Mirau-based full-field time-domain optical coherence tomography using Ce\textsuperscript{3+}:YAG crystal fiber light source

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ABSTRACT

Based on single-objective construction utilizing high brightness Ce\textsuperscript{3+}:YAG single-clad crystal fiber light source, this Mirau-based full-field time-domain optical coherence tomography with circular polarization incident light represents deeper penetration in scattering medium. Using objective-changeable ability of home-designed Mirau objective, this system provides different applications, like biological tissue and single cells, by different spatial resolution with corresponding dynamics. High quality image relying on less ghost image and near common-path interference was demonstrated under this compact and power-stable system.

Keywords: Optical coherence tomography, medical and biological imaging, interference microscopy

1. INTRODUCTION

Typically, fields of optical coherence tomography (OCT) can be divided into time-domain (TD) \cite{1} and frequency-domain (FD) OCT \cite{2}. FD-OCT is with good performance of high-speed scanning and high signal-to-noise ratio (SNR), but it has worse transversal resolution. TD-OCT can be operated by photo-detector \cite{1} and CCD camera \cite{3} as respectively single-point (SP) and full-field (FF) modes. Though SP-TD-OCT has higher dynamic range due to photodetector, but both the scan speed and transversal resolution are not enough. Via FF-TD-OCT, the scan speed and transversal resolution is highly improved. Compared to FD-OCT, FF-TD-OCT is much-suitable to be applied on bio-tissue and cellular imaging reconstruction \cite{3}.

In this article, a Ce\textsuperscript{3+}:YAG single-clad crystal fiber (SCF) pumped by 446 nm laser diode illuminates a broadband light at 560-nm central wavelength and with 95-nm bandwidth \cite{4}. The core and clad diameters of Ce\textsuperscript{3+}:YAG SCF are individually 70 \(\mu\)m and 330 \(\mu\)m. The Ce\textsuperscript{3+}:YAG single crystal, drawn by laser-heated pedestal growth (LHPG) \cite{5} and clad by borosilicate glass, provides high brightness and high numerical aperture output light. This is very suitable for FF-TD-OCT. In the experiment, the crystal-fiber-based FF-TD-OCT provides resolutions of 1.4 \(\mu\)m and 1 \(\mu\)m respectively in axial and transversal directions in air using 20X objective lens. Because of Gaussian-like broadband spectrum \cite{4}, the ghost image effect coming from side-band noise is suppressed, to produce high quality image. With circularly polarized light, the penetration depth becomes better \cite{6}.

2. OPTICAL SETUP AND WORKING PRINCIPLE

As shown in Fig. 1(a), the Ce\textsuperscript{3+}:YAG SCF pumped by 1.4 W blue laser diode illuminates a broadband light from the terminal of the core. After LWPF, the residual blue light was filtered out, and the broadband light was incident into the BPCB. The vertically polarized light became clockwise polarization light after AQWP. Via reciprocal path of reflection, the vertically polarized light became horizontal polarization, and then straight passed through the BPCB to the CCD after L3. Figure 1(b) shows the home-made Mirau-based objective lens. The 50/50 BBC was coated at the lower surface of the 2-nd GP. Surely, it is capable to use different ratio of BBC to enhance the weak signal for this objective lens. Besides, the natural 4\% reflection of 1-st GP from the reference arm can avoid from the power saturation of CCD, and ARCs of the GPs were to eliminate the stray light back to the CCD. 96\% transmitted light was absorbed by B, where B has refractive index near GP.
Following the properties of TD-OCT, assume the sampling points of single interferometric carrier wave are \( N \), the envelope intensity \( I(x,y,z_0)_{env} \) of the single carrier wave can be calculated according to Eq. (1).

\[
I(x,y,z_0)_{env} = \frac{\sqrt{2P}}{2N} \sum_{i,j=1}^{N/P} \left| I_i(x,y) - I_j(x,y) \right|^2, \tag{1}
\]

where \( z_0 \) is the central position of the calculated single carrier wave. \( I_i(x,y) \) and \( I_j(x,y) \) are two averaged sampling intensity of the \( N/P \) intervals. \( P \) is the sampling points in one interval. The calculated result of Eq. (1) is insensitive of the phase of the single carrier wave, which was derived from de-Movier’s and Phasor theorems, where \( N/P \geq 3 \) must be existed. That is why Eq. (1) can be applied on this continuous scanning TD-OCT without any Hilbert transform and band-pass filter to detect the envelope of interferometric carrier wave. As \( N \) increases, the SNR will correspondingly increases, as well. In this experiment, \( N/P=4 \) was used to reconstruct the 3-dimensional (3D) morphology of tissue and single cell, and the calculated four point for Eq. (1) was dominated from four equivalent intervals of one full carrier wave.

3. EXPERIMENTAL RESULTS

Figure 2 (a) shows the epidermal tissue of a human being (female, 1963) by transmission mode of Fig. 1(a). Transmission mode means a white light source is incident from top onto the sample, and \( L_2 \) collects the transmitting scatter light through the sample (see Fig. 1(a)). At this condition, the used \( L_2 \) was 10X objective lens, and the transversal resolution is about \( 1.4 \) \( \mu \)m. In Fig. 2(a), the image was filled with dead keratinocytes. At the central area of the sample, the cellular pile is quite loose; whereas, it becomes condensed when the position leaves from the central area. From traditional microscope, it is very difficult to find this morphologic phenomenon because the image is in a mass. The typical shapes of the aged keratinocytes are almost dish-like with rigid structure. For this sample, most of the aged keratinocytes are dead, where the nuclei are almost degraded. So, seldom nuclei can be found inside this sample. The positions pointed by the vectors in Figs. 2(c) and 2(d) are the same atrophied nucleus.

The nucleus of the aged keratinocyte versus dead one is contrastively obvious, even the nucleolus of Figs. 3(c) and 3(d) can be observed by logarithmic scale. Relying on the observation of nuclei in the cells, cellular state can be identified. This method may be useful for identifying the cell growth and death of keratinocytes.
Figure 2. (a) The schematic experimental setup of single lens FF-TD-OCT. LD: 445-nm laser diode; CM: collimation module; SCF: single-clad crystal fiber; L1: 60X objective lens; LWP: long-wave-pass filter; BPCB: broadband polarizing cubic beamsplitter; M: mirror; AQWP: achromatic quarter wave plate; PZT: piezo-electric transducer; L2: home-designed Mirau-based objective lens; S: formalin-immersed sample; GP: glass plate; LS: transversally moved linear stage; W: water; L3: tube lens; CCD: charge-coupled device camera. (b) The schematic figure of L2. OL: objective lens; GP: glass plate; BBC: broadband beamsplitter coating; ARC: anti-reflection coating; RH: ring holder; B: black blocker.

Figure 3. (a) The transmission image of segregated aged keratinocyte via FF-TD-OCT. (b) Oblique view of the 3D morphology of the sample (112 μm (L) X 85 μm (W) X 80 μm (H)), where (c) and (d) are respectively the en-face and tomographic images of (b). All the scale bars are 20 μm. The nucleus of the segregated aged keratinocyte (pointed position) is at the depth of 8.4 μm.

4. CONCLUSION

This Mirau-based FF-TD-OCT was successfully applied on the bio-tissue and single cell 3D reconstruction. With home-made Ce\(^3+:\)YAG SCF broadband light source and lens-selectable Mirau-based objective lens, this FF-TD-OCT system...
become compact and easily used. It only takes 2 minutes to scan and reconstruct cuboid (225 μm (L) X 170 μm (W) X 80 μm (H)) morphology with 1 μm isotropic resolution in tissue. The growth and death of keratinocytes are also verified by this system.

REFERENCES


