complex form as

\[ \hat{I}_{\text{int}}(k) = K \cdot \hat{S}(k) \cdot \left[ \int_{-\infty}^{\infty} r_S(l_D) \exp(ikl_D)dl_D \right] \]  

Equ. (5) is now in the form of the Fourier transformation and can be written as

\[ \hat{I}_{\text{int}}(k) = K \cdot \hat{S}(k) \cdot [\Im \{r_S(l_D)\}] \]  

Consequently, the inverse Fourier transform of Equ. (6) yields OCT signal as

\[ I_{\text{OCT}}(l_D) = K \cdot \Im^{-1}\{\hat{S}(k)\} \ast r_S(l_D). \]  

Equ. (7) states that the sample reflectivity profile along depth, i.e. \( r_S(l_D) \), can be reconstructed by the inverse Fourier transform of the measured spectral interference signal. The term \( \Im \{\hat{S}(k)\} \) is known as the temporal coherence of the light source [8], which also serves as an axial point spread function of the FD-OCT system [3]. Combining this concept with the lateral scanning, 2D and 3D OCT images can be constructed.

3. SYSTEM AND METHOD

An OCT system used for collecting data in our early development of OCT-based flow imaging is a swept-source based FD-OCT system that is custom designed and built at the Optical Diagnostics and Applications Laboratory (ODALab) at the Institute of Optics, University of Rochester [10, 11, 13, 15]. The system is built on a fiber-based Mach-Zehnder interferometer as shown in Fig. 1 [11, 16]. Light from the laser is split by a 80/20 fiber coupler and then delivered to a sample and reference arms of the interferometer. Light in the sample arm is focused into a sample through the objective lens. Backscattered light from the sample is then collected and recombined with light from the reference arm at the 50/50 coupler.

The 3D scanning scheme is implemented using a dual axis galvanometer beam steering (VM500, GSI Lumonics). The spectral interference at the output of the interferometer is recorded while scanning the sample beam across the 2D surface of the sample. The captured interference signal is then streamed to the computer memory for processing. The data processing involved signal pretreatment and then Fourier transform to obtain depth profile, representing sample microstructure along the beam path. From the capturing FD-OCT dataset, flow information, such as location, velocity, direction, and profile, can be extracted through the detection of Doppler phase shift of two interference signals obtained at the same location [10, 11].

Two modes of operation of Doppler imaging are normally performed in DOCT. One is a brightness mode or B-Mode Doppler, in which multiple axial scans (A-scans) are collected while performing a lateral scan (B-scan). An intensity map generated in

**Fig.3:** Doppler color map of flow activity inside the heart chamber of an African frog tadpole superimposed with the structural map of the sample. The flow velocity corresponds with different color is designated by the color bar.

**Fig.4:** Graphic User Interface of the M-mode Doppler shows measurement of flow profile as a function of time, which can be served as optical cardiogram.
Fig. 5: The system layout of the new spectrometer-based FD-OCT at Suranaree University of Technology.

Fig. 6: 3D images of a cucumber section taken by the developed spectrometer-based FD-OCT at its current stage. (a) Volumetric rendering of the 3D dataset obtained with the system. (b) An example of en face reconstruction from the same 3D dataset, showing cellular level structure of the sample.

B-mode represents the cross-sectional image of the sample structure. Corresponding to the structural map, the magnitude of the local phase shift is represented in 2D color mapping. Therefore, B-Mode Doppler is useful for locating the flow location inside the mainly static structure.

The other is a motion mode or M-Mode Doppler, in which multiple A-scans are collected at a fixed position of the sample beam. M-Mode Doppler generates a 2D map of Doppler signal, in which one axis is a depth profile and the other axis represents the time evolution of the flow. M-Mode Doppler is useful when the location of the flow is known, and one wants to monitor the flow changes as a function of time.

4. RESULTS

The results demonstrated in this paper are progress results over the past three years of the development of OCT-based flow detection system at the ODALab at the University of Rochester. The first dataset demonstrates the capability of the system to optically and noninvasively perform depth-sectioning the sample at microns resolution. With the developed FD-OCT system, we acquired a 3D dataset of an African frog tadpole over a lateral scanning field-of-view (FOV) of 4 mm × 2 mm. The imaging FOV along the depth is about 1 mm. From the 3D OCT dataset, a series of en face images of the sample was reconstructed as shown in Fig. 2(a-h). Each en face image represents sample structure analogous to that observed under a light microscope. However, unlike a conventional microscope, OCT is capable of virtual depth-sectioning of living sample, i.e. microscopy-like image at different depth of up to 2 mm from the...
sample surface, nondestructively.

The second dataset demonstrates the performance of the system in detection of microscopic flow information. In FD-OCT, the phase information is immediately obtained after the Fourier transform, allowing the ease of determination of the amount of Doppler phase shift. One commonly used algorithm is the technique of modified Kasai autocorrelation [11, 17].

Fig. 3(a-c) shows Doppler signal, representing flow activity at different states of the contraction of the heart chamber of an African frog tadpole captured by our developed method [11]. The Doppler phase shift was displayed in colors map, ranging from -2.7 mm/s to +2.7 mm/s, as designated by the color bar. The plus and minus signs represent flow in opposite direction, and hence red and blue regions in Fig.3(a-c) represent flow activity in opposite directions (i.e. inflow and outflow). It should be noted that, with high speed imaging capability, the system is capable of real time acquisition and display of flow activity at currently about 3-4 frame/s.

Moreover, in a similar manner with ultrasound Doppler, M-mode Doppler OCT was performed by fixing the lateral position of the sample beam as shown in Fig. 4(a) and acquiring multiple depth scans over time and then computing the Doppler phase shift between consecutive scans as shown in Fig. 4(b). From the M-mode Doppler map in Fig. 4(b), a flow profile as a function of depth (Fig. 4c) and a flow profile as a function of time (Fig. 4d) were extracted. The flow profile as a function of time as shown in Fig. 4(d) can be used as optical cardiogram for monitoring flow activity of in vivo biological samples.

5. DEVELOPMENT OF NEW FD-OCT SYSTEM

As part of a plan to improve Doppler flow imaging using FD-OCT, particularly for real time flow monitoring purpose, we are developing a new FD-OCT system at Suranaree University of Technology. The new system is a spectrometer-based FD-OCT as shown in Fig. 5. The light source is a super luminescent diode (SLD) that emits a broad spectral light, expanding from 800-900 nm output wavelength. An interferometer is a fiber-based Michelson interferometer with 50/50 split ratio. The detector is a custom built spectrometer that was designed and built in our laboratory, utilizing a high speed CMOS line camera with data capturing speed of over 70,000 lines/second. A 3D data acquisition is achieved by a dual mirror galvanometer beam steering, which is synchronizes with data capturing from the CMOS camera through a PCI express interface high speed frame grabber device. The overall imaging speed is currently 20 frame/second for a frame size of 500 spectra/frame (i.e. about 10,000 spectra/second). The operated speed is currently limited by hardware synchronization, which will be further optimized to achieve maximum speed as provided by the camera. Both lateral and axial resolutions of the system are currently about 20 microns. A scanning field of view is up to 10 mm × 10 mm and about 2 mm imaging depth.

Fig. 6 shows an early result on 3D microscopic imaging of biological sample, which is a cucumber section, using the developed spectrometer-based FD-OCT. An imaging FOV was about 10 mm × 10 mm, consisting 500 × 500 depth scans. Even though, the new system is currently operated at 80% of its full performance, the microscopic structure at cellular level is readily observed. Further improvement in term of imaging speed and resolution is under investigation. Fig. 6(a) shows volumetric rendering of the 3D dataset acquired by the new system, demonstrating its capability for 3D visualization at microscopic level. Fig. 6(b) is an en face reconstruction from the same 3D dataset, showing structural information at certain depth beneath the sample surface without actual sectioning. The en face reconstruction provides an image similar to that can be obtained by a confocal microscope. However, with high resolution depth sectioning capability of OCT, multiple en face reconstructions at different depths can be digitally obtained at the depth resolution of less than 10 microns.

6. SUMMARY

OCT technology, particularly FD-OCT, has been proven to be a useful tool for not only in vivo visualization of microstructure of biological sample but also for in vivo monitoring of flow activity within the sample. Flow activity serves as valuable information in diagnosis of the functionality and abnormality of in vivo biological tissue since most pathological development is related to the change in blood circulation system. The technique demonstrated here is only one of the methods of OCT-based flow imaging techniques. Over the past several years, there are many techniques have been developed. Combining of multiple techniques will allow for faster, more sensitive, and more precise detection of flow activity in living tissue, enabling a path for early diagnosis of many pathological development. In addition, we reported the progress of the development of our new spectrometer-based FD-OCT system. The developing system has potential for high speed Doppler flow imaging that will be particularly useful for application that requires real time monitoring of blood flow, such as monitoring a circulation system in biological samples.

Most typical commercial OCT FD-OCT systems are currently operated at imaging speed of 20 30 frames per second, which is still far from ideal for a snap-shot of 3D imaging. In this work, the proposed FD-OCT was designed to be capable of high resolution 3D imaging at high speed data acquisition of up to 200 frames per second, enabling by a high speed and high throughput line-scan CMOS sensor technology. Nevertheless, the first implemented prototype is currently operated at acquisition speed of
about 20 frames per second for up to 1000 depth scans per frame, which is limited by the triggering speed of the scanning waveform generator for driving the 3D scanning unit at the sample arm of the prototype. This speed will be improved in the future by using a higher performance device for the waveform generator to control the 3D sample scanning. At its maximum potential speed, the prototype is expected to be able to capture a single 3D dataset within about 3 seconds.

7. ACKNOWLEDGEMENT

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References


